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Carbohydrate Chemistry

VOLUME 13

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Preface

This Report, the thirteenth in the Series, covers the new literature available to us between January and December, 1979. Again we have continued to produce a comprehensive survey of publications on carbohydrate chemistry in a concise and readable form.

The absence of response to our previous invitations for constructive comments on the content of the Report we hope indicates a general satisfaction. Nevertheless, we have taken very seriously the legitimate comments received about delays in publication of current volumes of the Series. Whereas apportionment of blame in retrospect is neither appropriate nor productive, it is to be hoped that Volumes 13, 14, and (maybe even) 15 will have been published in 1982. Volume 15 will be published in Parts I and II, corresponding to the present Parts I and II, which will appear as separate books, produced from camera ready copy.

It is a pleasure to welcome Dr A. C. Richardson as a reporter for Part I and we wish to thank Dr R. Gigg for assistance with the literature survey. It is also a pleasure to acknowledge the willing secretarial and administrative assistance of Miss Vivienne J. Hunt.

Finally, the services provided by Dr P. G. Gardam (Manager, Books) and his staff, particularly Mr K. J. Wilkinson, at the Royal Society of Chemistry in the production of this Report are much appreciated.

November 1981

JOHN F. KENNEDY
N. R. WILLIAMS

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Abbreviations

The following abbreviations have been used:

ADP	adenosine diphosphate
ATP	adenosine triphosphate
c.d.	circular dichroism
CDP	cytidine diphosphate
CMP	cytidine monophosphate
DBU	1,5-diazobicyclo[5,4,0]undec-5-ene
DCC	dicyclohexylcarbodi-imide
DEAE	diethylaminoethyl
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dpm	dipivaloylmethanato
e.s.r.	electron spin resonance
fod	2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedionato
g.l.c.	gas–liquid chromatography
HMPT	hexamethylphosphotriamide
i.r.	infrared
NBS	*N*-bromosuccinimide
n.m.r.	nuclear magnetic resonance
o.r.d.	optical rotatory dispersion
py	pyridine
RNA	ribonucleic acid
THF	tetrahydrofuran
Thp	tetrahydropyranyl
TMS	trimethylsilyl
UDP	uridine diphosphate

Part I

MONO-, DI-, AND TRI-SACCHARIDES AND THEIR DERIVATIVES

By
B. E. Davison
R. J. Ferrier
A. C. Richardson
N. R. Williams

1

Introduction

Interest in the chemistry of mono- and di-saccharides continues unabated, with nearly 1300 references being mentioned in Part I of this report. Glycosides alone account for nearly 200 references, in part reflecting both the expanding range of glycosidic natural products being discovered, and the increasing study of di- and tri-saccharides following the advent in recent years of several efficient methods for the synthesis of α-glycosides. Likewise the range of complex carbohydrate-containing antibiotics shows no signs of reaching a limit, posing fresh challenges to synthesis which chemists have eagerly accepted; two elegant syntheses of spectinomycin are reported in Chapter 18. There is also great interest being shown in glucose as a convenient asymmetric source material for the synthesis of a wide range of chiral natural products, which is reflected in Chapter 23.

A text on aspects of asymmetry in carbohydrates has appeared, which includes reviews on the composition of reducing sugars in solution, asymmetric reactions of carbohydrates containing carbonyl groups, and prochirality and pseudoasymmetry in carbohydrate biochemistry, besides articles on nitro-sugar stereochemistry, chiral sulphur derivatives, and carbohydrate crown-ether compounds.[1] A general survey of organic chemistry includes an extensive account of monosaccharide chemistry.[2]

The latest issue in the series 'Advances in Carbohydrate Chemistry and Biochemistry' contains appreciations of the late Professors J. A. Mills[3] and J. V. Karabinos,[4] and includes a review on the chemistry of glycosiduronic acids.[5]

[1] 'Asymmetry in Carbohydrates', ed. R. E. Harmon, R. E. Dekker, New York, 1979.
[2] L. Hough and A. C. Richardson, in 'Comprehensive Organic Chemistry', vol. 5, ed. E. Haslam, Pergamon, Oxford, 1979, p. 687.
[3] S. J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 1979, **36**, 1.
[4] W. W. Binkley, *Adv. Carbohydr. Chem. Biochem.*, 1979, **36**, 9.
[5] D. Keglević, *Adv. Carbohydr. Chem. Biochem.*, 1979, **36**, 57.

2
Free Sugars

The composition of reducing sugars in aqueous solution has been surveyed.[1] A review on the structural properties of the anomeric centre in pyranoses and pyranosides has appeared.[2] Three reviews concerning properties of fructose have been published, dealing with its metabolism,[3] its chemical structure and properties,[4] and its chemistry with some emphasis on the relationship between molecular structure and calculated and experimental values of optical activity, and such properties as taste.[5] The production of nutritive sweeteners by acidic and enzymic hydrolysis of starch has been reviewed.[6]

1 Isolation and Synthesis

Analyses by t.l.c. and g.l.c. of honeys from Robinia spp. and honeydew have shown that the former contains evlose and the latter melezitose as well as the usual mono- and di-saccharides.[7] 3-Deoxytetrulose, useful for synthesis of 2-hydroxybutanoic acid, has been isolated in 4% yield from sodium bicarbonate treatment of xylan.[8] Incubation of *Aspergillus fumigatus* with inulin produces di-D-fructofuranose 1,2′:2,1′-dianhydride.[9] An improved method for the preparation of crystalline β-lactose from heating a supersaturated solution of α-lactose hydrate has been reported. Differential scanning calorimetry was used to determine its true melting point.[10] Improved yields of β-lactose have also been claimed from treatment of α-lactose hydrate with 0.02% sodium hydroxide in alcohols for 2 h. Methanol gave the best yields provided water content was below 10%.[11] A blue-green alga, *Agmenellum quadruplicatum*, has been used to prepare ^{13}C-labelled D-glucose, 2-*O*-α-D-glucosyl glycerol, 2-*O*-α-D-glucosyl glyceric acid, and sucrose by photobiosynthesis from 20 mole % $^{13}CO_2$ as carbon foodstock.[12]

[1] S. J. Angyal, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, 6, 15.
[2] G. A. Jeffrey, in 'Anomeric Effect', American Chemical Society Symposia, No. 87, 1979, p. 50.
[3] M. Chen and R. L. Whistler, *Adv. Carbohydr. Chem. Biochem.*, 1977, 34, 286.
[4] K. Heyns, *Staerke*, 1978, 30, 345 (*Chem. Abs.*, 1979, 90, 23 416*n*).
[5] R. S. Shallenberger, *Pure Appl. Chem.*, 1978, 50, 1409 (*Chem. Abs.*, 1979, 91, 157 986*c*).
[6] R. V. MacAllister, *Adv. Carbohydr. Chem. Biochem.*, 1979, 36, 15.
[7] F. Belliardo, M. Buffa, A. Patetta, and A. Manino, *Carbohydr. Res.*, 1979, 71, 335.
[8] L.-Å. Lindström, *Carbohydr. Res.*, 1979, 69, 269.
[9] K. Tanaka, K. Sonobe, T. Uchiyama, and T. Matsuyama, *Carbohydr. Res.*, 1979, 75, 340.
[10] T. Ito, M. Kato, and S. Adachi, *J. Dairy Res.*, 1978, 45, 363 (*Chem. Abs.*, 1979, 90, 121 902*n*).
[11] A. Olano, *J. Dairy Sci.*, 1978, 61, 1622 (*Chem. Abs.*, 1979, 90, 104 240*c*).
[12] V. H. Kollman, J. L. Hanners, R. E. London, E. G. Adame, and T. E. Walker, *Carbohydr. Res.*, 1979, 73, 193.

A further paper on the cold-plasma decomposition of methane–water mixtures in the presence of apatite has appeared (see vol. 12, p. 6). Glucose 6-phosphate and ribose 5-phosphate were found among the products.[13]

Reports on further studies of the formose reaction continue to appear. An investigation of solid catalysts has shown that selectivity can be achieved using a tobermorite or calcium oxalate; the major products were branched-chain sugar alcohols, 2-hydroxymethylglycerol, and 2,4-bis(hydroxymethyl)- and 3-hydroxymethyl-pentitol.[14] The same group has studied the effect of different solvent systems on the reaction. The calcium oxide-catalysed reaction was found to require a hydroxylic solvent and the induction period was shown to be related to the concentration of dissolved Ca^{2+} ion. The reaction in 90% and 100% methanol was analysed in detail; the formation of formaldehyde hemiacetal was shown to be essential, and competition between the Cannizzaro reactions and sugar formation was the important factor affecting sugar yield.[15,16] High temperatures have been shown to produce hexoses from formaldehyde–calcium hydroxide. At 98 °C the induction period was 15 s and the reaction gave 84% hexoses at 18% formaldehyde conversion, of which 90% was glucose. No branched-chain sugars were detected. Conversion was complete within 3 min. Higher conversions or lower temperatures gave more complex mixtures.[17] The calcium hydroxide-catalysed reaction has been studied to determine the effects of pH and catalyst–formaldehyde ratio; a greater proportion of carbohydrate is produced when the latter is higher. With lower catalyst concentrations Cannizzaro products become dominant.[18] Three 2-deoxy-sugars were formed in a one-step crossed formose reaction of acetaldehyde and formaldehyde.[19]

High performance liquid chromatography has been used to study the mechanism and kinetics of the formose reaction. The mechanism proposed is shown in Scheme 1. Previous emphasis on mixed aldol condensations between C_2 and C_3 fragments is not supported, these providing only minor pathways.[20]

The Fischer–Kiliani synthesis has been used to prepare ^{13}C-enriched carbohydrates.[21–23] The nitriles formed using Na ^{13}CN were reduced over Pd–$BaSO_4$ at pH 4.2 and 25 °C to give pentoses and hexoses in 60–90% yield. 1-Amino-1-deoxyalditols were produced in ~10% yield and their formation was favoured

[13] C. O. Simionescu, S. Dumitriu, V. Bulacovschi, and V. I. Popa, *Cellulose Chem. Technol.*, 1978, **12**, 585 (*Chem. Abs.*, 1979, **90**, 72 394*n*).

[14] Y. Shigemasa, H. Komaki, E. Waki, and R. Nakashima, *Tottori Daigaku Nogakubu Kenkyu Hokoku*, 1978, **9**, 116 (*Chem. Abs.*, 1979, **91**, 57 347*b*).

[15] Y. Shigemasa, T. Taji, C. Sakazawa, R. Nakashima, and T. Matsuura, *J. Catal.*, 1979, **58**, 296 (*Chem. Abs.*, 1979, **91**, 123 942*v*).

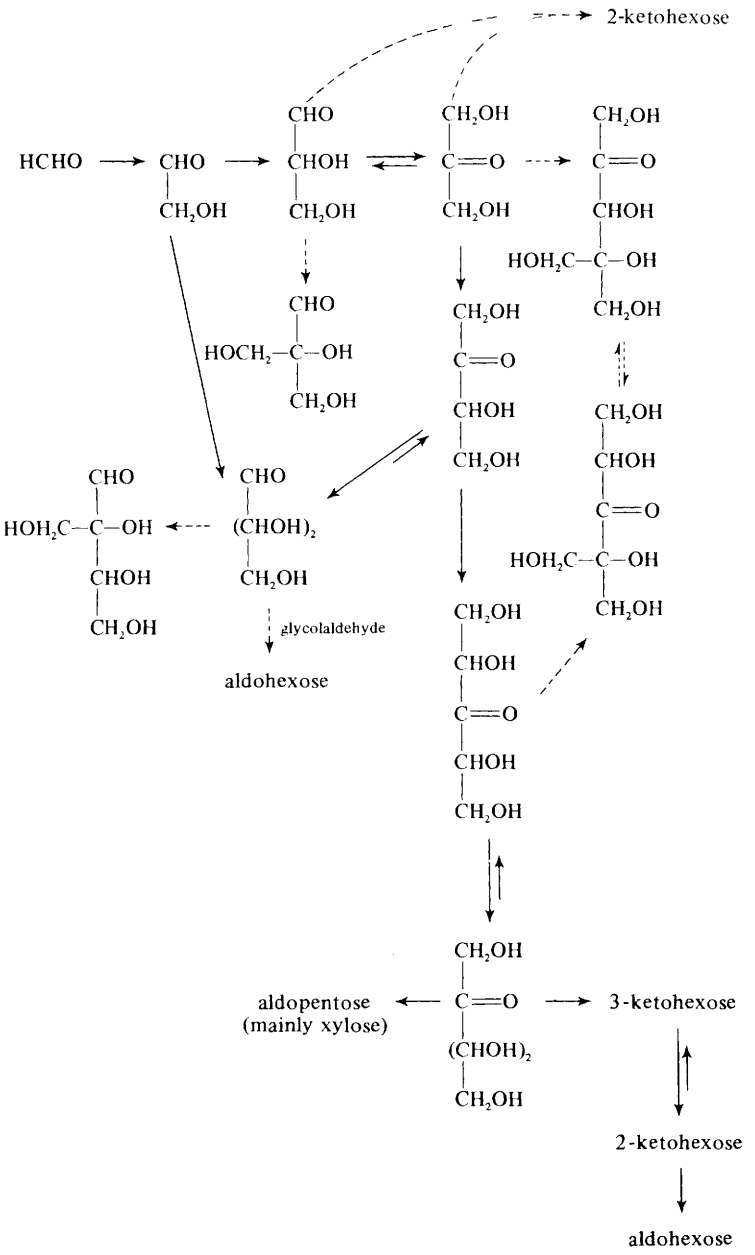
[16] Y. Shigemasa, Y. Matsuda, C. Sakazawa, R. Nakashima, and T. Matsuura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1091.

[17] V. A. Likholobov, A. H. Weiss, and M. M. Sakharov, *React. Kinet. Catal. Lett.*, 1978, **8**, 155 (*Chem. Abs.*, 1979, **90**, 23 425*g*).

[18] A. H. Weiss, V. A. Seleznev, and R. Partridge, *Catal. Org. Synth.* [*Conf.*] 6th, 1976, ed. G. V. Smith, Academic, New York, 1977, p. 153.

[19] T. Mizuno, S. Goda, K. Kanemitsu, and K. Utumi, *Shizucka Daigaku Nogakubu Kenkyu Hokoku*, 1977, **27**, 91 (*Chem. Abs.*, 1979, **90**, 138 108*f*).

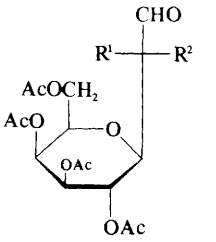
[20] G. Harsch, W. Voelter, and H. Bauer, *Z. Naturforsch., Teil B: Anorg. Chem., Org. Chem.*, 1977, **32**, 826.



Main routes, ——→ ; minor routes, - - - →

Scheme 1

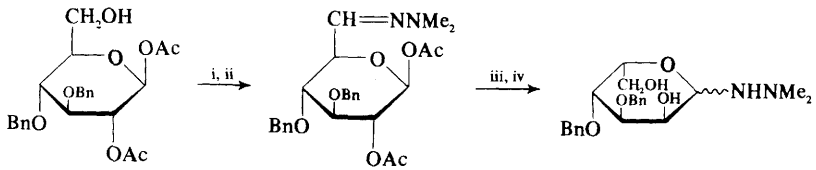
when hemiacetal formation was hindered in the parent aldoses.[21] Preparation of glycolaldehyde, glyceraldehyde, erythrose, and threose with ^{13}C-enrichment at various specific positions for use in determining proportions of cyclic hemiacetal and linear *gem*-diol by means of n.m.r., has been reported.[22] The same group has described systems for multilabelling of aldoses using K ^{13}CN and K ^{14}CN by sequential addition and reduction of the nitrile with deuterium gas. Compounds prepared include DL-[1-^{14}C,^{2}H]glyceraldehyde, D-[1-^{13}C,^{2}H]erythrose, D-[1-^{13}C,^{2}H]threose, D-[2-^{13}C,^{2}H]ribose, D-[2-^{13}C,^{2}H]arabinose, methyl α- and β-D-[2-^{13}C,^{2}H]ribofuranoside, DL-[3-^{2}H]erythrose, DL-[3-^{2}H]threose, DL-[1-^{13}C,^{2}H]glyceraldehyde 3-phosphate, and D-[1-^{13}C,^{2}H]ribose 5-phosphate.[23] The cyanohydrin synthesis has been used in the preparation of 2,4,5,6,8-penta-*O*-acetyl-3,7-anhydro-D-*threo*-L-*talo*-(1) and -L-*galacto*-octose (2), utilizing the reduction procedure due to Moffatt *et al.* (see vol. 7, p. 27).[24]



(1) R^1 = OAc, R^2 = H
(2) R^1 = H, R^2 = OAc

Aldol condensations analogous to the formose reaction have been utilized to synthesise mixtures of trioses, tetroses, pentoses, and hexoses from 3-oxo compounds. The same paper describes the synthesis of hexoses from barium hydroxide treatment of acrolein dibromide.[19]

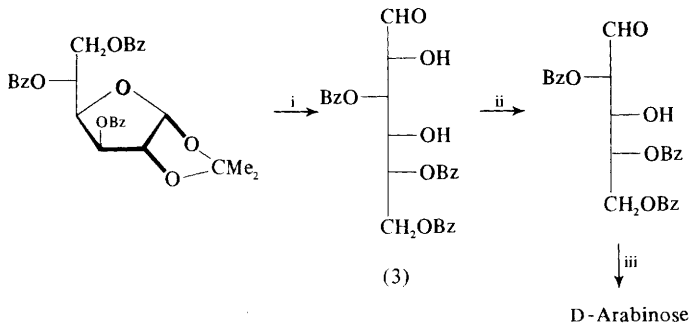
L-Gulose, required for synthetic work associated with bleomycin, has been prepared by a new route from D-glucose (Scheme 2).[25] A new synthesis of D-arabinose from D-glucose involves periodate oxidation of the glucose derivative (3) (Scheme 3). The reaction is thought to be general for all aldoses that form 1,2-*O*-isopropylidene derivatives.[26]



Reagents: i, NCS–Me_2S; ii, NH_2NMe_2; iii, NaOMe; iv, $NaBH_4$

Scheme 2

[21] A. S. Serianni, H. A. Nunez, and R. Barker, *Carbohydr. Res.*, 1979, **72**, 71.
[22] A. S. Serianni, E. L. Clark, and R. Barker, *Carbohydr. Res.*, 1979, **72**, 79.
[23] A. S. Serianni and R. Barker, *Can. J. Chem.*, 1979, **57**, 3160.
[24] H. Fritz, J. Lehmann, and P. Schlesselmann, *Carbohydr. Res.*, 1979, **74**, 309.
[25] D. K. Minster and S. M. Hecht, *J. Org. Chem.*, 1978, **43**, 3987.
[26] M. A. E. Sallam, S. Singaram, and U. Hornemann, *Carbohydr. Res.*, 1979, **73**, 320.



Reagents: i, AcOH–HCl; ii, $NaIO_4$; iii, NaOMe

Scheme 3

The formation of higher-sugar dialdose derivatives by dimerization of terminal acetylenic sugars and polymerization of enosuloses is covered in Chapter 12.

2 Physical Measurements

In an attempt to correlate gross structure of solute–water with packing and stereochemistry, the partial molal volumes and isentropic partial molal compressibilities were measured for sugars, uronic acids, and some di- and trisaccharides in water at 25 °C. Attempts to systematize the results were unsuccessful.[27] In a different approach, the structural transitions at saturation temperature using Arrhenius plots of results obtained by conductance measurements on electrolyte–sucrose–water solution were determined. These transitions were postulated to be due to the ability of the sucrose to form intermolecular hydrogen bonds with the solvent, so that at saturation temperature it was considered to have entered the total structure of the solution.[28]

The bonding of glucose to a yeast α-glucosidase has been studied using a fluorescent probe complexed to the enzyme.[29]

Isotope effects in the acid-catalysed mutarotation of D-glucose have been studied by polarography in the presence and absence of acetone with either water or deuterium oxide solvent. It was concluded that the difference in isotope effects for the acetone–water reaction from that in water alone was due to the rate-determining step in the former cleavage being of the C–H bond of the enol form of acetone, whereas in water alone the rate determining step involved cleavage of the O–H bond at C-1.[30] Two papers on the effect of Cu^{2+} and Ca^{2+} ions on the rate of mutarotation of D-glucose have appeared. The first-order rate constant was shown to be independent of pH in the range 3.0 to 6.2, but increasing the temperature changed the equilibrium concentrations.

[27] H. Hoeiland and H. Holvik, *J. Solution Chem.*, 1978, **7**, 587 (*Chem. Abs.*, 1979, **90**, 39 157*q*).

[28] Vishnu and A. K. Singh, *Carbohydr. Res.*, 1979, **70**, 13.

[29] M.-R. Siro and T. Lövgren, *Acta Chem. Scand., Ser. B*, 1978, **32**, 451.

[30] A. Nimara, A. Balint, E. Princz, A. Calusaru, and C. Dragulescu, *Rev. Roum. Chim.*, 1979, **24**, 337 (*Chem. Abs.*, 1979, **81**, 39 745*a*).

The calculated thermodynamic parameters indicated that Cu^{2+} complexed at C-1.[31,32] Studies on the mutarotation of α- and β-D-glucose in gels of poly(2-hydroxyethylmethacrylate) using polarimetry have been reported. The effect of incorporation of 4-vinylpyridine as co-polymer was investigated. With the co-polymer the rate approximated to that of the reaction in bulk water; in its absence the rate was essentially zero. The results were interpreted as arising from the difference in activity of water in the gels with or without 4-vinylpyridine.[33] The ionization and mutarotation of D-glucose, D-mannose, and D-fructose in aqueous alkali has been examined using ^{13}C n.m.r. By means of curves of ^{13}C n.m.r. shifts with increasing pH (^{13}C-n.m.r. 'titration') pK_a values and differences in acid strengths of the α- and β-anomers were determined. The shifts were not explainable by simple ionization but probably originate in a rapid equilibrium between cyclic and acyclic rotamer ionized forms; base catalysis thus causes a weakening of the ring C–O bond in the generated sugar anion.[34] As models for the analysis of oligosaccharides, the ^1H n.m.r. spectra of many pentoses, hexoses, glucosamine, galactosamine, fucose, and several di- and tri-saccharides in D_2O have been determined.[35] (For an account of the application of the method to oligosaccharide analysis see Chapter 20.)

The proportions of open-chain forms in fructose and sorbose in aqueous solution have been determined at 80 °C using ^{13}C n.m.r. to detect the carbonyl group. The proportions were 3 and 2% respectively.[36] Laser Raman spectroscopy has been shown to distinguish the five- and six-membered rings of D-fructose in aqueous solution. In agreement with other methods the furanose–pyranose ratio was found[37] to be 41:59. The temperature-dependence of the pyranose–furanose equilibria in aqueous solution has been studied by g.l.c. Equilibration at 0 °C gave the following proportions: β-pyranose, 84.8%; α-furanose, 4.1%; β-furanose, 11.1%; at 50 °C the proportions were 55.8, 12.3, and 31.9% respectively. Similar isomerizations were found to occur on melting.[38]

The irradiation effects of ^{60}Co γ-rays on crystalline mannose have been studied by e.s.r. to investigate how the nature and concentration of unpaired electron species trapped in the solid vary with dose and period of storage at room temperature. The results were compared with those of lyoluminescence studies.[39] Sucrose and rhamnose crystals have been shown to trap electrons

[31] P. A. Bobbio, F. O. Bobbio, L. R. Rodrigues, and F. G. R. Reyes, *An. Acad. Bras. Cienc.*, 1978, **50**, 255 (*Chem. Abs.*, 1979, **90**, 39 134*e*).

[32] P. A. Bobbio, F. O. Bobbio, L. R. Rodrigues, and A. P. Chagas, *An. Acad. Bras. Cienc.*, 1978, **50**, 315 (*Chem. Abs.*, 1979, **90**, 87 765*h*).

[33] D. G. Mercer and K. F. O'Driscoll, *J. Mol. Catal.*, 1978, **4**, 217 (*Chem. Abs.*, 1979, **90**, 55 173*x*).

[34] G. deWit, A. P. G. Kieboom, and H. Van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 1979, **98**, 355 (*Chem. Abs.*, 1979, **91**, 91 860*y*).

[35] J. H. Bradbury and J. G. Collins, *Carbohydr. Res.*, 1979, **71**, 15.

[36] W. Funcke, C. von Sonntag, and C. Triantaphylides, *Carbohydr. Res.*, 1979, **75**, 305.

[37] M. Mathouthi, C. Luu, and D.-V. Luh, *C.R. Hebd. Seances Acad. Sci., Ser. C*, 1979, **288** 359.

[38] E. O. Farhoudi and W. Mauch, *Forschringsber.-Inst. Zuckerind. Berlin*, 1976, **4**, 1 (*Chem. Abs.*, 1978, **89**, 163 862*j*).

[39] D. T. Bartlett and J. K. Brown, *Radiat. Eff.*, 1979, **41**, 43 (*Chem. Abs.*, 1979, **91**, 123 943*w*).

intermolecularly when *X*-irradiated at low temperature. The hyperfine couplings of the electron with the protons of hydroxy-groups forming the trap and the relative stabilities of the trapped electron were determined.[40] A similar study was performed on dulcitol and L-arabinose at 4.2 K.[41] Sugar radicals, formed by u.v. irradiation between 200 and 300 nm, have been shown to be stable at room temperature and were able to initiate polymerization of acrylamide in water. The e.s.r intensity was in the order sucrose > methyl α-D-glucopyranoside ⩾ cellobiose > D-xylose > D-glucose ≈ D-fructose and this was reflected in their relative abilities to initiate the acrylamide polymerization.[42]

The rate of protonation of the hydroxy-groups of D-glucose in DMSO have been measured by following the chemical shifts of the protons of all hydroxy-groups and the coupling $J_{OH,CH}$ for α- and β-D-glucose. The results showed $k_{O-6} \approx k_{O-2} > k_{O-3} > k_{O-4} > k_{O-1}$.[43] A kinetic investigation of the formose reaction has shown that the reaction is first order in formaldehyde ($k_1 \triangleq 10^{-7}$–10^{-6} s^{-1}).[44]

The difference in solubility of α- and β-D-glucose in water has been ascribed to the fact that the α-anomer can form a hydrogen bond with water at the ring oxygen giving intermolecular linkages, thus aiding nucleation. This bonding is diminished by the presence of the equatorial hydroxy-group of the β-anomer.[45] G.c. and n.m.r. have been used to study the retention of alcohols by anhydrous α-lactose and the results compared with those obtained in the vapour phase or by freeze-drying. The retention decreased in the order MeOH > EtOH > PrOH ≈ BuOH.[46]

3 Isomerizations

The kinetic parameters for aldose and ketose transformations in the D-glucose–D-mannose–D-fructose system at 27 °C on aluminate resin and hydroxide resin have been obtained. On both resins hydroxide ion catalyses isomerization. By complexation, resin-bound aluminate stabilizes D-fructose so that the equilibrium mixture contains 72% of the ketose. Lower temperatures were found to give the highest yields of D-fructose. A similar study on maltose gave a yield of 63% maltulose at 24 °C, but higher temperatures caused elimination of D-glucose at C-4.[47] In a study of isomerization, enolization, *etc.* of mono-

[40] E. E. Budzinski, W. R. Potter, G. Potienko, and H. C. Box, *J. Chem. Phys.*, 1979, **70**, 5040 (*Chem. Abs.*, 1979, **91**, 141 138*s*).

[41] H. C. Box, E. E. Budzinski, H. G. Fruend, and W. R. Potter, *J. Chem. Phys.*, 1979, **70**, 1320 (*Chem. Abs.*, 1979, **91**, 20 924*m*).

[42] H. Kubota, Y. Ogiwara, and K. Matsuzaki, *J. Appl. Polym. Sci.*, 1978, **22**, 3327 (*Chem. Abs.*, 1978, **89**, 215 856*g*).

[43] B. Gillet, D. Nicole, and J.-J. Delpuech, *Tetrahedron Lett.*, 1979, 1219.

[44] A. A. Morozov, O. E. Levanevskii, and T. K. Tamashaeva, *Izv. Akad. Nauk Kirg. S.S.R.*, 1978, 72 (*Chem. Abs.*, 1979, **90**, 55 185*c*).

[45] I. A. Maslov and E. G. Martsinovskaya, *Zh. Fiz. Khim.*, 1978, **52**, 2580 (*Chem. Abs.*, 1979, **90**, 104 230*z*), *Russ. J. Phys. Chem. (Eng. Transl.)*, 1978, **52**, 1485.

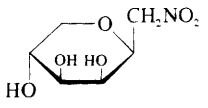
[46] F. W. Parrish, P. E. Pfeffer, K. D. Ross, D. P. Schwartz, and K. M. Valentine, *J. Agric. Food Chem.*, 1979, **27**, 56 (*Chem. Abs.*, 1979, **90**, 72 399*t*).

[47] J. A. Rendleman and J. E. Hodge, *Carbohydr. Res.*, 1979, **75**, 83.

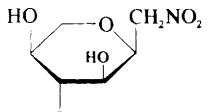
saccharides in aqueous alkali, u.v. spectroscopy has been used to obtain reaction rate constants for the scheme:

$$\text{sugar} + OH^- \rightleftharpoons \text{sugar anion} \rightleftharpoons \text{enediol anion} \rightarrow \beta\text{-elimination products} \rightarrow \text{final products.}$$

The reaction was carried out with the exclusion of oxygen to prevent oxidation of the enediol. The band at 310 nm was assigned to the enediol anion.[48] A report of an investigation of the suitability of several bases as catalysts for preparing D-psicose from D-fructose has appeared. The yields were determined by l.c. and pyridine was found to be the most effective base studied. Mixtures formed by boiling a concentrated solution of D-fructose (1 kg l^{-1}) in pyridine under reflux contained 12.4% D-psicose, 25.8% D-fructose, and lesser quantities of D-glucose and D-mannose. The D-psicose was isolated by removal of solvent, and fermentation of other sugars by bakers' yeast. The entire process was completed within three days to give gram quantities in 6.8% overall yield.[49] Dipotassium hydrogen phosphate isomerizes pent-2-uloses to mixtures of pentoses and isomeric pent-2-uloses. The product equilibrium depends on initial concentrations and temperature.[50] L-Rhamnose and L-fucose have been shown to isomerize under alkaline conditions. With pyridine or DCC, 6-deoxy-L-*ribo*-pentulose, 6-deoxy-L-*arabino*-pentulose, and L-quinovose were produced from L-rhamnose although almost half the starting material was recovered. With calcium hydroxide and triethylamine 6-deoxy-L-*arabino*-pentulose was the only product. Similar results were reported for L-fucose.[51] The molybdate-catalysed epimerization of heptoses has been shown to yield, in addition to the expected C-2 epimers, C-3 epimers and some heptuloses. The reaction was applied to D-*glycero*-D-*galacto*, -D-*talo*-, -D-*gulo*-, and -D-*ido*-heptose.[52] Molybdate has been used to isomerize 2,6-anhydro-1-deoxy-1-nitro-D-galactitol (4) to the corresponding L-iditol (5) which could be converted to L-xylose by treatment with alkaline hydrogen peroxide.[53]



(4)



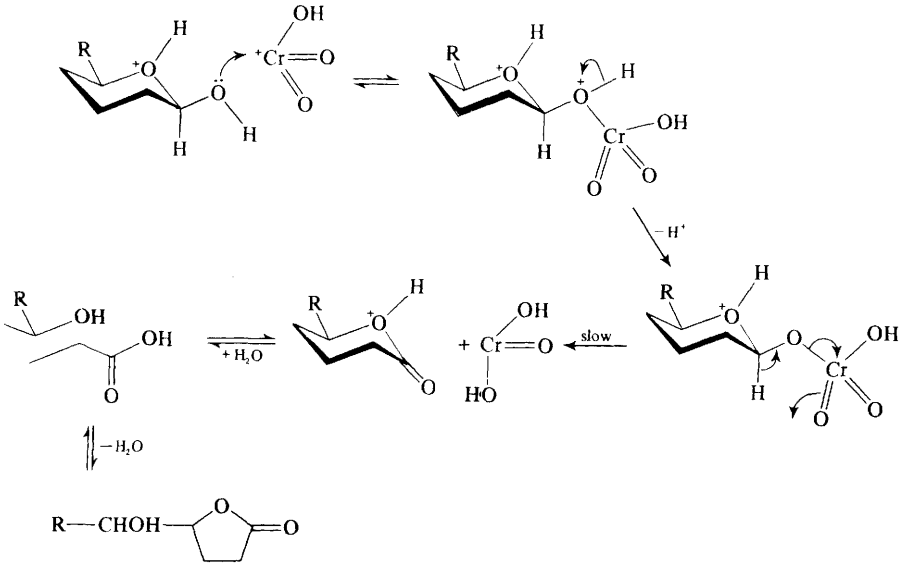
(5)

4 Oxidation

This section is concerned with oxidations of free sugars where the primary study is the kinetic or mechanistic interpretation. Preparative studies leading to alduloses, uronic acids, aldonic acids *etc.* will be found in chapters 14 and 15.

[48] G. de Wit, A. P. G. Kieboom, and H. van Bekkum, *Carbohydr. Res.*, 1979, **74**, 157.
[49] L. W. Doner, *Carbohydr. Res.*, 1979, **70**, 209.
[50] L. Stankovic and J. Konigstein, *Chem. Zvesti*, 1979, **33**, 397.
[51] S. Kučár, J. Zámocký, and J. Zemek, *Collect. Czech. Chem. Commun.*, 1979, **44**, 3119.
[52] L. Petruš, V. Bílik, D. Anderle, and F. Janeček, *Chem. Zvesti*, 1979, **33**, 636.
[53] V. Bílik, L. Petruš, M. Mišíková, and V. Sutoris, *Chem. Zvesti*, 1979, **33**, 114.

The kinetics of chromic acid oxidation of D-galactose, D-mannose, L-arabinose, and D-xylose in perchloric acid media have been measured. The reaction was found to be first-order with respect to Cr^{VI} and to [aldose] but showed a complex dependence on $[H^+]$. The activation parameters were obtained and the mechanism shown in Scheme 4 was proposed.[54] The kinetic



Scheme 4

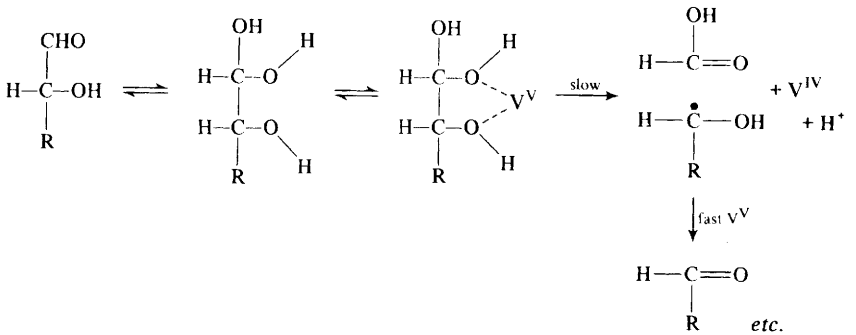
parameters of first-order oxidations of L-arabinose by $Hg(NO_3)_2$, $Hg_2(NO_3)_2$, and $AgNO_3$,[55] of D-galactose and L-arabinose by $CuSO_4$, $Fe_2(SO_4)_3$, and $Ce(SO_4)_2$,[56] and of D-glucose by $CuSO_4$, $Fe_2(SO_4)_3$, $Ce(SO_4)_2$, $Hg(NO_3)_2$, $Hg_2(NO_3)_2$, and $AgNO_3$ in aqueous media[57] have been determined. In general the oxidations proceeded destructively to give some of the corresponding aldonic acids, but mainly two- and three-carbon fragments. Copper(III), prepared from $CuSO_4$ and potassium periodate, has been used to oxidize L-arabinose, D-ribose, D-xylose, D-glucose, D-galactose, D-fructose, and di- and tri-ethanolamine. The kinetics were fast and estimated by potentiometry.[58] Three papers on the oxidation of sugars by vanadium(V) have been published.[59–61] The kinetics of

[54] K. K. S. Gupta, S. S. Gupta, and S. N. Basu, *Carbohydr. Res.*, 1979, **71**, 75.
[55] V. I. Krupenskii, *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.*, 1979, **22**, 134 (*Chem. Abs.*, 1979, **91**, 39 742*x*).
[56] V. I. Krupenskii, *Zh. Obshch. Khim.*, 1979, **49**, 457 (*Chem. Abs.*, 1979, **91**, 39 743*y*).
[57] V. I. Krupenskii, *Zh. Obshch. Khim.*, 1978, **48**, 2228 (*Chem. Abs.*, 1979, **90**, 121 892*j*).
[58] M. Bhatnagar, R. P. Bhatnagar, and K. C. Grover, *Cellulose Chem. Technol.*, 1978, **12**, 283 (*Chem. Abs.*, 1979, **90**, 55 177*b*).
[59] R. P. Bhatnagar and A. G. Fadnis, *J. Indian Chem. Soc.*, 1978, **55**, 357.
[60] K. K. S. Gupta and S. N. Basu, *Carbohydr. Res.*, 1979, **72**, 139.
[61] S. C. Pati and M. Panda, *Int. J. Chem. Kinet.*, 1979, **11**, 731 (*Chem. Abs.*, 1979, **91**, 91 868*g*).

oxidation of aldopentoses in aqueous sulphuric acid were shown to be first-order with respect to $[V^{5+}]$ and substrate. In acid of between 2.0 and 4.5 M the rate increased with $[H^+]$; in more concentrated acid the dependence of rate on $[H^+]^2$ became apparent. There was also an increase in rate with increasing $[HSO_4^-]$ at constant $[H^+]$. The thermodynamic parameters were calculated. A mechanism for the reaction was proposed:

$$CHO(CHOH)_3CH_2OH + V^{5+} \rightarrow CHO(CHOH)_2\dot{C}HOH + H_2CO + H^+ + V^{4+}$$

with the resulting radical giving $HCO_2H + 3H_2CO$ with more V^{5+}. The formaldehyde was further oxidized to give formic acid.[59] A similar mechanism has been proposed for vanadium(V) oxidation of aldoses in perchloric acid media. The reaction is acid-catalysed and addition of sodium perchlorate accelerates the rate. The fast-step, reaction with the vanadium species, is followed by the slow decomposition to give products. The rates of oxidation proceeded in the order xylose > arabinose > galactose > mannose.[60] Binary solvent mixtures have been used to determine the dependence of the rate of V^{5+} oxidation of sugars on the acidity function, H_0. D-Fructose was found to have unit dependence whereas D-galactose, D-glucose, D-mannose, D-arabinose, and D-xylose all have a 0.5 dependence. The mechanism proposed differs from that above (refs. 59 and 60) in that a chelate complex of the vanadium(V) forms with the aldehydrol of C-1 and the 2-OH, which breaks down to give a free radical, formic acid, and vanadium(IV) (Scheme 5).[61] A free radical mechanism has also been proposed for cerium(IV) oxidation of D-xylose in aqueous sulphuric acid. The rate was first-order in concentration of oxidant and substrate and was retarded by sulphate and bisulphate ions but accelerated by manganese(II) ions.[62] The kinetics and activation energies of the same reaction on L-arabinose have been measured. The Arrhenius energy, E_a, was 0.53 kJ mol^{-1} and $\Delta S^{\ddagger} - 23$ e.u.[63]



Scheme 5

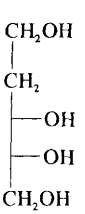
Mechanistic studies of hexacyanoferrate(III) oxidation of lactose and maltose in alkaline media have been reported. In the presence of ammonium hydroxide,

[62] R. P. Bhatnagar and A. G. Fadnis, *Cienc. Cult. (Sao Paulo)*, 1978, **30**, 1227 (*Chem. Abs.*, 1979, **90**, 55 188*f*).

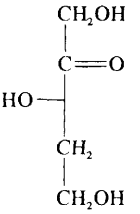
[63] V. I. Krupenskii, *Izv. Vyssh. Uchebn. Zaved., Lesu. Zh.*, 1979, 136 (*Chem Abs.*, 1979, **91**, 211 696*y*).

both sugars follow zero-order kinetics in $[[Fe(CN)_6]^{3-}]$ and first-order kinetics in [substrate]. The rates were proportional to $[OH^-]$. The mechanism suggested the intermediacy of the 1,2-enediol anion; it is likely that the rate of oxidation is equal to the rate of enolization.[64] The same group has examined the oxidation of the same disaccharides by iodine in alkaline media, and has concluded that the oxidizing species is hypoiodate.[65] Phenyl iodosoacetate, $PhI(OAc)_2$, has been examined as an oxidant for D-fructose[66] and for aldoses in the presence of perchloric acid,[67] both in aqueous acetic acid. A complex is formed between the substrate and oxidant in each case.

Biological dehydrogenation of 2-deoxy-D-*erythro*-pentitol (6) by *Gluconobacter oxydans* has been used to prepare 4-deoxy-L-*glycero*-pentulose (7), characterized by m.s. of its tri(trimethylsilyl) derivative.[68]



(6)



(7)

5 Other Reactions

The decomposition of D-glucose in the presence of $CrCl_3$, $AlCl_3$, or $TiCl_3$ has been shown to proceed by a first-order reaction to give $MeCOCH_2CH_2CO_2H$ (10%), HCO_2H (3%), CO_2 (3%), $OHCCO_2H$ (6%), and $HO_2CCH_2CH_2CO_2H$ (7%).[69]

An h.p.l.c. study of the initial stages of heat-induced decomposition of dextrose in neutral solution has shown that two intermediates with identical u.v. spectra consistent with 3-deoxyhexosone structure are formed. After about 20 min, 5-hydroxymethylfurfural appeared.[70] 5-Hydroxymethylfurfural has also

[64] R. Singh and E. B. Singh, *Indian J. Chem., Sect. A*, 1978, **16**, 654 (*Chem. Abs.*, 1979, **90**, 23 453*x*).

[65] V. P. Singh, B. P. Singh, A. K. Singh, and R. Singh, *Indian J. Chem. Soc., Sect. A*, 1979, **17**, 184.

[66] S. C. Pati and R. C. Mahapatro, *Natl. Acad. Sci. Lett. (India)*, 1978, **1**, 325 (*Chem. Abs.*, 1979, **90**, 23 444*v*).

[67] S. C. Pati and R. C. Mahapatro, *Proc. Indian Acad. Sci., Sect. A*, 1979, **88**, 203 (*Chem. Abs.*, 1979, **91**, 158 004*t*).

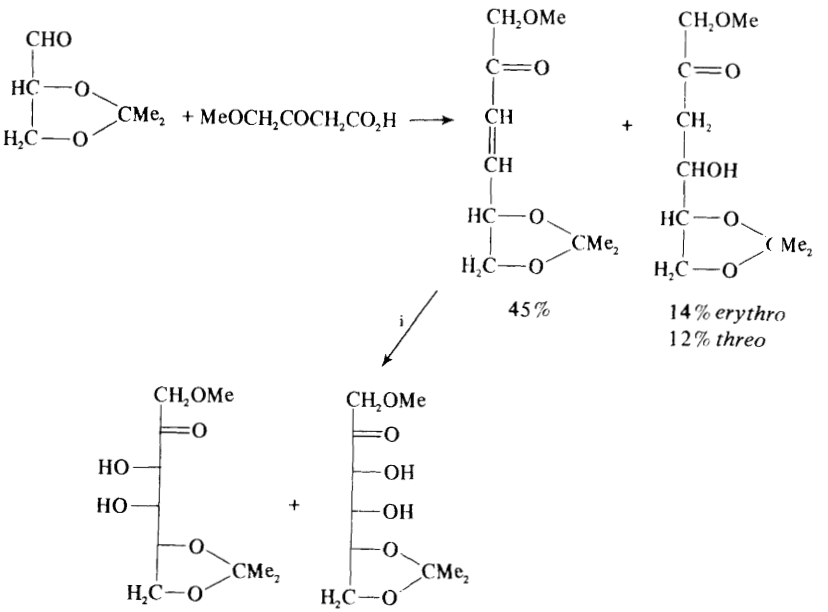
[68] K. Linek, R. Sandtnerova, T. Sticzay, V. Kováčik, M. Kulhánek, and M. Tadra, *Carbohydr. Res.*, 1979, **76**, 290.

[69] V. I. Krupenskii, *Khim. Drev.*, 1979, 96 (*Chem. Abs.*, 1979, **91**, 141 125*k*).

[70] R. B. Taylor and V. C. Sood, *J. Pharm. Pharmacol.*, 1978, **30**, 510 (*Chem. Abs.*, 1979, **90**, 72 397*r*).

been detected, together with furfural, as a decomposition product of heating D-glucose at 100 °C in aqueous solution for five days under oxygen.[71]

The Knoevenagel reaction of 2,3-*O*-isopropylidene-D-glyceraldehyde with active methylene compounds has led to derivatives of D-fructose and D-sorbose (Scheme 6).[72] Similar condensations with other β-dicarbonyl compounds led to furan derivatives on acidic hydrolysis.[73] In the self-condensation of D-fructose



Reagent: i, OSO_4

Scheme 6

in aqueous acid, levanobiose, inulobiose, and fructopyranosyl fructose have been identified.[74] A study of the side-reactions in the acid hydrolysis of sucrose has shown that self-condensation of fructose is predominant. Fructosyl glucose is the major by-product with lesser amounts of trisaccharide ketoses, fructopyranosyl fructose, and inulobiose.[75]

Reference to the synthesis of 1-deoxy-D-*altro*-heptulose, which is accumulated by transketolase mutants of *Bacillus pumilus*, is made in Chapter 11.

[71] H. Ogata, T. Shibazaki, A. Ejima, T. Matsuura, and F. Shimada, *J. Pharm. Pharmacol.*, 1978, **30**, 668 (*Chem. Abs.*, 1979, **90**, 192 430*t*).

[72] F. J. Lopez Aparicio, M. Gomez Guillen, and I. Izquierdo Cubero, *Anales Quim.*, 1977, **73**, 1168 (*Chem. Abs.*, 1979, **90**, 23 427*s*).

[73] F. J. Lopez Aparicio, F. J. Lopez Herrera, and J. Sanchez Ballesteros, *Carbohydr. Res.*, 1979, **69**, 55.

[74] B. Krol, *Acta Aliment. Pol.*, 1978, **4**, 287 (*Chem. Abs.*, 1979, **90**, 152 487*k*).

[75] B. Krol, *Acta Aliment. Pol.*, 1978, **4**, 373 (*Chem. Abs.*, 1979, **81**, 39 752*a*).

Chapter 15 contains a report of a study of the products of the application of Fehling's reaction to free sugars, while their complexation with borate ions is referred to in Chapter 21.

3

Glycosides

1 *O*-Glycosides

A book (210 pp.) has been published by Russian authors entitled 'Chemistry of the *O*-Glycosidic Bond',[1] and a symposium sponsored by the American Chemical Society on the anomeric effect yielded several relevant reports, including one on the influence of reactant structure and solvent on glycosidation reactions.[2] A further review has appeared on the Koenigs–Knorr reaction applied to the synthesis of both pyranosides and non-pyranoid glycosides,[3] and a more specific one on glycosidation processes applied to drugs.[4]

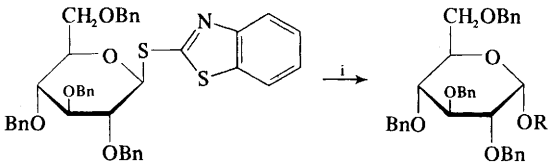
Synthesis of Monosaccharide Derivatives. – Fischer glycosidation continues to be of interest and has been surveyed in a Polish review,[5] and, specifically, it has been applied to the preparation of the methyl glycosides of 2-amino-2-deoxy-D-glucose. Heating glucosamine in methanol in the presence of acidic resin in a sealed tube at 120 °C initially gave rise to the anomeric furanosides but then, as occurs with simple sugars, the pyranosides were produced (ultimately 58% α, and 22% β).[6] In similar fashion, the methyl glucosides of 2-acetamido-2-deoxy-D-galactose were prepared and were separated by chromatography on resins,[7] and the ethylene glycol pyranosides of 2-acetamido-2-deoxy-D-glucose have also been made, the α by the Fischer procedure and the β by use of the oxazoline procedure. The pure anomers were isolated as their peracetates.[8] Ethylene glycol, glycerol, and related diols and triols have also been condensed with D-glucose to give glycosides which were then used to initiate the polymerization of propylene oxide and thus afford polyether glycosides.[9] More specifically, 2-deoxy-D-*arabino*-hexose gave, in 58% yield, the α-benzyl glycoside when treated in benzyl alcohol with hydrogen chloride at low temperature.[10] An enzymic method for the preparation of methyl and ethyl β-D-fructofuranoside involves incubation of sucrose and the alcohol with invertase and isolation of the glycosides by chromatographic methods.[11] The methyl α- and β-furanosides and the β-pyrano-

[1] A. F. Bochkov and G. E. Zaikov, 'Chemistry of the *O*-Glycosidic Bond', Pergamon Press, Oxford, N.Y., 1979.
[2] C. Schuerch, *Amer. Chem. Soc. Symp. Ser.*, 1979, No. 87, 80.
[3] K. Karashi, *Adv. Carbohydr. Chem. Biochem.*, 1977, **34**, 243.
[4] L. Brown and R. Thomas, *Aust. J. Pharm. Sci.*, 1979, **8**, 1 (*Chem. Abs.*, 1979, **91**, 74 789*q*).
[5] W. Szeja, *Wiad. Chem.*, 1979, **33**, 7 (*Chem. Abs.*, 1979, **90**, 152 477*g*).
[6] T. Tsuchiya, T. Usui, T. Kamiya, and S. Umezawa, *Carbohydr. Res.*, 1979, **77**, 267.
[7] M. Sarkar and E. A. Kabat, *Carbohydr. Res.*, 1979, **69**, 143.
[8] S. R. Sarfati and P. Szabó, *Carbohydr. Res.*, 1979, **72**, 297.
[9] F. Stoenescu, M. Ionescu, V. Dumitriu, and I. Mihalache, *Matoi. Plast. (Bucharest)*, 1979, **16**, 4 (*Chem. Abs.*, 1979, **91**, 21 482*c*).
[10] S. R. Sarfati and P. Szabó, *Carbohydr. Res.*, 1979, **68**, 155.
[11] S. Hisajima and Y. Arai, *Dempun Kagaku*, 1977, **24**, 45 (*Chem. Abs.*, 1979, **91**, 193 576*c*).

side of D-psicose have been obtained by methanolysis of the sugar during an extensive study of its derivatives.[12]

Sinay has provided a useful survey of the use of imidates in the synthesis of glycosides, including disaccharides. The work covers the preparation of α-D-glucopyranosides, α-D-galactopyranosides, and α-L-fucopyranosides especially in the context of human blood antigenic determinants.[13] In parallel fashion, Perlin has focused attention on glycosyl sulphonates as glycosylating agents, and in particular on their use in disaccharide synthesis.[14] A related report describes the treatment of tetra-*O*-benzyl-α-D-glucopyranose with equimolar proportions of methanol and cyclohexanol in the presence of chlorosilanes and silver sulphonates. Although good yields of glycosides were obtained under some conditions, the results suggested that stereospecificity was not good and, furthermore, α,α- and α,β-trehalose ethers were produced as by-products.[15] A further approach to the problem of specific glycoside synthesis has adopted the Vilsmeier–Haack reagent ($Cl-CH=\overset{+}{N}Me_2$) with DMF in chloroform in the presence of silver tosylate and the alcohol. From 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose, α-glycosides were obtained in high yields using simple primary, secondary, and tertiary alcohols and also a sterol and protected monosaccharides containing primary or secondary alcohol groups.[16]

The glycosidation procedure which involves a mercury-activated phenylthio-group as leaving group is reported to be facilitated by addition of perchloric acid and some other reagents,[17] and a new, but related process utilizes the crystalline thioglycoside (1), prepared from tetra-*O*-benzyl-α-D-glucopyranosyl chloride, as indicated in Scheme 1. Very high yields were reported for both simple and complex alcohols (including partially protected sugar derivatives with secondary alcohol groups), and α-:β-product ratios were between 2:1 and 5:1.[18] In parallel fashion, the same group of Japanese workers prepared the α-pyridyl glycoside (2) from tetra-*O*-benzyl-α-D-glucopyranose and 2-chloro-3,5-dinitropyridine and used it in the synthesis of β-glucopyranosides (Scheme 2). High yields were again reported, and 5–15% of α-anomers were present amongst the products.[19]

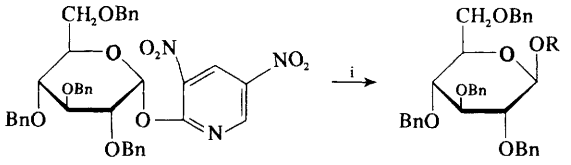


(1)

Reagents: i, ROH–$Cu(O_3S\cdot CF_3)_2$

Scheme 1

[12] P. C. M. Herve du Penhoat and A. S. Perlin, *Carbohydr. Res.*, 1979, **71**, 149.
[13] P. Sinay, *Pure Appl. Chem.*, 1978, **50**, 1437.
[14] A. S. Perlin, *Pure Appl. Chem.*, 1978, **50**, 1401 (*Chem. Abs.*, 1979, **91**, 157 985*b*).
[15] S. Koto, N. Morishima, and S. Zen, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 784.
[16] V. Dourtoglov and J.-C. Ziegler, *Tetrahedron Lett.*, 1979, 4371.
[17] J. W. Van Cleve, *Carbohydr. Res.*, 1979, **70**, 161.
[18] T. Mukaiyama, T. Nakatsuka, and S. Shoda, *Chem. Lett.*, 1979, 487.
[19] S. Shoda and T. Mukaiyama, *Chem. Lett.*, 1979, 847.

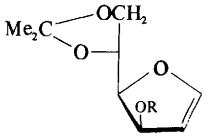


(2)

Reagents: i, $ROH–BF_3·Et_2O$

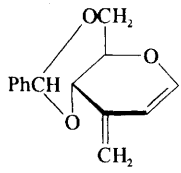
Scheme 2

The glycal-2,3-unsaturated glycoside conversion, which is also referred to in Chapter 12, has been utilized in the preparation of several complex glycosides, and the direct treatment of the furanoid glycals (3) and (4) with *m*-chloroperbenzoic acid in ethanol afforded substantial proportions of ethyl 5,6-*O*-isopropylidenehexofuranosides.[20] The synthesis of sucrose which utilizes compound (5) has been described in detail (see vol 9, p. 13) and, by use of cholesterol, higher yields of α-glycosides were shown to be obtainable by this procedure than by the methods using tri-*O*-acetyl-D-glucal and either boron trifluoride or nitrosyl chloride.[21]



(3) R = H

(4) R = 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranosyl



(5)

Considerable attention has been given to aryl glycosides. A new procedure for synthesizing nitrophenyl compounds involves the use of the phenol bound to a resin and treatment with acetylated glycosyl bromides. High yields of β-products were reported for *o*-, *m*-, and *p*-nitrophenyl-D-glucosides and D-galactosides.[22] Cadmium carbonate can be used as an acid acceptor during the condensation between acylated glycosyl bromides and phenols, but products of hydrolysis and elimination are also formed, and the efficiency of the salt varies substantially with the sample.[23] By use of silver oxide and pyridine (or quinoline) as bases, and tetra-acetylglucosyl bromide, 4-*O*-β-D-glucopyranosyl derivatives of vanillic and coumaric acids have been produced.[24] A modification of these procedures involves the condensation between tetra-*O*-benzyl-α-D-glycopyranosyl bromide in methylene chloride and phenols in sodium or potassium hydroxide solutions in the presence of phase transfer reagents. Substituted phenyl com-

[20] K. Bischofberger, S. J. Eitelman, and A. Jordaan, *Carbohydr. Res.*, 1979, **74**, 145.
[21] B. Fraser-Reid and D. E. Iley, *Can. J. Chem.*, 1979, **57**, 645.
[22] T. Iversen and R. Johansson, *Synthesis*, 1979, 823.
[23] W. E. Dick, *Carbohydr. Res.*, 1979, **70**, 313.
[24] A. B. Durkee and I. R. Siddiqui, *Carbohydr. Res.*, 1979, **77**, 252.

pounds and β-glycosides of 1-naphthol, 8-hydroxyquinoline, and of complex phenols including morphine were prepared and, following debenzylation, were oxidized to the corresponding uronic acid glycosides.[25] Similar oxidation of *p*-nitrophenyl α-D-glucopyranoside gave the uronoside, some spectroscopic and chromatographic properties of which were compared with those of the β-anomer.[26]

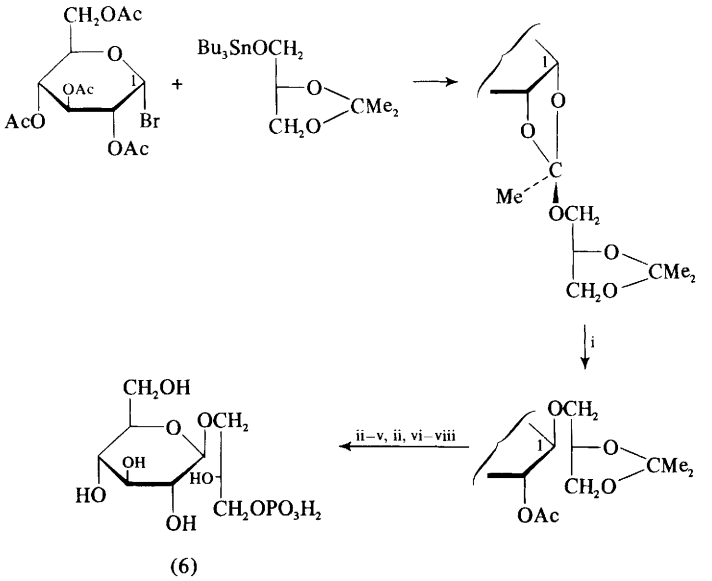
Diethyl azodicarboxylate and triphenylphosphine permit the condensation between aldose derivatives with a free reducing centre and phenols. 2,3 : 5,6-Di-*O*-isopropylidene-D-mannose gave 67% of the phenyl α-D-mannofuranoside derivative and 16% of the β-anomer,[27] while 2,3 : 4,6-di-*O*-cyclohexylidene-α-D-mannose afforded access to *p*-nitrophenyl β-D-mannopyranoside in 58% yield[28] and other, substituted aryl analogues.[29] Unsubstituted sugars can be subjected to the procedure, D-glucose giving mainly the phenyl β-pyranoside.[27]

High resolution n.m.r. studies have shown that the phenyl D-glucosides produced by the micro-organism *Mucor liemales* from phenols are β-pyranosyl compounds in the expected $^4C_1$ D-conformation.[30]

Several studies of natural products have led to the need to synthesize glycosylated acyclic compounds, and the L-glycerol phosphate (6), the repeating unit of a bacterial teichoic acid, was obtained as shown in Scheme 3.[31] In related work 'seminolipid' (7), a lipid of mammalian testes and spermatazoa,[32] and 'glucosylphosphatidylglycerol' (8), a component of the phospholipid pool of some Gram-negative bacteria,[33] have each been synthesized by a complex set of steps based on glycosylation of disubstituted glycerol derivatives. The 1-glyceryl glycosides (9) and (10) were used in an investigation of the role of the hydroxy-group at C-2 of D-galactose on the action of β-galactosidase. Use of the inhibitors (11) and (12) indicated that relative binding strengths did not account for the fact that the 2-deoxy-compound (10) hydrolysed much more slowly than did compound (9), and neighbouring group participation by the hydroxy-group under examination was invoked to account for the ease of hydrolysis of the galactoside.[34] 2-*O*-α-D-Glucopyranosyl-D-glyceric acid, a terminal unit of a bacterial polysaccharide, has been prepared from leucrose (5-*O*-α-D-glucopyranosyl-D-fructose) by reduction of the ketose unit and standard oxidative degradations.[35]

β-D-Xylopyranosides with complex haloacetamidoalkyl aglycones, for use in cytotoxicity studies, were prepared from 2′-hydroxyethyl glycosides *via*

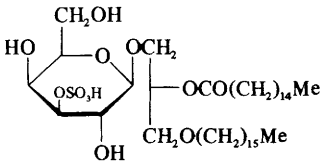
[25] K. Brewster, J. M. Harrison, and T. D. Inch, *Tetrahedron Lett.*, 1979, 5051.
[26] L. P. Johnson and C. Feneselau, *Carbohydr. Res.*, 1979, **73**, 255.
[27] G. Grynkiewicz, *Pol. J. Chem.*, 1979, **53**, 1571.
[28] P. J. Garegg, T. Iversen, and T. Norberg, *Carbohydr. Res.*, 1979, **73**, 313.
[29] K. Akerfeldt, P. J. Garegg, and T. Iversen, *Acta Chem. Scand., Ser. B*, 1979, **33**, 467.
[30] P. Granger, R. Herber, J.-P. Villoutreix, G. Hajdukovic, and Y. Chapleur, *Carbohydr. Res.*, 1979, **71**, 43.
[31] T. Ogawa, K. Katano, and M. Matsui, *Carbohydr. Res.*, 1979, **70**, 37.
[32] R. Gigg, *J. Chem. Soc., Perkin Trans. I*, 1979, 712.
[33] C. A. A. van Broeckel and J. H. van Boom, *Tetrahedron Lett.*, 1979, 3561.
[34] M. Brockhaus, H.-M. Dettinger, G. Kurz, J. Lehmann, and K. Wallenfels, *Carbohydr. Res.*, 1979, **69**, 264.
[35] B. K. Hunter, S. L. Mowbray, and D. J. Walton, *Biochemistry*, 1979, **18**, 4458.



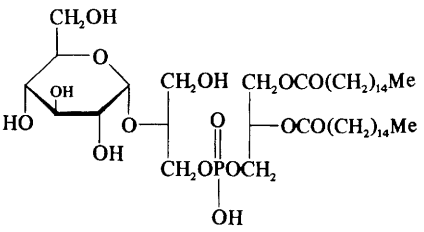
(6)

Reagents: i, $HgBr_2$; ii, H^+; iii, TrCl-py; iv, TsCl-py; v, KOAc–crown ether; vi, $(PhO)_2POCl$; vii, H_2–Pt; viii, MeO^-

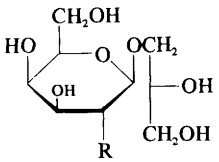
Scheme 3



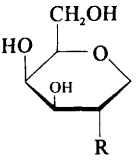
(7)



(8)



(9) R = OH
(10) R = H



(11) R = OH
(12) R = H

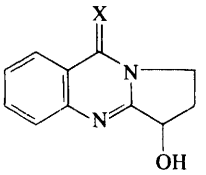
2′-azidoethyl compounds and the corresponding amines which were acylated by use of haloacetyl halides and dihaloacetyl halides.[36] A similar approach provided 2′-(*p*-nitrophenyl)ethyl β-D-xylopyranosides and -glucopyranosides and *p*-amino- and *p*-hydroxy-analogues required for pharmacological purposes.[37] Three reports have described improvements in the preparation of octyl β-D-glucopyranoside which is a good non-ionic detergent used in membrane studies[38–40] and for solubilizing enzymes.[41] The nonyl, decyl, and undecyl analogues and dodecyl-β-D-maltoside were also described.[38] For bonding carbohydrates to macromolecules functionalized aglycones are required, and 6-aminohexyl glycosides have been used by two groups of workers. In the first study a range of hexoses and disaccharides were bonded to 6-(trifluoroacetylamino)-hexanol and the products, after deprotection, were attached to solid matrices for the isolation of proteins which bind to carbohydrates;[42] in the second, a similar range of compounds (but including the β-D-xylopyranoside and β-L-fucopyranoside) were made and were coupled to agarose to isolate lectin from wheat germ. During these synthesis 1,2-orthoester products were also formed.[43]

Several of the syntheses already mentioned involve the use of glycosyl halide derivatives, but the Koenigs–Knorr reaction has been studied and used in many other specific cases. The presence of silver trifluoromethanesulphonate permits excellent yields of β-glycosides when benzoylated α-D-glucopyranosyl, -galactopyranosyl, and -xylopyranosyl bromides are condensed with complex alcohols including monosaccharide derivatives,[44] but tetrahydrofuran can divert the reaction to give orthoesters or, in the absence of participating groups at C-2, 1,2-*cis*-related glycosidic products.[45] A wide range of glycosylated natural products have been prepared by use of glycosyl halides: α- and β-menthyl glucosides were made to solubilize the terpene and for the production of food additives and medicines; they can be used to resolve dl-menthol. Disaccharide derivatives were also produced.[46] Several steroidal α-L-rhamnosides, β-D-fucopyranosides, and β-D-maltosides have been made for examination of their haemolytic properties,[47] and glucosides of diosgenin and neotigogenin[48] as well as

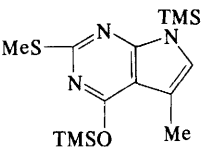
[36] A. F. Hadfield, J. S. Lazo, and A. C. Sartorelli, *Carbohydr. Res.*, 1979, **77**, 51.
[37] B. Borkowski, Z. Kolodynska, E. Lada, and W. Wieniawski, *Acta Pol. Pharm.*, 1977, **34**, 251 (*Chem. Abs.*, 1978, **89**, 24 701*t*).
[38] W. J. de Grip and P. H. M. Bovee-Geurts, *Chem. Phys. Lipids*, 1979, **23**, 321 (*Chem. Abs.*, 1979, **90**, 168 921*h*).
[39] J. F. W. Keana and R. B. Roman, *Membrane Biochem.*, 1978, **1**, 323 (*Chem. Abs.*, 1979, **90**, 56 732*r*).
[40] J. T. Lin, S. Riedel, and R. Kinne, *Biochim. Biophys. Acta*, 1979, **557**, 179.
[41] A. Radomińska-Pyrek, M. Pilarska, and P. Zimniak, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 1074.
[42] P. H. Weigel, M. Naoi, S. Roseman, and Y. C. Lee, *Carbohydr. Res.*, 1979, **70**, 83.
[43] C. K. Chiang, M. McAndrew, and R. Barker, *Carbohydr. Res.*, 1979, **70**, 93.
[44] P. J. Garegg and T. Norberg, *Acta Chem. Scand., Ser B.*, 1979, **33**, 116.
[45] G. Wulff, U. Schröder, and J. Wichelhaus, *Carbohydr. Res.*, 1979, **72**, 280.
[46] I. Sakata and H. Iwamura, *Agric. Biol. Chem.*, 1979, **43**, 307.
[47] R. Segal, F. Shud, and I. Milo-Goldzweig, *J. Pharm. Sci.*, 1978, **67**, 1589 (*Chem. Abs.*, 1979, **90**, 121 956*h*).
[48] A. P. Schelochkova, N. Sh. Pal'yants, K. K. Koshoev, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 1979, 193 (*Chem. Abs.*, 1979, **91**, 74 842*b*).

glucosides, glucuronides, and lactosides of glycyrrhetic acid and other complex triterpenes[49] have been reported.

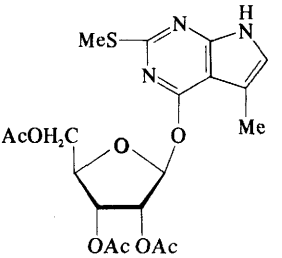
Newer methods for the selective glycosylation of flavonoid compounds have been reviewed,[50] and β-D-glucopyranosides have been prepared from 5,7,2′-trihydroxyisoflavone (7-derivative),[51] 5,7-dihydroxy-2-methylchromone (7-derivative),[52] and various xanthones.[53,54] Nitrogen derivatives that have been subjected to condensation with glycosyl halide derivatives include vasicine (13) and vasicinone (14),[55] 5-dimethylamino-6-azauracil (the initial main product was the 2-*O*-α-D-glucoside, but this gave way to the 1-*N*-isomer which, on further glycosylation gave the 1-*N*-,-4-*O*-disubstituted product),[56] and the deazapurine derivative (15) which gave mainly the *O*-bonded β-D-ribofuranosyl compound (16) rather than the nucleoside analogue.[57] Other compounds to have been subjected to glycosylation by this approach are tryptophol [2-(indol-3-yl)-ethanol][58] and ε-rhodomycinone (tetrahydropyranyl derivatives).[59]



(13) X = 2H
(14) X = O



(15)

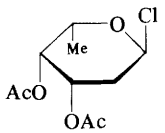


(16)

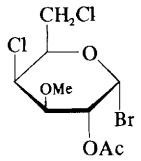
3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl bromide, prepared from 1,6-anhydro-4-*O*-benzyl-2-deoxy-2-fluoro-β-D-glucose has been used in the synthesis of several 2-deoxy-2-fluoro-β-D-glucopyranosides with phenol, 1,2 : 3.4-di-*O*-isopropylidene-D-galactose and 17-oxoestra-1,3,5(10)-trien-3-ol as agly-

[49] C. H. Brieskorn and J. Lang, *Arch. Pharm. (Weinheim, Ger.)*, 1978, **311**, 1001 (*Chem. Abs.*, 1979, **90**, 121 962*g*).
[50] L. Farkas and B. Vermes, *J. Indian Chem. Soc.*, 1978, **55**, 1192 (*Chem. Abs.*, 1979, **91**, 141 110*b*).
[51] S. Bhanumati, S. C. Chhabra, and S. R. Gupta, *Natl. Acad. Sci. Lett. (India)*, 1979, **2**, 53 (*Chem. Abs.*, 1979, **91**, 74 844*d*).
[52] S. R. Gupta and K. S. Verma, *Natl. Acad. Sci. Lett. (India)*, 1978, **1**, 328 (*Chem. Abs.*, 1979, **90**, 72 431*x*).
[53] V. M. Chari, R. Klapfenberger, and H. Wagner, *Z. Naturforsch., Teil B: Anorg. Chem., Org. Chem.*, 1978, **33**, 946 (*Chem. Abs.*, 1978, **89**, 215 711*j*).
[54] V. M. Char, R. Klapfenberger, and H. Wagner, *Helv. Chim. Acta*, 1979, **62**, 678.
[55] S. Johne, K. Seifert, and S. Haertling, *Pharmazie*, 1979, **34**, 197.
[56] I. V. Alekseeva, A. S. Shalamai, V. I. Kobylinskaya, and V. P. Chernetskii, *Ukr. Khim. Zh. (Russ. Ed.)*, 1979, **45**, 162 (*Chem. Abs.*, 1979, **90**, 187 272*p*).
[57] U. Lüpke and F. Seela, *Chem. Ber.*, 1979, **112**, 799.
[58] V. Magnus, *Carbohydr. Res.*, 1979, **76**, 261.
[59] H. S. El Khadem and T. S. Winowiski, *Carbohydr. Res.*, 1979, **76**, 265.

cones.[60] Other deoxyglycosides to have been synthesized were prepared by use of the glycosyl halides (17)[61] and (18).[62]

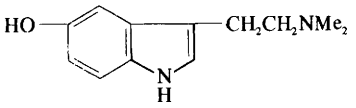


(17)

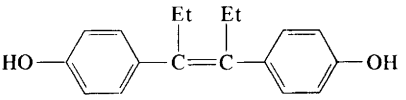


(18)

The glycosyl bromide method was favoured in the preparation of the β-D-glucuronide of bufotenine (19),[63] but the corresponding glycosyl acetate was preferred for obtaining the mono- and di-substituted derivatives of diethylstilbestrol (20).[64] A second report refers to the glucuronides of diethylstilbestrol and of hexosterol,[65] and a partially purified glucuronosyl transferase immobilized on sepharose was used in the preparation of uronosides of cannabinol and several of its derivatives.[66]



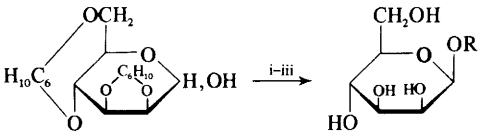
(19)



(20)

The preparation of aryl mannosides is mentioned above, and several other reports have focused on the synthesis of glycosides of this sugar, in particular the β-pyranosides. Treatment of 3,4,6-tri-*O*-benzyl-D-mannose with dibutyltin oxide gave a stannylene derivative which with methyl iodide or allyl bromide opened, as expected, to give the products of equatorial etherification, *i.e.* the 2-hydroxy-β-glycosides, in near quantitative yields. With the D-glucose analogue, methyl iodide gave mainly the 2-*O*-methylglucose derivative but also some of the α-glucoside.[67] A more general synthesis of β-D-mannopyranosides involves the use of tetra-*O*-benzyl-α-D-mannopyranosyl bromide in ether with alcohols and silver salicylate. Cholesteryl and glucose-6-yl products were obtained, and

[60] J. Pacák, Z. Köllnerová, and M. Černý, *Collect. Czech. Chem. Commun.*, 1979, **44**, 933.
[61] D. Horton and W. R. Turner, *Carbohydr. Res.*, 1979, **77**, C8.
[62] H. Redlich and W. Roy, *Carbohydr. Res.*, 1979, **68**, 275.
[63] L. S. Krasavina, M. M. Vigdorchik, K. F. Turchin, and N. N. Suvorov, *Zh. Org. Khim.*, 1979, **15**, 431.
[64] R. Maurwald and W. Krueger, *Hoppe-Seyler's Z. Physiol. Chem.*, 1978, **359**, 1803 (*Chem. Abs.*, 1979, **90**, 168 909*k*).
[65] K. Krohn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1977, **358**, 1551 (*Chem. Abs.*, 1978, **88**, 191 330*g*).
[66] M. A. Lyle, S. Pallante, K. Head, and C. Fenselau, *Biomed. Mass Spectrom.*, 1977, **4**, 190 (*Chem. Abs.*, 1978, **88**, 51 121*q*).
[67] V. K. Srivastava and C. Schuerch, *Tetrahedron Lett.*, 1979, 3269.

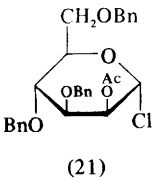


Reagents: i, MsCl, Et_3N; ii, ROH; iii, H^+

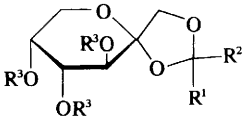
Scheme 4

α-mannopyranosides could be produced by use of 2,6-lutidine as acid acceptor.[68] An alternative approach to the β-anomers is illustrated in Scheme 4; simple glycosides and that derived from 1,2 : 3,4-di-*O*-isopropylidene-α-D-galactopyranose were obtained in high yield.[69] Compound (21), prepared from the corresponding methyl orthoacetate, alternatively affords access to 3,4,6-tri-*O*-benzyl-α-D-mannopyranosides (including disaccharides which were made in high yield). These, after deacetylation, oxidation of the hydroxy-group, and reduction afford α-D-glucopyranosides.[70]

1,2-Orthoesters were encountered in the methanolysis of tetra-*O*-(*p*-nitrobenzoyl)-β-D-fructopyranosyl bromide; while the main product (60%) was the result of direct inversion (α-pyranoside), the β-anomer and the bicyclic compounds (22) and (23) were all formed to the extent of about 12%.[71] The orthoester glycosylation method has been used to obtain a β-D-glucopyranosyl porphyrin derivative[72] and β-D-ribopyranosides of strophanthidin and digitoxigenin.[73]



(21)



$R^3 = p\text{-}NO_2 \cdot C_6H_4CO$

	R^1	R^2
(22)	OMe	$p\text{-}NO_2 \cdot C_6H_4$
(23)	$p\text{-}NO_2 \cdot C_6H_4$	OMe

The synthesis of 2-acylamino-2-deoxy-β-D-glucopyranosides can be effected from the acetylated glycosyl acetates by reaction with alcohols in the presence of iron(III) chloride. The procedure is satisfactory with primary, secondary, or tertiary alcohols, and with a range of *N*-acyl substituents. It is believed that the

[68] G. Wulff and J. Wichelhaus, *Chem. Ber.*, 1979, **112**, 2847.
[69] P. J. Garegg and T. Iversen, *Carbohydr. Res.*, 1979, **70**, C13.
[70] P. J. Garegg and L. Maron, *Acta Chem. Scand., Ser. B*, 1979, **33**, 39.
[71] H. Steinlin, L. Camarda, and A. Vasella, *Helv. Chim. Acta*, 1979, **62**, 378.
[72] A. F. Mironov, G. M. Isaeva, V. I. Shvets, R. P. Evstigneeva, A. N. Stepanov, A. A. Perov, and S. E. Kupriyanov, *Bioorg. Khim.*, 1978, **4**, 1410 (*Chem. Abs.*, 1979, **90**, 121 955*g*).
[73] N. Sh. Pal'yants and N. K. Abubakirov, *Khim. Prir. Soedin.*, 1978, 656 (*Chem. Abs.*, 1979, **90**, 152 538*c*).

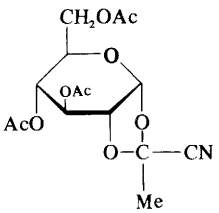
1,2-oxazolines are reaction intermediates.[74] The oxazoline method of obtaining glycosides of 2-acylamino-2-deoxy sugars is also referred to in Chapter 8.

Synthesis of Disaccharides and their Derivatives. – A survey of methods of synthesis of oligosaccharides has appeared,[75] as has a brief review of the general chemistry of these compounds.[76]

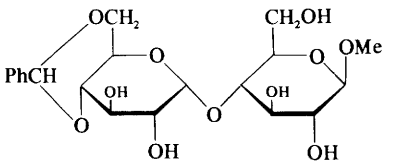
In the following section disaccharides will be categorized according to the nature of the non-reducing terminal sugar: little has been reported on trehalose although deoxyfluoro-derivatives are referred to in Chapter 7. Several glucosylating procedures have been used to obtain reducing disaccharide derivatives, notably the imidate procedure which is concluded to be perhaps the most effective α-glucosidation method so far developed; it was applied to the preparation of five disaccharides.[77] Alternatively, several disaccharides containing the 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl unit on deacetylation, oxidation with chromium(III) oxide, pyridine and acetic anhydride, and reduction of the ketonic products gave α-glucopyranosyl compounds. Excellent stereoselectivity was obtained by reductions using diborane.[78] Russian workers, using the cyano-derivative (24) with tritylated alcohols and trityl perchlorate, prepared various 1,1-, 1,3-, 1,4-, 1,5-, and 1,6-linked β-D-glucopyranosyl disaccharides[79] as well as galactosyl, mannosyl, and rhamnosyl 1,4-linked compounds.[79a] A paper from Japan described three methods for preparing 2-*O*-β-D-glucopyranosyl-D-glucose (sophorose), one a Koenigs–Knorr approach and the others involving Brigls' anhydride,[80] while hepta-*O*-acetyl sophorosyl bromide has been used to obtain corresponding glycosides.[81] A standard glycosylation process has been used to obtain 3-*O*-β-D-glucopyranosyl-D-glucose (laminaribiose),[82] and a yeast enzyme gave this disaccharide in 47% yield from laminarin.[83] In the area of 1,4-linked glucobioses, methyl β-D-cellobioside on deuteriation with D_2O in the presence of Raney nickel gave the C-2,3,3′,4,6,6,6′,6′ labelled compound which was examined in elegant proton-coupled ^{13}C studies. Three bond ^{13}C–^{1}H coupling across the interunit glycosidic bond led to the determination of the involved torsion angles.[84] The acetylated glycosyl bromides of cellobiose, maltose, and lactose gave *trans*-related glycosides of 8-ethoxycarbonyloctanol with silver trifluoromethanesulphonate and

[74] M. Kiso and L. Anderson, *Carbohydr. Res.*, 1979, **72**, C12.
[75] H. M. Flowers, *Methods Enzymol.*, 1978, **50**, 93.
[76] L. Hough and A. C. Richardson, in 'Comprehensive Organic Chemistry', ed. E. Haslam, Pergamon, Oxford, 1979, vol. 5, p. 749 (*Chem. Abs.*, 1979, **91**, 39 735x).
[77] J. R. Pougny, M. A. M. Nassr, N. Naulet and P. Sinay, *Nouv. J. Chim.*, 1978, **2**, 289 (*Chem. Abs.*, 1979, **90**, 104 283a).
[78] P. J. Garegg and L. Maron, *Acta Chem. Scand., Ser. B*, 1979, **33**, 453.
[79] Y. V. Wozney, L. V. Backinowsky and N. K. Kochetkov, *Carbohydr. Res.*, 1979, **73**, 282.
[79a] V. I. Betaneli, M. V. Ovchinnikov, L. V. Backinowsky, and N. K. Kochetkov, *Carbohydr. Res.*, 1979, **76**, 252.
[80] S. Hirano, R. Yamasaki, S. Koto, and H. Iwaki, *Carbohydr. Res.*, 1979, **68**, 147.
[81] M. Nozaki, T. Ogawa, and M. Matsui, *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, 21st, 1978, 213 (*Chem. Abs.*, 1979, **90**, 168 908j).
[82] K. Takeo, *Carbohydr. Res.*, 1979, **77**, 245.
[83] T. G. Villa, H. J. Phaff, and V. Notario, *Carbohydr. Res.*, 1979, **74**, 369.
[84] G. K. Hamer, F. Balza, N. Cyr, and A. S. Perlin, *Can. J. Chem.*, 1978, **56**, 3109.

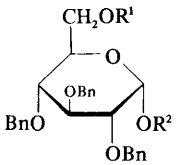
tetramethylurea as acid acceptors, but with 2,4,6-trimethylpyridine the corresponding orthoesters were obtained.[85] On the other hand, the β-octa-acetates of these sugars with this alcohol in the presence of tin(IV) chloride gave initially the β-glycosides which then underwent anomerization to give the α-anomers.[86] A convenient route to 6-substituted methyl β-maltosides utilizes the benzylidene derivative (25) which affords the 6-tosyl ester in good yield.[87] From the glycosyl tosylate (26) the 6-hydroxy-compound (27) was obtained, and the combination of these two gave access to the isomaltose derivative (28). Repetition gave tri- and tetra-saccharides which were coupled to proteins for biological studies.[88] Thymidine and 2′-deoxycytidine 5′-β-D-glucopyranosides were made using the 3′-acetyl-5′-trityl derivatives and tetra-*O*-acetyl-α-D-glycosyl bromide. Glycosylation of the untritylated analogues gave rise to the 1,2-orthoester analogues.[89]



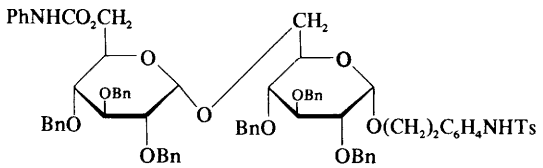
(24)



(25)



	R^1	R^2
(26)	PhNHCO	Ts
(27)	H	$O(CH_2)_2C_6H_4NHTs$



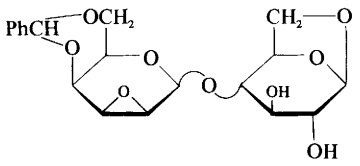
(28)

In the field of D-galactose-containing disaccharides 2-*O*-β-D-galactopyranosyl-D-galactose was prepared by glycosylation of methyl 4,6-*O*-benzylidene-α-D-galactoside,[90] and hepta-*O*-acetyl-D-lactosyl bromide was used in the preparation of the β-glycoside of 8-ethoxycarbonyloctanol (*cf.* ref. 85) which was converted to the corresponding 8-azidocarbonyl compound and coupled to proteins to afford an artificial disaccharide antigen.[91] Methods for the isolation of α- and

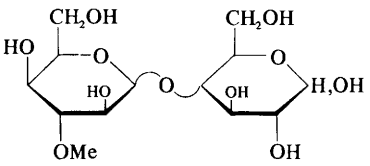
[85] J. Banoub and D. R. Bundle, *Can. J. Chem.*, 1979, **57**, 2091.
[86] J. Banoub and D. R. Bundle, *Can. J. Chem.*, 1979, **57**, 2085.
[87] K. Takeo, *Carbohydr. Res.*, 1979, **69**, 272.
[88] R. Eby, *Carbohydr. Res.*, 1979, **70**, 75.
[89] N. D. Chkanikov, V. N. Tolkachev, and M. N. Preobrazhenskaya, *Bioorg. Khim.*, 1978, **4**, 1620 (*Chem. Abs.*, 1979, **90**, 204 408*g*).
[90] T. Toba and S. Adachi, *Carbohydr. Res.*, 1979, **75**, C24.
[91] D. R. Bundle, *Can. J. Biochem.*, 1979, **57**, 367.

β-lactose from octa-acetates have been described,[92] and the reversion of D-galactose (and D-mannose and L-rhamnose) to give disaccharides has been studied. The hexoses gave 6- and 4-linked products (both anomers) in the approximate ratio 1.5 : 1, while rhamnose gave mainly 1,4-products but some 1,2-linked isomers.[93]

2-*O*-α-D-Mannopyranosyl-β-D-mannopyranose and 6-*O*-α-D-mannopyranosyl-α-D-mannopyranose have been prepared by Koenigs–Knorr procedures as their octa-acetates and fused with *p*-nitrophenol to afford the α-aryl glycosides,[94] and methyl 3-*O*-α-D-mannopyranosyl-α-D-mannopyranoside was made *via* the 4,6-*O*-benzylidene acetal.[95] Standard reactions applied to the dianhydride (29) afforded means of obtaining the unusual 3-*O*-methyl-β-D-idopyranosyl-D-glucose (30) as well as the 3′-deoxy analogue.[96]



(29)

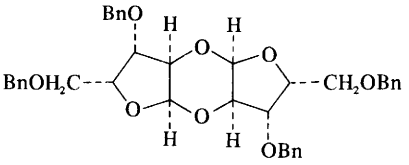


(30)

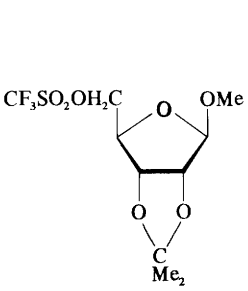
In the pentose series 4-*O*-β-D-xylopyranosyl-D-xylose has been made by glycosylation of benzyl 2,3-anhydro-β-D-ribopyranoside,[97] and 3,5-di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose was converted in high yield to the tricyclic bisglycoside (31) on treatment with boron trifluoride in benzene[98] (*cf.* below). A novel approach to glycoside synthesis with D-ribofuranose compounds involves the use of the trifluoromethanesulphonyl leaving group in compound (32) and the anomerically specific metalated anions (33) and (34), both of which were available from 2,3-*O*-isopropylidene-D-ribofuranoses, to give α- and β-linked 1,5-disaccharides, respectively.[99] Ribose oligosaccharides which are 1,5-α linked have also been obtained using the glycosylating agent (35), which gave mainly the α-glycoside, followed by lithium aluminium hydride reduction of the ester group (see later).[100]

Considerable attention has been given to deoxydisaccharides, mainly because of their biological significance. Benzyl 2,3-dideoxy-β-D-*glycero*-pentopyranoside gave compound (36) by appropriate glycosylation,[101] but most attention has

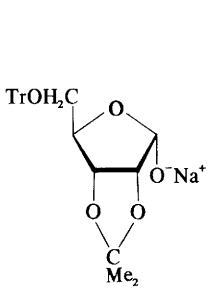
[92] F. W. Parrish, K. D. Ross, and T. D. Simpson, *Carbohydr. Res.*, 1979, **71**, 322.
[93] A. Soler, P. A. Garcia Ruiz, and A. Tarraga Tomas, *An. Quim.*, 1978, **74**, 1097 (*Chem. Abs.*, 1979, **91**, 91 877*j*).
[94] C. M. Reichert, *Carbohydr. Res.*, 1979, **77**, 141.
[95] E. E. Lee and J. O. Wood, *Carbohydr. Res.*, 1979, **75**, 322.
[96] T. Chiba and S. Tejima, *Chem. Pharm. Bull.*, 1978, **26**, 3426.
[97] P. Kováč, *Chem. Zvesti*, 1979, **33**, 365.
[98] P. H. A. Zollo, J.-R. Pougny, and P. Sinay, *Tetrahedron Lett.*, 1979, 2447.
[99] R. R. Schmidt and M. Reichrath, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 466.
[100] R. R. Schmidt and P. Hermentin, *Chem. Ber.*, 1979, **112**, 2659.
[101] H. S. El Khadem and D. Matsuura, *Carbohydr. Res.*, 1979, **75**, 331.



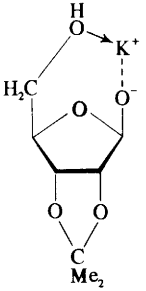
(31)



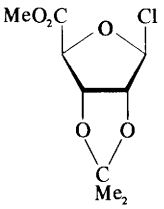
(32)



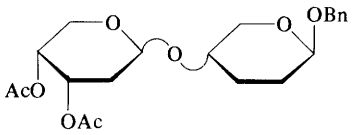
(33)



(34)



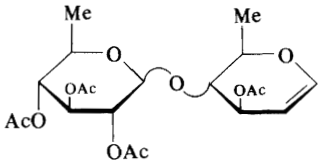
(35)



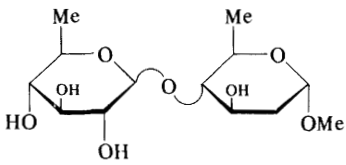
(36)

centred on dihexose derivatives. Several procedures were used to prepare 6,6′-dideoxy-6,6′-dihalogenocellobiosides which were reduced to give compounds from which the glycal (37) was obtained and hence the trideoxy glycoside (38).[102] A further 6,6′-dideoxy-D-glucobiose compound was obtained from the unsaturated derivative (39) which was the unexpected product formed on treatment of the 5-enoside (40) with β-glucosidase. The 1,4-linked analogue of the dimer (39) was prepared by standard methods for comparison.[103] Also in the 6-deoxyhexose series 2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl-, 2-*O*-α-L-rhamnopyranosyl-β-D-galactopyranosyl-, and 2-*O*-α-L-quinovopyranosyl-β-D-glucopyranosyl-units have been bonded to a diterpene to give analogues of stevioside,[104] and acetylated and *p*-nitrobenzoylated esters of α-L-fucopyranosyl bromide were used in the preparation of α- and β-linked fucosyl disaccharides.[105]

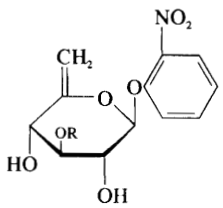
[102] J. Thiem, *Carbohydr. Res.*, 1979, **68**, 287.
[103] J. Lehmann and E. Schröter, *Carbohydr. Res.*, 1979, **71**, 65.
[104] S. Kamiya, F. Konishi, and S. Esaki, *Agric. Biol. Chem.*, 1979, **43**, 1863.
[105] H. M. Flowers, *Carbohydr. Res.*, 1979, **74**, 177.



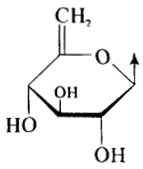
(37)



(38)

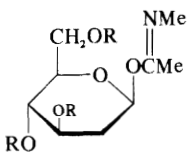


(39) R =

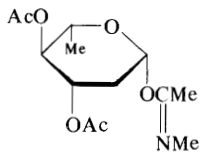


(40) R = H

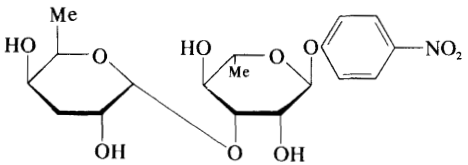
The imidates (41) and (42) have been used to obtain 2-deoxy- and 2,6-dideoxy compounds,[106] and the trideoxy-disaccharide glycoside (43), used in the preparation of artificial antigens, was made by glycosylation of the appropriate dibenzoylated rhamnoside.[107] In related work, the tetradeoxy compounds (44) and (45) were obtained from appropriate monohydroxy derivatives following glycosylations with tri-*O*-acetyl-D-glucal. While the intermediate from which the former was prepared was a 2-deoxy-2-iodo-glycosyl compound,[108] that for the latter contained a 2,3-epoxide ring in each unit.[109] An alternative route to compound (45) involved the use of the appropriate 6-deoxyglycal derivative in the presence of *N*-iodosuccinimide and the production of another iodinated disaccharide.[110]



R = *p*-$NO_2C_6H_4CO$ (41)

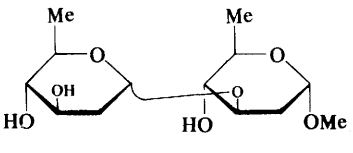


(42)

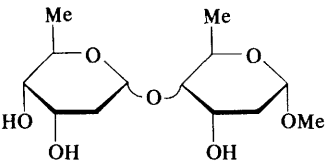


(43)

[106] J. Szymoniak and P. Sinaÿ, *Tetrahedron Lett.*, 1979, 545.
[107] P. J. Garegg and H. Hultberg, *Carbohydr. Res.*, 1979, **72**, 276.
[108] J. Thiem, H. Karl, and U. Ellermann, *Chem. Ber.*, 1979, **112**, 3139.
[109] J. Thiem and J. Schwentner, *Chem. Ber.*, 1979, **112**, 3126.
[110] J. Thiem, P. Ossowski, and J. Schwentner, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 222.



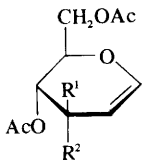
(44)



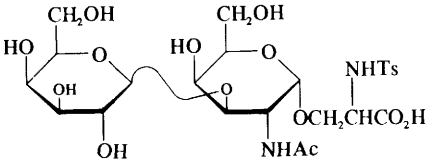
(45)

Considerable attention has been given to amino-sugar disaccharides. The 2-azido-2-deoxyglycosyl chloride procedure has been applied to obtain 2-amino-2-deoxy-α-D-glucopyranosyl α-D-glucopyranoside (trehalosamine),[111] and the 2-deoxy-2-phthalamidoglycosyl bromide method to produce 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose.[112] Otherwise, 2-amino-2-deoxy-D-glucosyl disaccharides can be prepared by direct condensation between appropriate glycosyl acetates and monohydroxy sugar derivatives in the presence of iron(III) chloride. The procedure was used to obtain monoamino-compounds and 2-amino-2-deoxy-D-glucose dimers.[113] The following disaccharides which are common to the O-antigen determinants of *Shigella flexneri* were prepared for immunological purposes: 2-acetamido-2-deoxy-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucose, 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-L-rhamnose, and 2-*O*-(α-L-rhamnopyranosyl)-α-L-rhamnose.[114] Compounds (46) and (47) have been used together with the nitrosyl chloride addition procedure to yield a series of diaminoglycosides, and a 2,3-diamino-2,3-dideoxy-D-allosyl disaccharide.[115]

Condensation between tetra-*O*-acetyl-α-D-galactopyranosyl bromide and a suitably protected 2-acetamido-2-deoxy-α-D-galactopyranosyl-L-serine derivative gave access to compound (48) which is the *N*-tosyl derivative of the carbohydrate core of mucins and was required for studies of haemagglutination.[116] Evidence that the major glycoprotein of TA3-Ha tumour cells contains the same disaccharide was produced by the isolation, after alkaline degradation and sodium borohydride reduction, of 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactitol.[117] In order to determine whether *N*-acetylhyalobio-

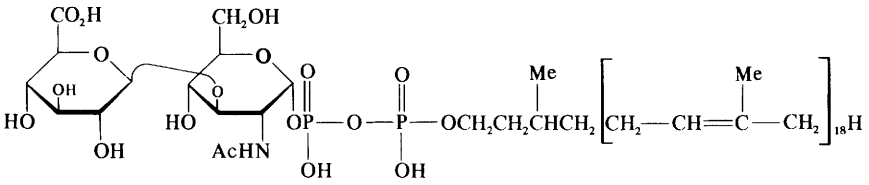


	R^1	R^2
(46)	N_3	H
(47)	H	N_3



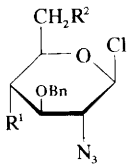
(48)

[111] H. Paulsen and B. Sumfleth, *Chem. Ber.*, 1979, **112**, 3203.
[112] B. Y. Chung and Y. K. Sim, *Taehan Hwahakhoe Chi*, 1979, **23**, 46 (*Chem. Abs.*, 1979, **91**, 91 890*h*).
[113] M. Kiso and L. Anderson, *Carbohydr. Res.*, 1979, **72**, C15.
[114] D. R. Bundle and S. Josephson, *Can. J. Chem.*, 1979, **57**, 662.
[115] K. Heyns and R. Hohlweg, *Chem. Ber.*, 1978, **111**, 3912.
[116] R. Kaifu and T. Osawa, *Carbohydr. Res.*, 1979, **69**, 79.
[117] J. F. Codington, T. Yamazaki, D. H. van den Eijnden, N. A. Evans, and R. W. Jeanloz, *FEBS Lett.*, 1979, **99**, 70.

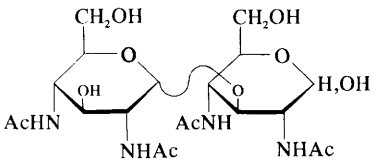


(49)

syluronic dolichyl diphosphate (49) is an intermediate in the biosynthesis of hyaluronic acid it was synthesized by use of a corresponding oxazoline derivative.[118] α-D-Glucosylation, by use of the imidate method, of appropriate 2-acetamido-2-deoxy-D-glucose derivatives gave access to 1,4-linked disaccharides.[119] In the area of disaccharides composed of two amino-sugars, Durette and co-workers have synthesized chitobioses with dipeptide chains linked through a lactyl group bonded at C-3[120] and at C-3′[121] for studies of bacterial cell-wall peptidoglycans. By use of the glycosylating agents (50) and (51) 2,4-diamino- and 2,6-diamino-glucosyl units were bonded to various amino-sugar derivatives to afford tri- and tetra-deoxyamino disaccharides, *e.g.* (52).[122]



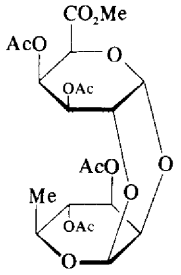
	R¹	R²
(50)	N_3	OAc
(51)	OBn	N_3



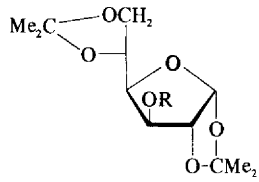
(52)

In the field of aldobiouronic acid derivatives an ingenious method has been devised for the preparation of radioactive disaccharides for use as substrates for α-L-iduronidase, β-D-glucuronidase, and 2-sulpho-L-iduronate 2-sulphatase. Heparin was cleaved by the deamination procedure and the products were reduced using sodium borotritide to afford access to α-L-idopyranosyluronic and β-D-glucopyranosyluronic acid derivatives of 2,5-anhydro-D-[1-^{3}H]mannitol.[123] Acidic treatment in methanol of the water-soluble polysaccharides of wobaku wood, followed by acetylation, have led to the isolation of the anhydride derivative (53)[124,125] which was concluded to arise by way of the corresponding aldo-

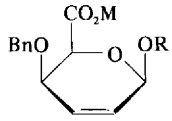
[118] E. Walker-Nasir and R. W. Jeanloz, *Carbohydr. Res.*, 1979, **68**, 343.
[119] M. A. M. Nassr, J.-C. Jacquinet, and P. Sinaÿ, *Carbohydr. Res.*, 1979, **77**, 99.
[120] P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Carbohydr. Res.*, 1979, **77**, C1.
[121] P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Tetrahedron Lett.*, 1979, 4013.
[122] H. Paulsen, O. Lockhoff, B. Schröder, and W. Stenzel, *Carbohydr. Res.*, 1979, **68**, 239.
[123] J. J. Hopwood, *Carbohydr. Res.*, 1979, **69**, 203.
[124] T. Fujiwara and K. Arai, *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu*, 21st, 1978, 206 (*Chem. Abs.*, 1979, **91**, 20935*r*).
[125] T. Fujiwara and K. Arai, *Carbohydr. Res.*, 1979, **69**, 97.



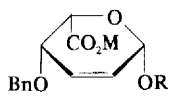
(53)



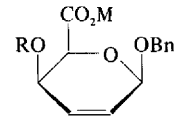
(54) R = —CH=CH—CH=CHOBn



(55)

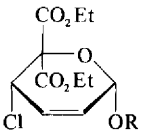


(56)

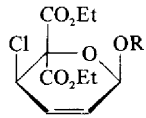


(57)

M = L-menthyl
R = di-*O*-isopropylidene-D-glucofuranes-3-yl



(58)



(59)

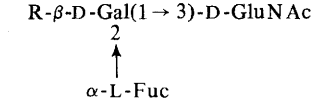
biouronic acid, since this also afforded the anhydride on similar treatment.[126] David and Eustache have continued their novel work on building up disaccharide derivatives from monosaccharides, and from the diene (54), by a Diels–Alder addition, have prepared the unsaturated uronic acids (55)–(57) and hence have produced 3-*O*-β-D-gulopyranosyl- and 3-*O*-β-L-gulopyranosyl-D-glucose derivatives as well as a gulose–glucose disaccharide linked by a 4–3 bond.[127] Compounds (58) and (59) were also obtained, and various 3,4-unsaturated derivatives were isolated after allylic rearrangement processes.[128] Standard procedures were used for β-D-xylopyranose substitution at O-2 of D-galacturonic acid, and various derivatives of the resultant disaccharide were reported.[129]

Synthesis of Tri- and Tetra-saccharides. — Kováč and co-workers have produced several D-xylose trisaccharides related to structural components of xylans. Direct D-xylosylation of methyl 2-*O*-benzyl-β-D-xylopyranoside gave access to the methyl glycoside of 3,4-di-*O*-(β-D-xylopyranosyl)-β-D-xylose,[130] and the 2,4-

[126] T. Fujiwara and K. Arai, *Carbohydr. Res.*, 1979, **69**, 107.
[127] S. David and J. Eustache, *J. Chem. Soc., Perkin Trans. I*, 1979, 2230.
[128] S. David and J. Eustache, *J. Chem. Soc., Perkin Trans. I*, 1979, 2521.
[129] S. B. Tjan, D. J. Poll, and T. Doornbos, *Carbohydr. Res.*, 1979, **73**, 67.
[130] P. Kováč, *Collect. Czech. Chem. Commun.*, 1979, **44**, 928.

substituted isomer, as well as the β-glycoside of *O*-β-D-xylopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-β-D-xylopyranose, were also prepared[131] as was a related branched-chain tetrasaccharide derivative.[132] In the D-glucose oligomer series standard β-D-glucosylation of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside afforded both anticipated disaccharides, but also some 3,6-disubstituted-D-glucose derivative which, the author concluded, arose following migration of the acetal ring to the 2,4-sites. In view of the improbability of this, it is here suggested that the trimer may be a product of glucosylation of a partially hydrolysed intermediate.[133] A close isomer of this product, *O*-α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl-(1 → 6)-D-glucose, has been made using hepta-*O*-acetyl-β-kojibiosyl chloride or, less efficiently, the corresponding α-bromide.[134] In the homo-D-mannose series 2,6-di-*O*-(α-D-mannopyranosyl)-D-mannose was synthesized together with the component disaccharides and corresponding mixed D-glucose,D-mannose disaccharides by use of glycosyl tosylates.[135] Substitution of benzyl 2,4-di-*O*-benzyl-α-D-mannopyranoside, prepared by stannylation of the glycoside and conversion to the 3,6-di-*O*-allyl ether, afforded access to 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannose.[136] Simple heterotrisaccharides to have been isolated are 6-*O*-(α- and β-D-galactopyranosyl)-4-*O*-(α-D-glucopyranosyl)-D-glucose (by galactosylation of a maltose derivative),[137] and α-D-xylopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 4)-D-glucose (by cellulase degradation of an arabinoxyloglucan obtained from tobacco leaves). In addition a pentasaccharide containing this trisaccharide was obtained.[138]

Major stimulus has been given to the chemical synthesis of oligosaccharides by the desire to obtain the antigenic determinants of human blood group substances, and Lemieux has reported on his major achievements in the field in giving the Haworth Memorial Lecture.[139] Two groups have described the synthesis of the trisaccharide determinant of substance B, *i.e.* 2-*O*-(α-L-fucopyranosyl)-3-*O*-(α-D-galactopyranosyl)-D-galactose,[140,141] and Paulsen's group has reported the synthesis of the tri- and tetra-saccharides of substances H, A, and B (60)–(62) respectively.[142] Several syntheses of closely related tri- and tetra-



(60) R is absent
(61) R = α-D-GalNAc(1 → 3)
(62) R = α-D-Gal(1 → 3)

[131] J. Hirsch and P. Kováč, *Carbohydr. Res.*, 1979, **77**, 241.
[132] P. Kováč, J. Hirsch, and V. Kováčik, *Carbohydr. Res.*, 1979, **75**, 109.
[133] K. Takeo, *Carbohydr. Res.*, 1979, **77**, 131.
[134] V. Pozsgay, P. Nánási, and A. Neszmélyi, *Carbohydr. Res.*, 1979, **75**, 310.
[135] R. Eby and C. Schuerch, *Carbohydr. Res.*, 1979, **77**, 61.
[136] J. Arnarf and J. Lönngren, *Acta Chem. Scand., Ser. B*, 1978, **32**, 696.
[137] T. G. Chung and S. Tejima, *Chem. Pharm. Bull.*, 1978, **26**, 3562.
[138] M. Mori, S. Eda, and K. Kato, *Agric. Biol. Chem.*, 1979, **43**, 145.
[139] R. U. Lemieux, *Chem. Soc. Rev.*, 1978, **7**, 423.
[140] J. C. Jacquinet and P. Sinaÿ, *Tetrahedron Lett.*, 1979, 365.
[141] C. Augé and A. Veyrières, *J. Chem. Soc., Perkin Trans. I*, 1979, 1825.
[142] H. Paulsen and C. Kolář, *Chem. Ber.*, 1979, **112**, 3190.

saccharides have been carried out both in Paulsen's[143] and Sinay's[144–146] laboratory.

Repeating units of antigenic bacterial polysaccharides have also presented targets for oligosaccharide syntheses. The trisaccharide of a *Shigella flexneri* polymer, *O*-α-L-rhamnopyranosyl-(1→3)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-L-rhamnose, has been described as its δ-methoxycarbonyloctyl glycoside,[147] as has a related tetrasaccharide.[148] Part of a repeating unit of a pneumococcal polysaccharide, 2-*O*-(α-D-glucopyranosyl)-3-*O*-(α-L-rhamnopyranosyl)-L-rhamnose, was synthesized as its methyl α-glycoside together with some of the β-glucosyl isomer.[149] The disaccharide glycosidulose (63) on reduction with sodium borotritide and conversion into a glycosyl bromide derivative has been used to prepare the trisaccharide repeating unit of *Salmonella newington* O-specific polysaccharide *O*-β-D-mannopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-D-galactose with a tritium label in the mannose moiety.[150] The *p*-aminophenyl β-pyranoside was then synthesized and coupled to edestin to give an artificial antigen.[151]

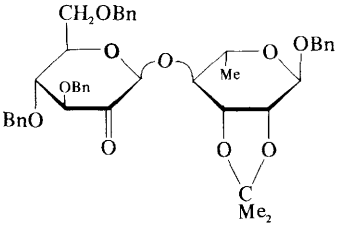
Medical biochemical work has provided the impetus for several other synthetic studies. Peracetylated *O*-α-D-galactopyranosyl-(1→4)-*O*-β-D-galactopyranosyl-(1→4)-α-D-glucopyranosyl chloride was used to condense the trisaccharide to ceramide to give access to a compound which accumulates in patients with Fabry's disease,[152] and in related work, *O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-L-serine, a proteoglycan fragment, was synthesized.[153] The following milk oligosaccharides have been prepared synthetically: *O*-β-L-fucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose,[154] *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose, this trimer with β-D-galactopyranose bonded to O-4 of the non-reducing moiety, and *O*-β-D-galactopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose.[155] Administration of D-galactose p.o. or i.v. to humans leads to urinary excretion of 3-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-2-*O*-(α-L-fucopyranosyl)-D-galactose, 3-*O*-(α-D-galactopyranosyl)-2-*O*-(α-L-fucopyranosyl)-

[143] H. Paulsen and C. Kolář, *Tetrahedron Lett.*, 1979, 2881.
[144] M.-L. Milat and P. Sinaÿ, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 464.
[145] J.-C. Jacquinet and P. Sinaÿ, *J. Chem. Soc., Perkin Trans. I*, 1979, 314.
[146] J.-C. Jacquinet and P. Sinaÿ, *J. Chem. Soc., Perkin Trans. I*, 1979, 319.
[147] D. R. Bundle and S. Josephson, *J. Chem. Soc., Perkin Trans. I*, 1979, 2736.
[148] S. Josephson and D. R. Bundle, *Can. J. Chem.*, 1979, **57**, 3073.
[149] D. Schwarzenbach and and R. W. Jeanloz, *Carbohydr. Res.*, 1979, **77**, C5.
[150] V. N. Shibaev, V. N. Chekonchikov, and N. K. Kochetkov, *Izv. Akad. Nauk, SSSR, Ser. Khim.*, 1978, 2124 (*Chem. Abs.*, 1979, **90**, 87 768*m*).
[151] N. K. Kochetkov, B. A. Dmitriev, A. Ya. Chernyak, V. I. Pokrovsky, and Yu. Ya. Tendetnik, *Bioorg. Khim.*, 1979, **5**, 217.
[152] D. Shapiro and A. J. Acher, *Chem. Phys. Lipids*, 1978, **22**, 197 (*Chem. Abs.*, 1979, **90**, 104 284*v*).
[153] P. J. Garegg, B. Lindberg, and T. Norberg, *Acta Chem. Scand., Ser. B*, 1979, **33**, 449.
[154] H. H. Baer and S. A. Abbas, *Carbohydr. Res.*, 1979, **77**, 117.
[155] S. E. Zurabyan, V. A. Markin, V. V. Pimenova, B. V. Rozynov, V. L. Sadovskaya, and A. Ya. Khorlin, *Bioorg. Khim.*, 1978, **4**, 928 (*Chem. Abs.*, 1979, **90**, 23 448*z*).

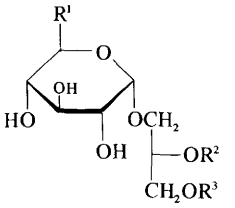
D-galactose, and 2-*O*-α-L-fucopyranosyl-D-galactose[156] (see blood group substances above).

Transglycosylation of sucrose catalysed by brewers' yeast α-glucosidase gives *O*-α-D-glucopyranosyl-(1 → 6)-*O*-β-D-fructofuranosyl-(2 → 1)-*O*-α-D-glucopyranoside (isomelezitose),[157] and in connection with plant glycoside studies an ester of *O*-α-L-rhamnopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranose was prepared and condensed with the triterpene oleanolic acid.[158] Many oligosaccharides have been characterized as components of natural products, but, as is customary, are not covered in this report.

O-Glycosides Isolated from Natural Products. – Amongst the plethora of such compounds which have been described, only a few are mentioned here; all have interesting carbohydrate components: steroidal glycosides containing 2,6-dideoxy-3-*O*-methyl-D-*ribo*-,[159] D-*lyxo*-, and L-*arabino*-hexopyranose[160] moeities, a flavonoid containing a β-D-allopyranosyl unit,[161] and α-D-glucos-6-ylsulphonic acid and α-D-*glycero*-D-*gluco*-heptose glycerol derivatives (64)[162] and (65).[163]



(63)



	R¹	R²	R³
(64)	CH_2SO_3H	H	Acyl
(65)	CHOH·CH_2OH	Acyl	Acyl

Hydrolysis and Other Reactions. – Further data have appeared on the relative rates of acid catalysed hydrolysis of methyl α-D-glucopyranoside, -galactopyranoside, and -mannopyranoside, and consequently, optimum conditions for their completion were proposed.[164] A detailed investigation of the acidic cleavage of substituted phenyl α-D-mannopyranosides indicated a probable mechanism; thermodynamic data were calculated and discussed.[165] The relative rates of hydrolysis of D-xylose oligosaccharides with 2–5 residues in aqueous sulphuric acid were determined, and one of the terminal bonds was found to cleave significantly faster than all of the other linkages.[166]

[156] M. A. Chester, P. Hallgren, A. Lundblad, and L. Messeter, *Eur. J. Biochem.*, 1979, **100**, 385.
[157] S. Chiba, M. Murata, K. Matsusaka, and T. Shimomura, *Agric. Biol. Chem.*, 1979, **43**, 775.
[158] S. Takabe, T. Takeda, and Y. Ogihara, *Carbohydr. Res.*, 1979, **76**, 101.
[159] K. Ito, J. Lai, and K. Usuda, *Chem. Pharm. Bull.*, 1978, **26**, 3189.
[160] F. Abe and T. Yamauchi, *Chem. Pharm. Bull.*, 1978, **26**, 3023.
[161] T. Okuyama, K. Hosoyama, Y. Hiraga, G. Kurono, and T. Takemoto, *Chem. Pharm. Bull.*, 1978, **26**, 3071.
[162] I. Kitagawa, Y. Hamamoto, and M. Kobayashi, *Chem. Pharm. Bull.*, 1979, **27**, 1934.
[163] S. G. Wilkinson and L. Galbraith, *Biochim. Biophys. Acta*, 1979, **575**, 244.
[164] M. A. L. Feijo, M. Iacomini, and J. H. Duarte, *Arg. Biol. Technol.*, 1978, **21**, 119 (*Chem. Abs.*, 1979, **91**, 158 038g).
[165] C. K. De Bruyne and A. De Bock, *Carbohydr. Res.*, 1979, **68**, 71.
[166] Y. Kamiyama and Y. Sakai, *Carbohydr. Res.*, 1979, **73**, 151.

An interesting study of eleven alkyl aldofuranosides (with the 1,2-*cis* configuration) in aqueous perchloric acid at different temperatures suggested that hydrolysis succeeded protonation of the ring oxygen atoms, followed by ring opening to give acyclic carbenium ions.[167] A further study of this reaction in the same laboratory involved alteration of the group at C-2; electron withdrawing groups were found to retard the hydrolysis.[168]

Trifluoroacetolysis provides a means of specifically cleaving monosaccharide and disaccharide moieties glycosidically linked to L-serine and L-threonine.[169] A further study of the reaction using trifluoroacetic anhydride–trifluoroacetic acid mixtures showed that 50:1 mixtures led to trifluoroacetylation of hydroxy-groups without glycosidic bond cleavage, whereas equimolar mixtures caused partial cleavage of the acid labile glycosidic bonds of 6-deoxyhexoses and pentoses.[170] Anomerization of alkyl per-*O*-benzyl-β-D-glucopyranosides is readily effected by use of titanium tetrachloride, but replacement of the benzyl group at C-6 by acetate caused appreciable reduction in the reaction rates which suggested that the Lewis acid co-ordinated with both the ring and C-6 oxygen atoms prior to ring opening. The observation that the isomerizations were quantitative led to the suggestion that O-1 and O-2 also became co-ordinated to titanium.[171]

Hydrolyses of the glycosidic linkages of phenyl α- and β-D-glucopyranoside were studied at different alkali concentrations, and the reaction rates increased rapidly above 2M. Mechanisms involving direct hydrolysis and the intermediacy of 1,6-anhydro-β-D-glucose and, with the β-anomer, the 1,2-α-anhydride were proposed.[172] Analogous work with *p*-acetylphenyl 3- and 5-*O*-methyl-β-D-xylofuranoside led to the conclusion that the 2-oxyanions (and presumably 1,2-anhydrides) were involved as intermediates.[173]

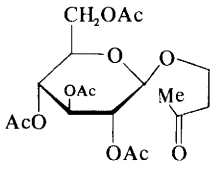
From study of the action of invertase on derivatives of sucrose and methyl β-D-fructofuranoside, it was concluded that small changes at positions 1′, 4′, or 6′ cause loss of enzymic acitivity; various azido-, deoxy, and halogenated-derivatives were examined.[174]

The thermal decomposition of a range of mono-, di-, and tri-saccharides related to the components of 4-*O*-methylglucuronoxylans was studied, and it was found that the thermal stability increased with increasing number of sugar units. The courses of the decompositions were affected by glycosidation of the reducing centres, and by the addition of ionic dehydrating agents, and it was concluded that the 4-*O*-methyl-α-D-glucopyranosiduronic acid bond is most

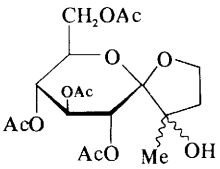
[167] H. Lonnberg and L. Valtonen, *Finn. Chem. Lett.*, 1978, 209 (*Chem. Abs.*, 1979, **90**, 39 192*x*).
[168] H. Lonnberg and M. Arminen, *Finn. Chem. Lett.*, 1978, 244 (*Chem. Abs.*, 1979, **90**, 138 141*m*).
[169] B. Lindberg, B. Nilsson, T. Norberg, and S. Svensson, *Acta Chem. Scand., Ser. B*, 1979, **33**, 230.
[170] B. Nilsson and S. Svensson, *Carbohydr. Res.*, 1979, **69**, 292.
[171] N. Morishima, S. Koto, and S. Zen, *Chem. Lett.*, 1979, 749.
[172] Y.-Z. Lai and D. E. Ontto, *Carbohydr. Res.*, 1979, **75**, 51.
[173] H. Lonnberg, *Finn. Chem. Lett.*, 1978, 213 (*Chem. Abs.*, 1979, **90**, 39 193*y*).
[174] R. D. Guthrie, I. D. Jenkins, P. J. Rogers, W. F. Sum, J. J. Watters, and R. Yamasaki, *Carbohydr. Res.*, 1979, **75**, C1.

thermally stable, and that the presence of acetyl ester groups does not cause acceleration of the decomposition.[175]

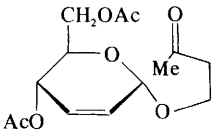
Novel spiro components are obtained by photolysis of 3-oxobutyl glycosides. Compound (66) was thus converted into the epimers (67), but when the reaction was applied to the unsaturated, α-compound (68), the product (69) was not stereochemically discrete at either the 'anomeric' centre or at the new asymmetric centre in the 5-membered ring. Because of this, together with the observation that the α-anomer of compound (66) did not cyclize, the stereospecificity in the first reaction was attributed to the presence of the ester group at C-2.[176] Irradiation of analogous 4-oxopentyl glycosides caused Norrish type II decomposition to the vinyl glycosides.[176a] *cf.* Chapter 12 for reactions of unsaturated 4-oxopentyl glycosides.



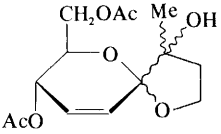
(66)



(67)



(68)



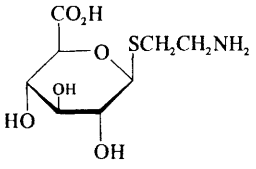
(69)

By use of e.s.r. and ENDOR techniques several radicals have been identified amongst the products of *X*-radiolysis of methyl α-D-glucopyranoside. These include the species formed by loss of hydrogen atoms bonded to O-2, O-6, C-1, and C-5.[177]

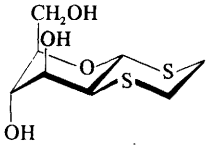
2 *S*-Glycosides

Ethyl 2,3,4-tri-*O*-acetyl-1-thio-β-L-rhamnopyranoside has been prepared by way of the diethyldithioacetal,[178] and the cysteamine *S*-glucuronide (70) was obtained both by use of a glyuronosyl bromide derivative and by treatment of a 1-thiosugar derivative with 2-bromoethylamine.[179] *S*-Benzyl thioglycosides of

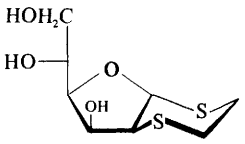
[175] M. Košík, V. Reiser, and P. Kováč, *Carbohydr. Res.*, 1979, **70**, 199.
[176] G. Remy, L. Cottier, and G. Descotes, *Tetrahedron Lett.*, 1979, 1847.
[176a] L. Cottier, G. Remy, and G. Descotes, *Synthesis*, 1979, 711.
[177] K. P. Madden and W. A. Bernhard, *J. Chem. Phys.*, 1979, **70**, 2431.
[178] T. Fujiwara and K. Arai, *Carbohydr. Res.*, 1979, **69**, 305.
[179] J. Oehlke, *Pharmazie*, 1978, **33**, 644 (*Chem. Abs.*, 1979, **90**, 55 199*k*).



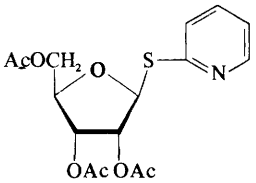
(70)



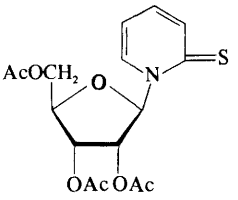
(71)



(72)



(73)



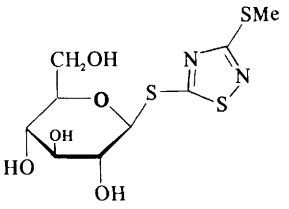
(74)

2-*O*- and 6-*O*-α-D-mannopyranosyl-D-mannose have been synthesized by conventional routes.[180] The unusual thioglycoside derivatives (71) and (72) are obtained on nitrous acid deamination of 2-amino-2-deoxy-D-glucose ethylenedithioacetal (see Chapter 8).[181]

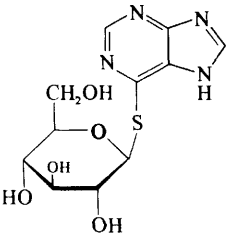
Thioglycosides of β-D-galactopyranose, β-D-glucopyranose, α-D-mannopyranose, and 2-acetamido-2-deoxy-β-D-glucopyranose having the aglycone $CH_2CONHCH_2CH(OMe)_2$ have been obtained by use of corresponding 1-thiosugar derivatives. The acetal function was hydrolysed to the aldehyde which was required for coupling to proteins.[182] In related work, 1-thio-α-D-mannopyranose was substituted at the sulphur atom with the group $CH_2(CH_2)_4CONHNH_2$, to give a thioglycoside suitable for bonding to cell membranes,[183] and with the groups $CH_2(CH_2)_5NH_2$ and $CH_2(CH_2)_5NHCO(CH_2)_5NH_2$ to give products which were bonded to cholesterol to afford synthetic glycolipids.[184]

Irradiation of the pyridyl thioglycoside (73) caused a complex isomerization to give the β-*N*-bonded compound (74) as well as the α- and β-*C*-nucleosides bonded through C-3 and C-5. All were isolated in low yield only.[185] The 1,2,4-thiadiazole thioglucoside (75) has been reported,[186] and the acid- and almond β-glucosidase-catalysed hydrolysis of the purine compound (76) and its 2′-deoxy analogue have been investigated. As expected, the latter is the more sensitive in acid solution, but the former is the better enzyme substrate.[187]

[180] P. L. Durette and T. Y. Shen, *Carbohydr. Res.*, 1979, **69**, 316.
[181] P. Angibeaud, C. Bosso, J. Defaye, D. Horton, C. Cohen-Addad, and M. Thomas, *J. Chem. Soc., Perkin Trans. I*, 1979, 1583.
[182] R. T. Lee and Y. C. Lee, *Carbohydr. Res.*, 1979, **77**, 149.
[183] R. R. Rando, G. A. Orr, and F. W. Bangerter, *J. Biol. Chem.*, 1979, **254**, 8318.
[184] G. A. Orr, R. R. Rando, and F. W. Bangerter, *J. Biol. Chem.*, 1979, **254**, 4721.
[185] J.-L. Fourrey and P. Jouin, *J. Org. Chem.*, 1979, **44**, 1892.
[186] G. Wagner and B. Dietzsch, *Pharmazie*, 1978, **33**, 764 (*Chem. Abs.*, 1979, **90**, 104 291*v*).
[187] P. A. Lartey and L. Fedor, *Carbohydr. Res.*, 1979, **69**, 89.



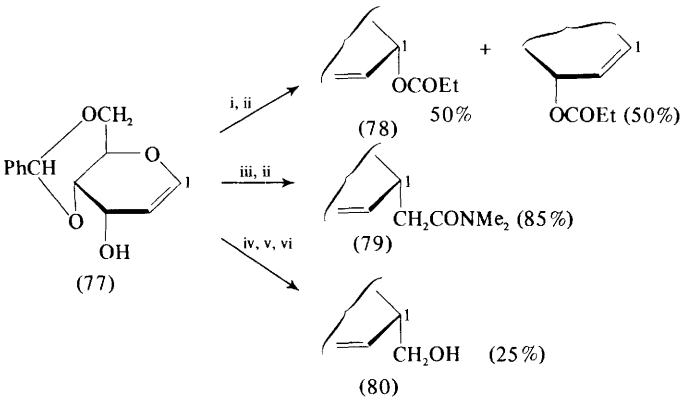
(75)



(76)

3 *C*-Glycosides

Two groups have reported on the use of the Claisen rearrangement to obtain 2,3-unsaturated *C*-glycosides. Fraser-Reid and colleagues found the standard reaction applied to derivatives of the allylic alcohol (77) inefficient because of the poor yields of the derived vinyl ethers or because allylic esters *e.g.* (78) predominated. They were successful, however, in obtaining compounds (79) and (80) by modified procedures (Scheme 5). An alternative synthesis of saturated, and hence unsaturated, *C*-glycosides was developed as illustrated in Scheme 6.[188] In parallel work Ireland and co-workers used the enol silyl ether approach as shown in Scheme 7 and employed the products in polyether antibiotic syntheses.[189]

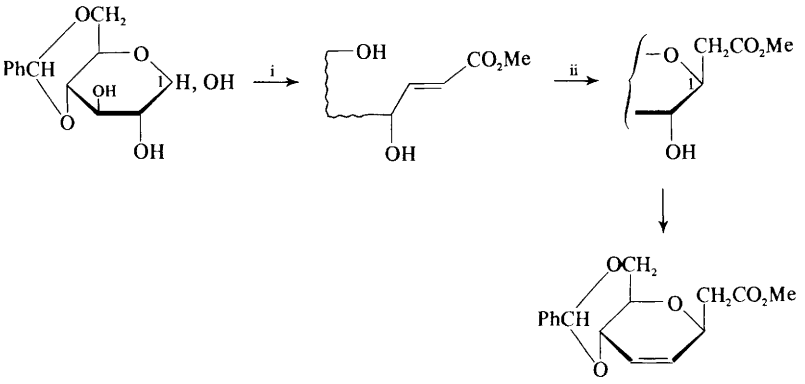


Reagents: i, $MeC(OEt)_3$–$EtCO_2H$; ii, Δ; iii, $MeC(OMe)_2NMe_2$; iv, NaH; v, Bu_3SnCH_2I; vi, BuLi

Scheme 5

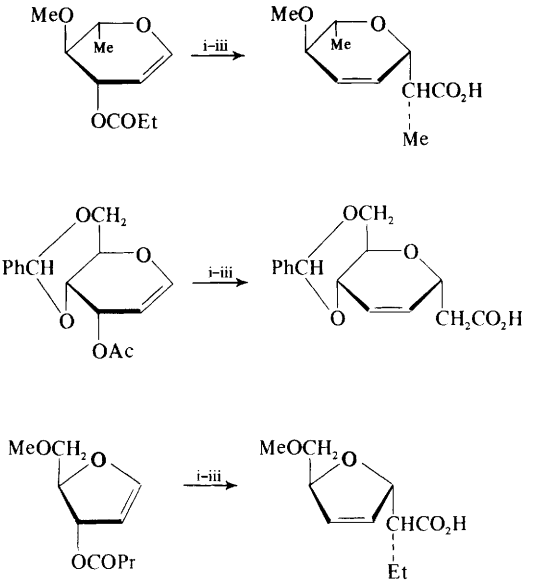
The diazo-compound (81), which is a 'suicide substrate' for β-galactosidase, decomposes in methanol or water to give compounds (82)–(84) together with the solvolysis products (85) or (86).[190]

[188] B. Fraser-Reid, R. D. Dawe, and D. B. Tulshian, *Can. J. Chem.*, 1979, **57**, 1746.
[189] R. E. Ireland, C. S. Wilcox, S. Thaisrivongs, and N. R. Vanier, *Can. J. Chem.*, 1979, **57**, 1743.
[190] M. Brockhaus, H. Fritz, and J. Lehmann, *Carbohydr. Res.*, 1979, **68**, 225.



Reagents: i, $Ph_3P{=}CHCO_2Me$; ii, $\bar{O}H$

Scheme 6

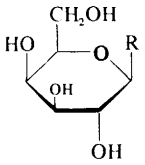


Reagents: i, $(Me_2CH)_2NLi$; ii, Bu^tMe_2SiCl; iii, Δ

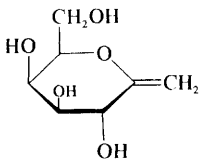
Scheme 7

Treatment of acylated β-D-ribofuranosyl cyanides with liquid hydrogen sulphide gives the corresponding thiocarboxamides,[191] while aldehydo-sugar derivatives when condensed with two equivalents of methyl nitroacetates

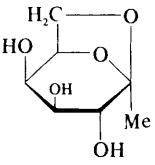
[191] M. V. Pickering, P. C. Srivastava, J. T. Witkowski, and R. K. Robins, *Nucleic Acid Chem.*, 1978, **1**, 145 (*Chem. Abs.*, 1979, **90**, 72 391*j*).



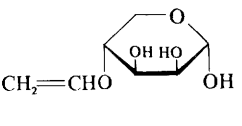
(81) R = CHN_2
(85) R = CH_2OMe
(86) R = CH_2OH



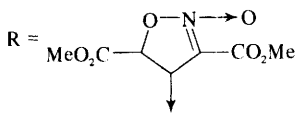
(82)

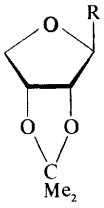


(83)

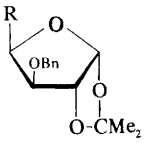


(84)

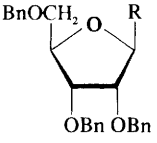




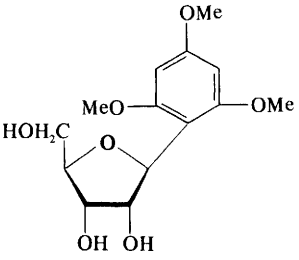
(87)



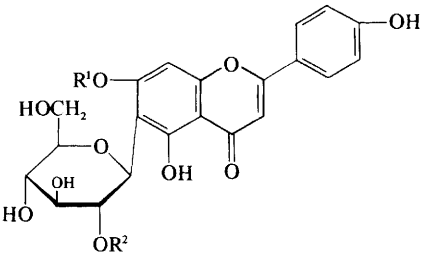
(88)



(89)



(90)



(91) R^1 = D-galactosyl, R^2 = D-glucosyl
(92) R^1 = D-galactosyl, R^2 = L-rhamnosyl
(93) R^1 = D-galactosyl, R^2 = H
(94) R^1 = H, R^2 = L-rhamnosyl

afforded the 4-substituted isoxazoline *N*-oxides (87)–(89); these are *C*-glycosides, whereas acyclic aldehydes led to heterocyclic compounds with acyclic carbohydrate substituents.[192]

2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl bromide condensed with 1,3,5-trimethoxybenzene in the presence of zinc oxide gave the triester of compound (90).[193]

New *C*-glycosides continue to be discovered in natural products; amongst those reported are: a 6,8-di-*C*-hexoside of apigenin (isolated together with

[192] E. Kaji, H. Ichikawa, and S. Zen, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2928.
[193] L. Kalvoda and J. Farkas, *Nucleic Acid Chem.*, 1978, **1**, 461 (*Chem. Abs.*, 1979, **90**, 104 254*k*).

several related *O*-glycosides from the moss *Hedwigia ciliata*),[194] 6-*C*-glucopyranosylnaringenin (the first *C*-glycosyl flavanone found in the *Liliaceae*),[195] and the mixed *C*-, *O*-glycosides (91)–(94) (from *Melandrium album*).[196] Several *C*-arabinosyl flavones have been isolated from a member of the *Rutaceae* family,[197] and 8-*C*-α-L-arabinopyranosylgenkwanin and the β-D-glucopyranosyl analogue and their 2″-rhamnosides have been obtained from *Mollugo distica*.[198]

[194] B.-G. Österdahl, *Acta Chem. Scand., Ser. B*, 1979, **33**, 119.
[195] J. Budzianowski and L. Skrzypczakowa, *Phytochemistry*, 1978, **17**, 2044 (*Chem. Abs.*, 1979, **91**, 16 636*b*).
[196] H. Wagner, G. Obermeier, O. Seligmann, and V. M. Chari, *Phytochemistry*, 1979, **18**, 907 (*Chem. Abs.*, 1979, **91**, 211 748*s*).
[197] M. Jay, J. Gleve, M. L. Bouillant, E. Stanislas, and C. Moretti, *Phytochemistry*, 1979, **18**, 184 (*Chem. Abs.*, 1979, **91**, 20 966*b*).
[198] J. Chopin, M. L. Bouillant, A. G. R. Nair, P. Ramesh, and T. J. Mabry, *Phytochemistry*, 1978, **17**, 299 (*Chem. Abs.*, 1978, **88**, 191 332*j*).

4

Ethers and Anhydro-sugars

1 Ethers

Methyl Ethers. – A further variation on the use of diazomethane for methylation has been described which involves carrying out the reaction in the presence of silica gel. Only one carbohydrate example was described, namely the conversion of methyl α-D-glucopyranoside into its tetra-*O*-methyl ether in 98% yield.[1] Further studies have been conducted on the use of methyl (and ethyl) triflate for alkylation; the reaction is best conducted using 2,6-di-t-butyl-4-methylpyridine as base and most other functional groups were found to be compatible with its use, although one case of acetyl migration and one case of *O*-isopropylidene cleavage were noted.[2] The use of potassium fluoride deposited in celite has been reported as an effective catalyst for *O*-alkylation, although no carbohydrate examples have been described.[3]

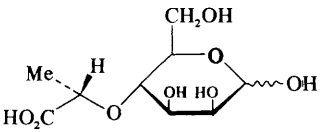
Phase transfer catalysed methylation of a variety of mono- and di-*O*-cyclohexylidene acetals have been described using dimethyl sulphate and benzyltriethylammonium chloride as catalyst. The yields were good in most cases, but 1,2:3,4-di-*O*-cyclohexylidene-α-D-galactopyranose was extremely slow to react under these conditions.[4] The selective methylation of benzyl 4-*O*-benzyl-α-L-rhamnopyranoside using phase transfer catalysis (MeI–Bu_4NBr) and the corresponding methyl glycoside gives mainly the 2-methyl ethers.[5] 3-*O*-Methyl-lactose has been prepared from α-lactose by sequential benzoylation (to give the heptabenzoate), methylation, and *O*-debenzoylation.[6] All the mono- and di-*O*-methyl ethers of methyl 2-acetamido-2-deoxy-α (and β)-D-glucopyranoside have been synthesized for n.m.r. studies[7] and the synthesis of the 2,4,6- and 2,3,4-tri-*O*-methyl ethers of methyl α-D-galactopyranoside have been reported.[8]

Application of the Fetizon reagent ($AgCO_3$–celite) to monomethyl aldoses has been proposed as a means of assigning the position of the ether group.[9] The reaction product is first reduced to the alditol, trimethylsilylated, and examined

[1] K. Ohno, H. Nishiyama, and H. Nagase, *Tetrahedron Lett.*, 1979, 4405.
[2] J. Arnarp and J. Lönngron, *Acta Chem. Scand., Ser. B.*, 1978, **32**, 465.
[3] T. Ando and J. Yamawaki, *Chem. Lett.*, 1979, 45.
[4] Yu. A. Zhdanov, Yu. E. Alekseev, S. S. Doroshenko, G. V. Bogdanova, T. P. Sudareva, and V. G. Alekseeva, *Dokl. Akad. Nauk, SSSR.*, 1978, **238**, 1102 (*Chem. Abs.*, 1978, **89**, 43 992*e*); Yu. A. Zhdanov, Yu. E. Alekseev, S. S. Doroshenko, T. P. Sudareva, G. V. Bogdanova, V. G. Alekseeva and V. A. Tyumenev, *Zh. Obshch. Khim.*, 1978, **48**, 2614 (*Chem. Abs.*, 1979, **90**, 152 481*d*).
[5] V. Pozsgay, *Carbohydr. Res.*, 1979, **69**, 284.
[6] M. Martinez-Pardo, P. G. Montes, M. Martin-Lomas, and A. Sols, *FEBS Lett.*, 1979, **98**, 99.
[7] A. S. Shashkov, A. Yu. Evstigneev, and V. A. Derevitskaya, *Bioorg. Khim.*, 1978, **4**, 1495 (*Chem. Abs.*, 1979, **90**, 168 904*e*).
[8] N. Roy, *Indian J. Chem., Sect. B.*, 1978, **16B**, 846 (*Chem. Abs.*, 1979, **90**, 104 292*w*).
[9] S. Morgenlie, *Carbohydr. Res.*, 1979, **73**, 315.

by g.l.c. The number of peaks is diagnostic of the number of oxidative degradations undergone by the aldose, and is hence related to the position of the methyl group. It is not possible to distinguish between 5- and 6-*O*-methyl aldohexoses.

Other Alkyl and Aryl Ethers. – Several long-chain ethers of glucose and galactose have been prepared and evaluated for surface-active properties. For example, 3-*O*-dodecyl-D-glucose and 6-*O*-dodecyl-D-galactose have been made from the corresponding di-*O*-isopropylidene derivatives,[10] and reaction of D-glucose with various alkyloxymethyl chlorides afforded mixtures of mono-, bis-, and tris-alkoxymethyl ethers of glucose with ether chain lengths of 6–36 atoms. The mono-decyloxymethyl and the mono-dodecyloxymethyl ethers had excellent detergent properties.[11] A series of 3-*O*-alkyl ethers of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose have been reported in which the alkyl substituents are n-pentyl, propenyl, propynyl, benzyl, carboxymethyl, and a series of dialkylamino ethyl groups.[12] The use of diazoethane–boron trifluoride has been described for the preparation of 6-*O*-ethyl-D-glucose.[13] The carboxyethyl ether isolated from the extracellular polysaccharide of *Mycobacterium lacticolum* has been identified as 4-*O*-[(*S*)-1′-carboxyethyl]-D-mannose (1).[14]



(1)

Further examples of the regioselective hydrogenolysis of benzylidene acetals have been reported by Lipták *et al.* using $LiAlH_4$–$AlCl_3$.[15–18] Benzyl (or methyl) 4,6-*O*-benzylidene-α(or β)-hexopyranosides which are etherified at O-2 and O-3 with an ethyl or larger group, give the 4-*O*-benzyl ether in high yield. For example, the transformation (2) → (3) (Scheme 1) is accomplished in 92% yield.[16] Application of the reaction to the *endo*- and *exo*-isomers of methyl 2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside resulted in selective cleavage of the 2,3-acetal to give, respectively, the 2- and 3-*O*-benzyl ethers.[17] Similarly the

[10] A. Blazej, M. Kosik, B. Havlinova, J. Zemanovic, and P. Hodul, *Tr.-Mezhdunar Kongr. Poverkhn. – Akt. Veshchestram*, 7th, 1976, **1**, 217 (*Chem. Abs.*, 1979, **91**, 22 809*b*).

[11] D. Biniakiewicz and J. Szymanowski, ref. 10 (*Chem. Abs.*, 1979, **91**, 22 806*g*).

[12] A. Goi, T. Bruzzese, A. F. Notarianni, M. Riva, and A. Ronchini, *Arzneim.-Forsch.*, 1979, **29**, 986 (*Chem. Abs.*, 1979, **91**, 211 692*u*).

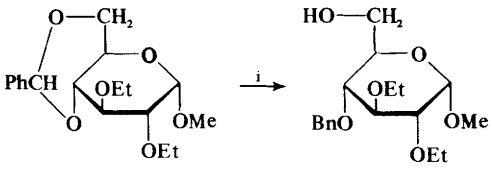
[13] Yu, L. Sebyakin, L. V. Volkova, and R. P. Evstigneeva, *Zh. Obshch. Khim.*, 1979, **49**, 221 (*Chem. Abs.*, 1979, **90**, 187 239*h*).

[14] Kh. A. Arifkhodshaev, A. F. Sviridov, A. S. Shashkov, O. S. Chizhov, and N. K. Kochetkov, *Izv. Akad. Nauk, SSSR, Ser. Khim.*, 1979, **438** (*Chem. Abs.*, 1979, **90**, 204 389*b*).

[15] A. Lipták, P. Fügedi, and P. Nánási, *Carbohydr. Res.*, 1979, **68**, 151.

[16] A. Lipták, F. Pekár, J. Jánossy, I. Jodál, P. Fügedi, J. Harangi, P. Nánási, and J. Szejtli, *Acta Chem. Acad. Sci. Hung.*, 1979, **99**, 201.

[17] A. Lipták, I. Czégény, J. Harangi, and P. Nánási, *Carbohydr. Res.*, 1979, **73**, 327.



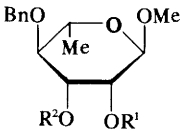
(2) (3)

Reagents: i, $LiAlH_4$–$AlCl_3$

Scheme 1

exo- and *endo*-isomers of benzyl 2,6-di-*O*-benzyl-3,4-*O*-benzylidene-β-D-galacto-pyranose afforded, respectively, the 2,3,6- and 2,4,6-tri-*O*-benzyl ethers in 76 and 82% yields.[18]

Selective benzoylation (and allylation) of methyl (and benzyl) 4-*O*-benzyl-α-L-rhamnopyranoside (4) has been studied by phase-transfer catalysis[5,19] and by activation *via* the tributyl stannate.[19] The former reaction gave the 2,4-dibenzyl ether (5) in high yield, whereas the latter process gave mainly the 3,4-diether (6). An alternative means of obtaining the 2,4-diether (5) involved selective tritylation to give (7) followed by benzylation and removal of the trityl group. These dibenzyl ethers have been employed in the synthesis of the tetrasaccharide repeating unit of the immunodeterminant of *Klebsiella* serotype K36 polysaccharide.[20] Similar results have been recorded for the selective benzylation of other 4,6-blocked α-mannosides.[19,21] The syntheses of 3-*O*-benzyl-D-ribose,[22] 2-*O*-benzyl-D-ribose,[23] and 1,6-anhydro-2,3,2′,4′-tetra-*O*-benzyl-β-lactose[24] have been recorded. The latter was employed in the synthesis of lacto-*N*-tetraose.[24]



(4) $R^1 = R^2 = H$
(5) $R^1 = Bn$, $R^2 = H$
(6) $R^1 = H$, $R^2 = Bn$
(7) $R^1 = H$, $R^2 = Tr$

Selective de-*O*-benzylation at primary positions by acetolysis has been described,[25] and the procedure (*cf.* M. M. Ponpipom, *Carbohydrate Res.*, 1977, **59**, 311) would appear to be a convenient means of obtaining benzylated carbohydrates with the primary hydroxy-groups free. Formerly, it was necessary to employ sequential tritylation, benzylation, and detritylation in order to achieve these blocked compounds. De-*O*-benzylation has also been described using $EtSH$–BF_3, under mild conditions, and although it has not yet been applied to carbohydrates, it would appear to be of potential value.[26]

[18] A. Lipták, L. Jánossy, J. Imre, and P. Nánási, *Acta Chem. Acad. Sci. Hung.*, 1979, **101**, 81.
[19] V. K. Handa, C. F. Piskorz, J. J. Barlow, and K. L. Matta, *Carbohydr. Res.*, 1979, **74**, C5.
[20] V. Pozsgay, P. Nánási, and A. Neszmélyi, *J. Chem. Soc., Chem. Commun.*, 1979, 828.
[21] V. K. Handa, J. J. Barlow, and K. L. Matta, *Carbohydr. Res.*, 1979, **76**, C1.
[22] R. R. Schmidt, A. Gohl, and J. Karg, *Chem. Ber.*, 1979, **112**, 1705.
[23] R. R. Schmidt and A. Gohl, *Chem. Ber.*, 1979, **112**, 1689.
[24] T. Takamura, T. Chiba, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.*, 1979, **27**, 1497.
[25] R. Eby, S. J. Sondheimer, and C. Schuerch, *Carbohydr. Res.*, 1979, **73**, 273.
[26] K. Fuji, K. Ichikawa, M. Node, and E. Fujita, *J. Org. Chem.*, 1979, **44**, 1661.

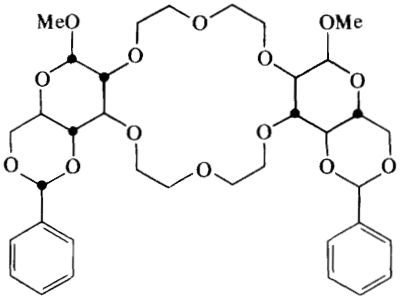
The selective tritylation of pentoses has been studied, and providing that the reaction is conducted at below 25 °C and with only one equivalent of trityl chloride, the 5-*O*-tritylate is the major product.[27] However, even under these conditions lyxose afforded substantial amounts of diethers, and the 5-monoether was isolated in only 32% yield. The monotritylation of D-xylose has been studied in detail at 50 °C in the presence of either pyridine or AgOAc–HMPT, and four of the monotrityl ethers were isolated as tetra-*O*-acetyl derivatives. The ratio of 1-, 3-, 4-, and 5-substitution was 36: ~0: 25: 100 (in pyridine) and 0: 49: 57: 100 (AgOAc–HMPT). The products were also prepared unequivocally by the tritylation of the appropriate D-xylose tetra-acetate.[28] The selective tritylation of methyl α- and β-L-rhamnosides has also been studied; the α-anomer giving the 3-, 4-, and 2-trityl ethers in 57, 3, and 1% yields respectively. The β-anomer afforded the 3- and 4-trityl ethers in 34 and 17% yields respectively.[29] Pyridinium perchlorate has been used for the selective de-*O*-tritylation of otherwise peracetylated methyl gluco- and manno-pyranosides.[30]

Some novel vinyl ethers of the type ROCH=CH.CHO, ROCH=CH.CH=CHCl, and ROCH=CH.CH=CHOBn where R is a sugar residue have been described. Reaction of the dienes with suitable dienophiles has been used by David's group as a method for synthesizing disaccharides.[31] (See Vol. 10, p. 92.)

It has been emphasized that care must be taken in the use of the t-butyldimethylsilyl group as a partial blocking group in nucleosides, because intramolecular migration between the 2′- and 3′-hydroxy-groups occurs in ribofuranoside and is very rapid ($t_{1/2}$ = 0.5 min) in the presence of triethylamine.[32]

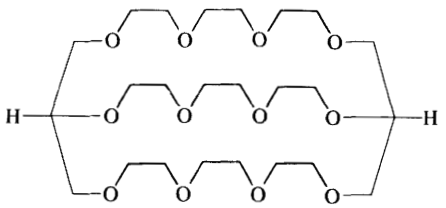
Stoddart and his co-workers have described the synthesis of numerous chiral crown ethers incorporating one or more carbohydrate moieties. 18-Crown-6 polyethers incorporating methyl 4,6-*O*-benzylidene-α(or β)-glucopyranoside (and the galacto-, manno-, and altro-isomers) have been made, for example (8).[33] Further 18-crown-6 polyethers incorporating 1,2:5,6-di-*O*-isopropylidene-D-mannitol and 2,5-anhydro-3,4-di-*O*-methyl-D-mannitol units have also been described.[34] All the polyethers complex with a variety of cations and show discrimination towards chiral ammonium salts, although polyethers incorporating methyl 4,6-*O*-benzylidene-α-D-altropyranoside complex only weakly because of the *trans* fusion. The two faces of some of the crown ethers also show discrimination towards complexation and complexes are formed more readily from one face than the other. Similarly, 20-crown-6 and 22-crown-6 polyethers have been made incorporating 1,3:4,6-di-*O*-methylene-D-mannitol, but the latter

[27] B. L. Kam and N. J. Oppenheimer, *Carbohydr. Res.*, 1979, **69**, 308.
[28] D. Dupeyre, J. P. Utille, and P. J. A. Vottero, *Carbohydr. Res.*, 1979, **72**, 105.
[29] T. Otake, T. Sonobe, and T. Suami, *Bull. Soc. Chem. Jpn.*, 1979, **52**, 3109.
[30] N. K. Kochetkov, B. A. Dmitriev, N. E. Bairamova, and A. V. Nikaloev, *Izv. Akad. Nauk, SSSR, Ser. Khim.*, 1978, 652 (*Chem. Abs.*, 1978, **89**, 24 655*f*).
[31] S. David and J. Eustache, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2230, 2521.
[32] S. S. Jones and C. B. Reese, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2762; W. Köhler and W. Pfleiderer, *Annalen*, 1979, 1855.
[33] D. A. Laidler, J. F. Stoddart, and J. B. Wolstenholme, *Tetrahedron Lett.*, 1979, 465.
[34] R. B. Pettman and J. F. Stoddart, *Tetrahedron Lett.*, 1979, 457, 461; J. A. Haslegrave, J. F. Stoddart, and D. J. Thompson, *Tetrahedron Lett.*, 1979, 2279.



(8)

show weak complexing properties.[35] The topic has been reviewed.[36] An interesting series of macrobicyclic polyethers have been made from glycerol derivatives, for example (9). Their fascination lies in the fact that they show isomerism about C-2 of the original glycerol molecules. The hydrogen at this position may exist either *exo* or *endo* with respect to the bicyclic system.[37]



(9)

2 Intramolecular Ethers (Anhydro-sugars)

Oxiranes. – A synthesis is reported of a 1,2-epoxide (11) by sequential treatment of 3,4,6-tri-*O*-benzyl-D-glucose (10) with hydrogen chloride in chloroform and then with ammonia.[38] The deamination of 1,6-anhydro derivatives of amino-deoxy glucoses has been examined in detail, and epoxides are formed in high yield when the amino-group is axial, for example, the 3-amine (12) affords the 1,6:2,3-dianhydride (13) and the 1,6:3,4-dianhydride (14) in the ratio of 31:69 (Scheme 2). On the other hand, the 4-amine yields only 1,6:3,4-dianhydro-β-D-galactopyranose. In the case of the 2-amine (15) a competing rearrangement involving attack by O-6 gives rise to 2,6-anhydro-D-mannose (17) in addition to the 1,6,2,3-dianhydride (16) (Scheme 2).[39]

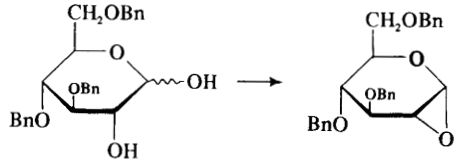
[35] D. A. Laidler and J. F. Stoddart, *Tetrahedron Lett.*, 1979, 453; D. G. Andrews, P. R. Ashton, D. A. Laidler, J. F. Stoddart, and J. B. Wolstenholme, *Tetrahedron Lett.*, 1979, 2629.

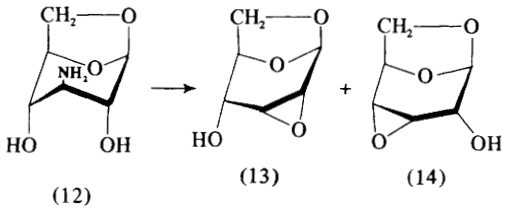
[36] A. C. Coxon, W. D. Curtis, D. A. Laidler, and J. F. Stoddart, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, **6**, 167.

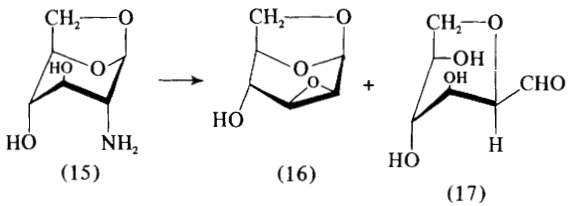
[37] A. H. Haines and P. Karntiang, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2577.

[38] S. J. Sondheimer, H. Yamaguchi, and C. Schuerch, *Carbohydr. Res.*, 1979, **74**, 327.

[39] T. Elbert, M. Černy, and J. Defaye, *Carbohydr. Res.*, 1979, **76**, 109.

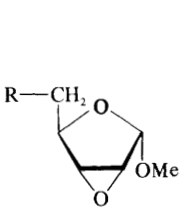




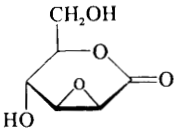


Scheme 2

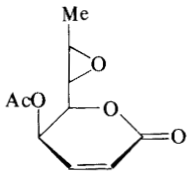
Methyl 2,3-anhydro-5-*O*-benzoyl-β(and α)-D-ribofuranoside (18) have been made from treatment of the corresponding 3-*O*-tosyl-D-xylofuranosides with tetrabutylammonium fluoride, although the yield of the α-anomer was only 22%.[40] The corresponding 5-deoxy derivative (19) was made in a similar manner.[41] The epoxides (20)[42] and (21)[43] have been reported, and (20) is of



(18) R = OBz
(19) R = H



(20)



(21)

[40] A. A. Akhrem, E. I. Kvasyuk, I. A. Mikhailopulo, G. Kowollik, P. Langen, and A. Otto, *Zh. Obshch. Khim.*, 1979, **49**, 1175.
[41] J. A. Montgomery, H. J. Thomas, and S. D. Clayton, *Nucleic Acid Chem.*, 1978, **1**, 195 (*Chem. Abs.*, 1979, **90**, 23 594*u*).
[42] J. Lehmann and B. Schwesinger, *Chem. Ber.*, 1978, **111**, 3961.
[43] S. Lesage and A. S. Perlin, *Can. J. Chem.*, 1978, **56**, 3117.

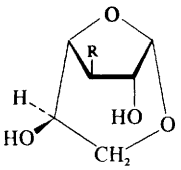
interest inasmuch as it acts as an irreversible inhibitor of the β-galactosidase from *E. coli*. 1,2-Anhydro-4-*O*-benzyl-L-threitol has been prepared from L-tartaric acid as a useful chiral intermediate in synthesis,[44] and 1,2-anhydro-3,4-*O*-isopropylidene-D-erythritol and -D-threitol have been reported from the reaction of 2,3-*O*-isopropylidene-D-glyceraldehyde with either diazomethane or sulphur ylides.[45] Condensation of several pentopyranosyl halides with benzyl 2,3-anhydro-D-ribopyranosides has produced a series of anhydro-disaccharide derivatives.[46]

Other Anhydrides. – The 1,6-anhydro derivatives of aldohexoses have been reviewed,[47] and the application of empirical force-field calculations to all eight of these anhydrides has closely reproduced the conformational variations observed in crystal structures, but the conformational differences in the five-membered ring are not so well predicted.[48] The yield of 1,6-anhydro-β-D-mannopyranose from the pyrolysis of ivory nut meal is improved by 50% by predigestion with acid.[49]

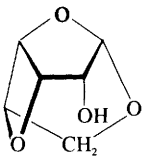
Selective tosylation of 1,6-anhydro-D-galactofuranose (22) gives mainly the 3-tosylate (23) which undergoes ring closure on treatment of KOAc in DMF to give 1,6 : 3,5-dianhydro-α-D-gulofuranose (25), the structure of which was established by *X*-ray crystallography.[50] The tritosylate of (22) is transformed into the corresponding 3-deoxy-hexos-2-ulose derivative with potassium hydroxide, and reduction with borohydride gave the 3-deoxy derivative (24).[51] Further studies have been conducted on 2,6-anhydrohexopyranosides; methyl 2,6-anhydro-β-D-gulopyranoside (26) has been converted into the *ido*- and *manno*-isomers by sequential reaction with α-acetoxyisobutyryl chloride, sodium iodide (to give the 3-iodo-idoside), and base.[52] The anhydride (26) was also converted into the 3,4-unsaturated derivative (27) which was hydroxylated with OsO_4 to give a mixture of (26) and the *altro* isomer. Epoxidation of the 3-ene (27) yielded a mixture of the two 2,6 : 3,4-dianhydrides, a new class of dianhydro-sugars.[53]

In a substantial paper, Sinclair[54] has examined the action of base on all six methyl di-*O*-mesyl-α-D-glucopyranosides. In all cases except one, a 2,3- or 3,4-anhydro mesylate is formed initially, but on further reaction (usually under more rigorous conditions) these are either isomerized to 3,6-anhydrides or converted into dianhydrides depending on the position of the remaining mesyl substituent. In the case of the dimesylate (28), a mixture of the 2,3- and

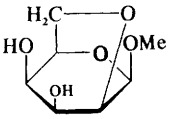
[44] E. Hungerbuhler, D. Seebach, and D. Wasmuth, *Angew Chem. Int. Ed. Engl.*, 1979, **18**, 958.
[45] S. Hagen, T. Anthonsen, and L. Kilaas, *Tetrahedron*, 1979, **35**, 2583.
[46] A. De Bruyn, R. Van Rijsbergen, M. J. O. Anteunis, M. Claeyssens, and F. Deleyn, *Bull. Soc. Chim. Belges.*, 1978, **87**, 783 (*Chem. Abs.*, 1979, **90**, 168917*m*).
[47] M. Černý and J. Staněk, *Adv. Carbohydr. Chem. Biochem.*, 1977, **34**, 24.
[48] G. A. Jeffrey and Y. J. Park, *Carbohydr. Res.*, 1979, **74**, 1.
[49] R. H. Furneaux and F. Shafizadeh, *Carbohydr. Res.*, 1979, **74**, 354.
[50] P. Köll, J. Schulz, and U. Behrens, *Chem. Ber.*, 1979, **112**, 2068.
[51] P. Köll, J. Schulz, and K. Heyns, *Chem. Ber.*, 1979, **112**, 2337.
[52] P. Köll, F. S. Tayman, and K. Heyns, *Chem. Ber.*, 1979, **112**, 2305.
[53] P. Köll and F. S. Tayman, *Chem. Ber.*, 1979, **112**, 2296.
[54] H. B. Sinclair, *J. Org. Chem.*, 1979, **44**, 3361.



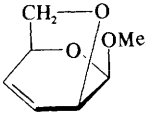
(22) R = OH
(23) R = OTs
(24) R = H



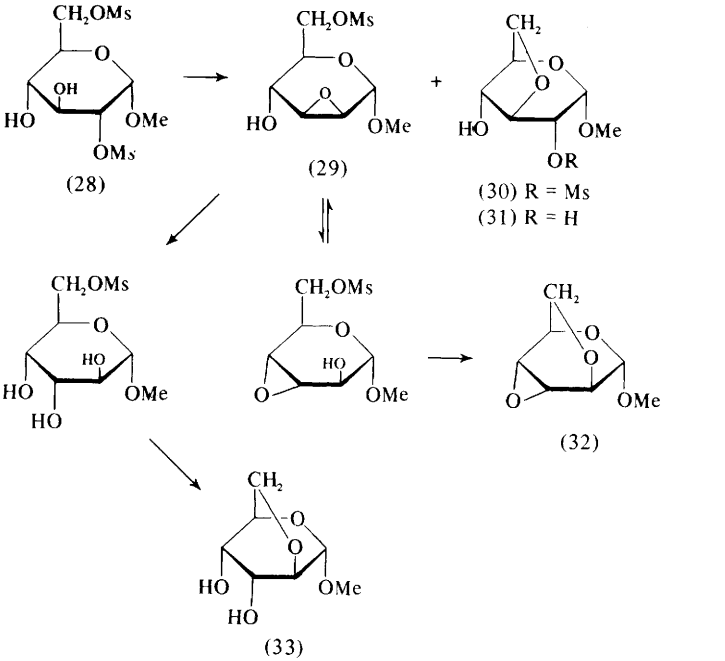
(25)



(26)



(27)



(28) (29) (30) R = Ms (31) R = H (32) (33)

Scheme 3

3,6-anhydrides [(29) and (30) respectively] was formed initially. The 2,3-anhydro-6-mesylate was then subsequently converted into a mixture of the 2,6 : 3,4-dianhydro-altroside (32) and 2,6-anhydro-altroside (33), whereas the 3,6-anhydro-2-mesylate (30) underwent hydrolysis of the sulphonyl ester group to give methyl 3,6-anhydro-α-D-glucopyranoside (31) (Scheme 3). It is noteworthy that of the two methyl 3,4-anhydro-2-*O*-mesyl-α-D-hexopyranosides isolated, only the *allo*-isomer was transformed into the 2,6 : 3,4-dianhydride [*e.g.*, (32)]. In the case of the *galacto* isomer, inhibition by the *β-trans*-axial group at C-3 is believed to be responsible for its inability to produce a dianhydride.

5

Acetals and Ketals

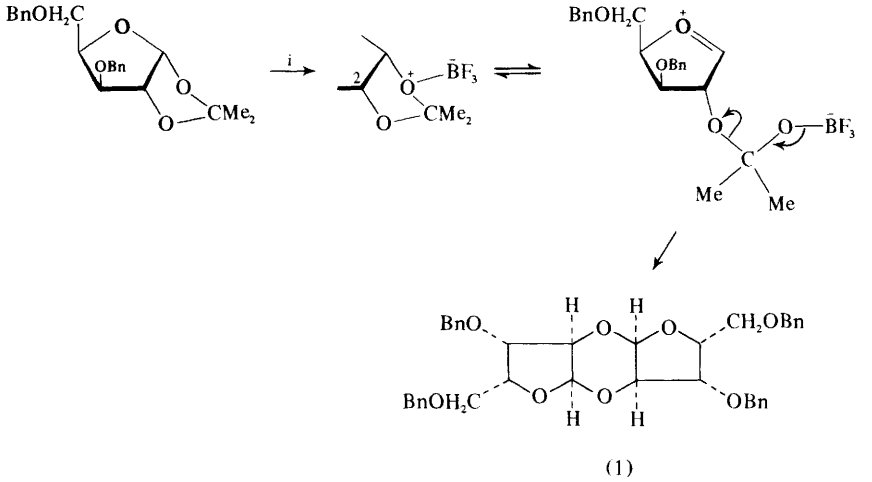
A review of the preparation, structures, and stereochemistry of cyclic acetals of the aldoses and aldosides has appeared.[1] Pyridinium toluene-*p*-sulphonate has been reported to be a mild catalyst for the formation and cleavage of dioxolane-type acetals, although no carbohydrate examples were quoted.[2] Acetals and ketals of carbohydrates have been used as co-agents to introduce chirality into the products of sodium borohydride reduction of acetophenone, propiophenone, *etc.*[3]

1 Isopropylidene Ketals

A synthesis of 1,2-*O*-isopropylidene-α-D-ribofuranose from 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose has been described.[4] The kinetic products of isopropylidenation of D-galactose, D-allose, and D-talose have been shown to be the 4,6-linked isomers in 67, 81, and 93% yield respectively.[5] A shorter synthesis of 1,2-*O*-isopropylidene-α-D-galactofuranose by hydrolysis of the 1,2 : 5,6-di-*O*-isopropylidene ketal with 40% acetic acid is reported. The product was converted into 1,2-*O*-isopropylidene-β-L-arabinofuranoside by sequential periodate oxidation and borohydride reduction.[6] Treatment of 1,2-*O*-isopropylidene-glycofuranoses with boron trifluoride etherate in benzene has been shown to lead to dimeric dianhydrides, *e.g.*, the 3,5-di-*O*-benzoyl-xylose derivative (1) (Scheme 1).[7]

A study of the oleum-catalysed acetonation of L-sorbose has shown that 71–83% of ketal mixtures containing 92–94% of 2,3 : 4,6-di-*O*-isopropylidene-L-sorbose can be obtained. Lowering the temperature from 0 to −10 °C increased the yield by 3%, but increasing the time of reaction above 20 min had little effect on purity or yield.[8] A continuous-flow method for production of sorbose acetonide has been optimized and yields of 11.6% obtained.[9] Room temperature reaction of lactose with 2,2-dimethoxypropane in DMF gives mainly 4′,6′-*O*-isopropylidene lactose,[10] whilst at reflux the same reaction gave 45% of the

[1] A. N. De Belder, *Adv. Carbohydr. Chem. Biochem.*, 1977, **34**, 179.
[2] R. Sterzycki, *Synthesis*, 1979, 724.
[3] A. Hirao, H. Mochizuki, S. Nakahama, and N. Yamazaki, *J. Org. Chem.*, 1979, **44**, 1720.
[4] H. Tsutsumi, Y. Kawai, and Y. Ishido, *Carbohydr. Res.*, 1979, **73**, 293.
[5] J. Gelas and D. Horton, *Carbohydr. Res.*, 1979, **71**, 103.
[6] M. M. A. Abdel Rahman, *Egypt. J. Chem.*, 1976 (Pub. 1978), **19**, 887 (*Chem. Abs.*, 1979, **91**, 211 603*v*).
[7] P. H. A. Zollo, J.-R. Pougny, and P. Sinay, *Tetrahedron Lett.*, 1979, 2447.
[8] S. H. Levi, G. Spirov, and E. Pencheva, *Khim.-Farm. Zh.*, 1978, **12**, 78 (*Chem. Abs.*, 1979, **90**, 87 759*j*).
[9] V. V. Kukhnov, Kh. I. Gurnikov, V. G. Nasonov, V. R. Pogosyants, T. K. Blokhina, I. N. Litvinov, V. I. Galkin, and L. F. Beshevli, *Khim.-Farm. Zh.*, 1979, **13**, 65 (*Chem. Abs.*, 1979, **91**, 20 918*n*).
[10] H. H. Baer and S. A. Abbas, *Carbohydr. Res.*, 1979, **77**, 117.



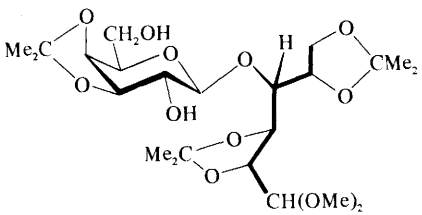
(1)

Reagents: i, BF_3–Et_2O–C_6H_6

Scheme 1

acyclic triketal (2), which was characterized by ^1H n.m.r., mass spectrometry and *X*-ray crystallography.[11] Selective deketalization of 1′,2:4,6-di-*O*-isopropylidenesucrose tetra-acetate using aqueous acetic acid, methanolic hydrogen chloride, or cation exchange resin leads to the 1′,2-ketal in each case.[12]

A new diketal, 2,3:5,6-di-*O*-isopropylidene-D-glucitol has been isolated from the reaction of D-glucitol with acetone in the presence of zinc chloride.[13]

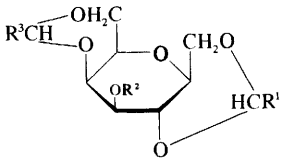


(2)

2 Benzylidene Acetals

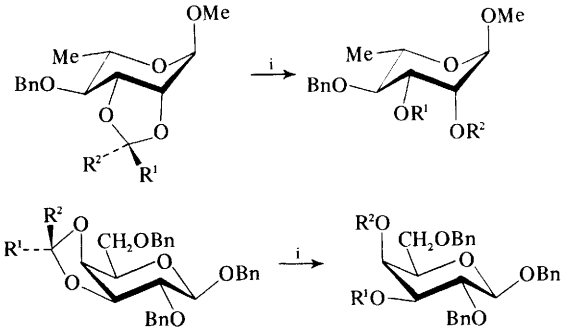
The crystal structures of the thermodynamically less stable (*R*)-diastereoisomers of methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-gluco- and -β-D-galacto-pyranosides have been reported. In each the 2-phenyl-1,3-dioxane ring adopts the expected (Eliel, *Pure Appl. Chem.*, 1971, **25**, 509) axial phenyl chair.[14] The

[11] L. Hough, A. C. Richardson, and L. A. W. Thelwall, *Carbohydr. Res.*, 1979, **75**, C11.
[12] R. Khan and H. Lindseth, *Carbohydr. Res.*, 1979, **71**, 327.
[13] J. Kuszmann and P. Sohár, *Carbohydr. Res.*, 1979, **74**, 187.
[14] J. C. Barnes, J. S. Brimacombe, B. H. Nichols, and T. J. R. Weakley, *Carbohydr. Res.*, 1979, **69**, 47.



(3) $R^1 = R^3 = Ph$; $R^2 = H$
(4) $R^1 = R^3 = Ph$; $R^2 = Ac$
(5) $R^1 = C_6H_4OMe\text{-}p$; $R^2 = H$; $R^3 = Ph$

tritium-labelled [1-^{3}H] 2,6-anhydro-heptitol di-acetals (3)–(5) have been used to show that the *trans*-fused benzylidene group is more reactive than the *cis*-acetal towards acid-hydrolysis.[15] Three papers on the regioselective reduction of benzylidene acetals by $LiAlH_4$–$AlCl_3$ to give β-hydroxybenzyl ethers have been published. Compounds studied were the two diastereomeric 2,3-acetals of L-rhamnose (Scheme 2),[16] 2,3-protected 4,6-*O*-benzylidene-hexopyranosides,[17] and 2,6-protected-3,4-benzylidene-galactosides (Scheme 2).[18] The 4,6-*O*-benzylidene-hexosides gave the 4-*O*-benzyl-6-hydroxy compounds specifically if the O-3 protecting group was ethyl or larger.[17]



$R^1 = Ph$, $R^2 = H$; $R^1 = H$, $R^2 = Ph$

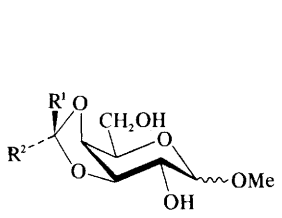
Reagents: i, $LiAlH_4$–$AlCl_3$

Scheme 2

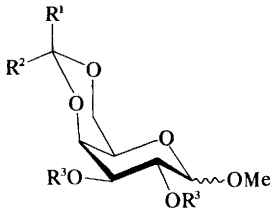
3 Other Acetals and Ketals

Conventional methods have been used to prepare the three series of ketals (6)–(8), and their ^{1}H and ^{13}C n.m.r. spectra were analysed. Chemical shift

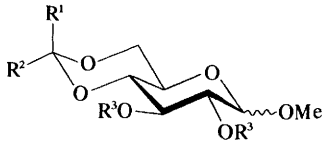
[15] J. Lehmann and B. Schwesinger, *Carbohydr. Res.*, 1979, **72**, 267.
[16] A. Lipták, A. Neszmélyi, and H. Wagner, *Tetrahedron Lett.*, 1979, 741.
[17] A. Lipták, F. Pekár, J. Jánossy, I. Jodál, P. Fügedi, J. Harangi, P. Nánási, and J. Szejtli, *Acta Chem. Acad. Sci. Hung.*, 1979, **99**, 201.
[18] A. Lipták, L. Jánossy, J. Imre, and P. Nánási, *Acta Chem. Acad. Sci. Hung.*, 1979, **101**, 81.



(6) R^1, R^2 = Me, $CH_2OH(OAc)$



(7)



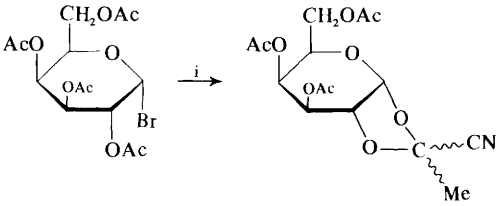
(8)

R^1, R^2 = Me, $CH_2OH(OAc)$; Me, $CO_2H(CO_2Me)$
R^3 = H
(9) = (8), R^1 = CO_2Me, R^2 = Me, R^3 = $COCF_3$

differences of diastereoisomeric pairs of ketal methyl groups allows the stereochemistry of pyruvic acid ketals in naturally occurring polysaccharides to be determined.[19] Compound (9) has been synthesized by another group of workers.[20]

A review of the chemistry of formamide acetals with *inter alia* some carbohydrate and nucleoside examples has appeared.[21]

Treatment of acylated 1,2-*cis*- and 1,2-*trans*-glycosyl bromides with KCN–MeCN at r.t. for 12–24 h gave 1,2-*O*-(1-cyanoalkylidene) sugars. The reaction was applied to D-glucopyranosyl, L-rhamnosyl, D-mannopyranosyl, L-arabinofuranosyl, and maltosyl bromides. With the 1,2-*cis*-bromide tetrabutylammonium bromide was included as catalyst, but no catalysis was necessary for the 1,2-*trans* analogues. An example of the reaction is given in Scheme 3.[22]



Reagents: i, $KCN–NBu_4Br$

Scheme 3

[19] P. J. Garegg, B. Lindberg, and I. Kvarnström, *Carbohydr. Res.*, 1979, **77**, 71.
[20] V. Dininno, E. L. McCandless, and R. A. Bell, *Carbohydr. Res.*, 1979, **71**, C1.
[21] R. F. Abdulla and R. S. Brinkmeyer, *Tetrahedron*, 1979, 1675.
[22] V. I. Betaneli, M. V. Ovchinnikov, L. V. Backinowsky, and N. K. Kochetkov, *Carbohydr. Res.*, 1979, **68**, C11.

6

Esters

1 Carboxylic Esters

The formylation reported by Hough and Lewis (see Vol. 9, p. 41) as a side-reaction during glycosidation in DMF has been further investigated. When benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside was treated with hepta-*O*-acetyl-α-kojibiosyl bromide in DMF in the presence of bromide ion, only the 6-*O*-formyl derivative of the former was obtained in 70% yield with no glycoside, whereas with tetra-*O*-acetyl-α-D-glucopyranosyl bromide, only the corresponding ortho-ester was formed; the authors suggest that initial epimerization to the β-bromide occurs, which, with a non-participating group at C-2, is sufficiently reactive to induce Vilsmeier formylation rather than glycosidation of the glycoside.[1]

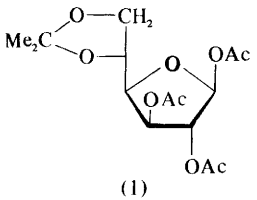
Selective acetylations of D-mannose and D-xylose have been carried out. The former with 5.7 mol Ac_2O–NaOAc gave 60% penta-acetate and 29% 1,2,3,6-tetra-*O*-acetyl-α,β-D-mannopyranose. The latter with 5.3 mol Ac_2O–NaOAc yielded 27% tetra-acetate and 7.2% 1,2,4-tri-*O*-acetyl-β-D-xylopyranose.[2] Benzyl β-maltoside with 20 mol Ac_2O at r.t. during 10 days gave 10% hepta-acetate, and 71% 2,6,2′,3′,4′,6′-hexa-acetate which was characterized by methylation followed by deacetylation and ^{13}C n.m.r.[3]

Photolysis of 3-esters of methyl 4,6-*O*-cyclohexylidene-2-deoxy-2-methoxycarbonylamido-α-D-glucopyranoside has been studied with a view to effecting efficient deoxygenation at C-3. The method was successful with the 3-*O*-acetyl derivative in aqueous HMPT on irradiation at 254 nm.[4]

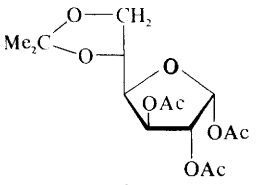
Cold acetic anhydride–pyridine has been shown to give the crystalline α-triacetate with 4,6-*O*-ethylidene-α-D-glucopyranose whereas previous work has given non-crystalline mixtures of α- and β-anomers or only β-anomer with hot acetic anhydride–sodium acetate. However, cold benzoyl chloride–pyridine gave α,β-anomeric mixtures.[5]

Using the Guthrie–Smith acetolysis of the methyl glycosides, all eight α- and β-D-aldopentofuranose tetra-acetates have been synthesized; all were separated, except the α,β-D-arabinoses, and characterized by ^1H and ^{13}C n.m.r.[6] When the same reaction was applied to methyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranoside, the anomeric 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-arabinoses were separable by chromatography on silica gel.[7]

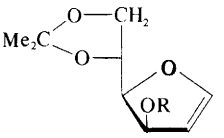
[1] V. Pozsgay and P. Nánási, *Carbohydr. Res.*, 1979, **68**, 157.
[2] E. E. Lee, A. Bruzzi, E. O'Brien, and P. S. O'Colla, *Carbohydr. Res.*, 1979, **71**, 331.
[3] E. E. Lee and J. O. Wood, *Carbohydr. Res.*, 1979, **75**, 317.
[4] T. Kishi, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 3015.
[5] I. Ziderman, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, **6**, 411.
[6] B. L. Kam, J.-L. Barascut, and J. L. Imbach, *Carbohydr. Res.*, 1979, **69**, 135.
[7] I. A. Korbukh, L. M. Abramova, and M. N. Preobrazhenskaya, *Nucleic Acid Chem.*, 1978, **1**, 151 (*Chem. Abs.*, 1979, **90**, 23 435*t*).



(1)



(2)



(3) R = H
(4) R = 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranosyl

The glucofuranose tri-acetates (1) and (2) have been prepared from the glycal (3) using osmium tetroxide–pyridine followed by sodium metabisulphite and acetylation. A similar sequence on the disaccharide glycal (4) gave a 7:1 ratio of the corresponding mannose and glucose disaccharides both obtained as anomeric mixtures.[8] Sequential treatment of β-D-mannosyl-(1→4)-β-D-*N*-acetyl-glucosaminyl-(1→4)-β-D-*N*-acetyl-glucosamine pyrophosphoryl dolichol with acid and β-mannosidase yielded *N*,*N*-diacetylchitobiose.[9]

Phase-transfer selective benzoylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside using tetrabutylammonium hydrogen sulphate, benzoyl chloride, and 5% aqueous sodium hydroxide during 15 min gave 50% 2-*O*-benzoyl derivative. The yields were increased to 85% by using benzene, benzoyl chloride, tetrabutylammonium iodide, and potassium carbonate for 48 h.[10]

Selective benzoylation of methyl 4,6-*O*-benzylidene-α-D-galactopyranoside using phase-transfer catalysis could be controlled by choice of solvent to yield primarily the 3-*O*-benzoyl (62%, in HMPT) or the 2-*O*-benzoyl derivative (78%, in benzene).[11]

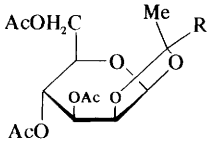
Partial benzoylation of methyl α-sophoroside with 7 mol benzoyl chloride in pyridine at −40 °C gave 11% heptabenzoate, 61% 4,6,2′,3′,4′,6′-hexabenzoate, and 18% 4,6,2′,3′,6′-pentabenzoate. A similar reaction with methyl α-laminaribioside gave 20% heptabenzoate, 45% 2,6,2′,3′,4′,6′-hexabenzoate, 16% 2,4,6,2′,3′,6′-hexabenzoate, and 9% 2,6,2′,3′,6′-pentabenzoate.[12]

Regioselective debenzoylation of nucleoside 2′,3′,5′-tri-*O*-benzoates using hydrazine hydrate has been reported. With 1 mol reagent in acetic acid–pyridine the 3′5′-di-*O*-benzoyl derivative was produced when the bases were adenine, guanine, or indole, whereas with excess hydrazine hydrate in 1:1 chloroform–methanol, 98% of the 5′-*O*-benzoyl derivative was produced (base = adenine or uracil). 3′,5′-Di-*O*-benzoyl-2-deoxy-nucleosides gave a 90% yield of a 1:1 mixture of 3′- and 5′-*O*-benzoyl nucleosides with 1 mol hydrazine hydrate in pyridine.[13]

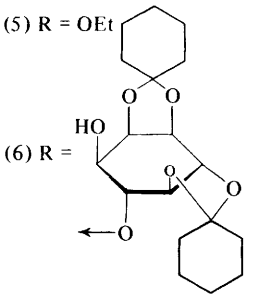
Treatment of the mannose orthoacetate (5) with benzoyl chloride in pyridine gave 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl chloride and ethylbenzoate, whereas the related orthoester of muco-inositol (6) gave 66% of the correspond-

[8] K. Bischofberger, S. J. Eitelman, and A. Jordaan, *Carbohydr. Res.*, 1979, **74**, 145.
[9] C. J. Waechter and J. B. Harford, *Arch. Biochem. Biophys.*, 1979, **192**, 380.
[10] L. L. Box, V. G. S. Box, and E. V. A. Roberts, *Carbohydr. Res.*, 1979, **69**, C1.
[11] W. Szeja, *Synthesis*, 1979, 821.
[12] K. Takeo, *Carbohydr. Res.*, 1979, **69**, 277.
[13] Y. Ishido, N. Nakazaki, and N. Sakairi, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2088.

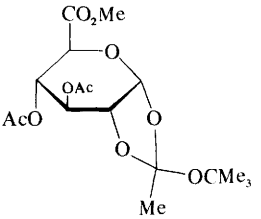
ing di-*O*-benzoyl di-*O*-cyclohexylidene inositol derivative.[14] When the methyl uronate orthoester (7) was treated with the appropriate carboxylic acid in chlorobenzene at reflux, the β-2,3,4-tri-*O*-acetyl glucosyl propanoate, benzoate, salicylate, cinnamate, *p*-nitrophenylacetate, hippurate, or nicotinate were obtained.[15]



(5) R = OEt



(6) R =



(7)

Ortho-α-dibromotoluate has been shown to be useful as a blocking group which is as stable to alkali as the acetyl group and is easy to remove by the sequence shown in Scheme 1.[16]

Methyl 2-(2-methylacrylyl)-β-L-arabinopyranoside has been prepared conventionally.[17]

All the isomeric 1α-,1β-,2-,3-,4-, and 6-mono-*O*-acyl-D-glucopyranose derivatives of the even-numbered carbon acids from decanoic acid to stearic acid have been synthesized and characterized by g.l.c., i.r., and ^{1}H n.m.r.[18] The synthesis of α,β-glycosyl esters of lysergic acid and dihydrolysergic acid by reaction of 2,3,5-tri-*O*-acetyl-α-D-xylopyranosyl and -β-L-arabinopyranosyl bromides with the acid has been reported. Similarly prepared were the corresponding glucosyl and galactosyl dihydrolysergates.[19]

The reaction of 2,3,4,6- and 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose and D-glucose itself with substituted phenylacetic acid and 3,4,5-trimethoxybenzoic acid in presence of DCC has been studied by n.m.r. spectroscopy.[20]

[14] A. P. Kaplun, V. N. Krylova, G. Yu. Soloveichik, and V. I. Shvets, *Zh. Org. Khim.*, 1979, **15**, 307 (*Chem. Abs.*, 1979, **91**, 57 346*a*).

[15] K. Honma and A. Hamada, *Carbohydr. Res.*, 1979, **73**, 297.

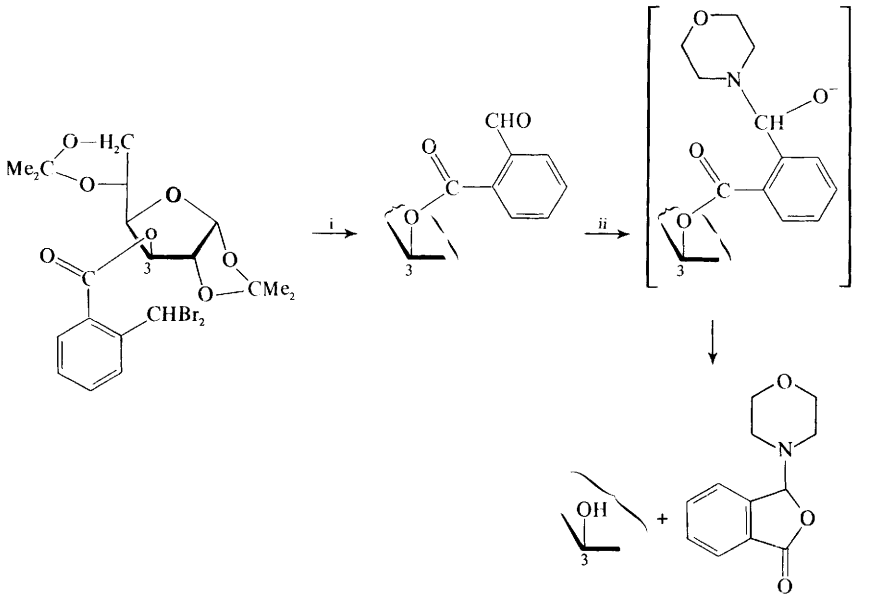
[16] J. B. Chattopadhyaya, C. B. Reese, and A. H. Todd, *J. Chem. Soc., Chem. Commun.*, 1979, 987.

[17] V. L. Lapenko, B. I. Mikhant'ev, and V. N. Prokina, *Izv. Vyssh. Uchebn. Zaved., Khim. khim. Tekhnol.*, 1978, **21**, 488 (*Chem. Abs.*, 1978, **89**, 110 257*j*).

[18] K. Yoshimoto, K. Tahara, S. Suzuki, K. Sasaki, Y. Nishikawa, and Y. Tsuda, *Chem. Pharm. Bull.*, 1979, **27**, 2661.

[19] K. Seifert, S. Johne, and H. Meyer, *J. Prakt. Chem.*, 1979, **321**, 171 (*Chem. Abs.*, 1979, **91**, 57 317*s*).

[20] D. Dziuviene and J. Degutis, *Zh. Obshch. Khim.*, 1978, **48**, 2607 (*Chem. Abs.*, 1979, **90**, 204 377*w*).



Reagents: i, $AgClO_4$–2,4,6-collidine–H_2O–Me_2CO; ii, morpholine

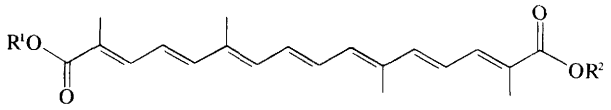
Scheme 1

Transesterification with methyl carboxylates and sucrose in DMF in the presence of potassium carbonate at 90–95 °C for 12–15 h led to 6-*O*-substituted sucrose derivatives of C_3–C_{18} carboxylic acids. The surface tension properties of the products were measured and the highest values were obtained with C_7–C_{12} derivatives.[21] The physical properties of nine sucrose octa-esters prepared from homogeneous fatty acids have been reported. The melt forms a stable phase with a diffraction pattern identical to that of the triglyceride α-phase. Evaporation of an acetone solution gave a material with a diffraction pattern identical with that of the triglyceride β-phase. The i.r. and diffraction pattern are consistent with vertical alignment of the fatty-acid chain in the α-phase giving a hexagonal subcell of methylene groups, and fatty acids extending from both sides of the disaccharide rings. Data on viscosity and miscibility and on the solid derivatives are also given.[22] Heating starch with polyols in the presence of sulphuric acid at 125 °C for a short time gave polyol monoglucosides which could be transesterified at 160–200 °C in the presence of 5–10% soaps and hydrophobic esters such as mono- and di-glycerides or esters of diethyleneglycol monomethyl ether. The free polyol or alcohol was removed by low pressure distillation and the glucosyl esters, which were obtained in good yield, were found to be highly surface active.[23]

[21] M. I. Goryaev, A. F. Artamonov, L. P. Petelina, and B. Bektursynov, *Vestn. Akad. Nauk Kaz. SSR*, 1978, 31 (*Chem. Abs.*, 1979, **90**, 204 387*z*).

[22] R. J. Jandacek and M. R. Webb, *Chem. Phys. Lipids*, 1978, **22**, 163 (*Chem. Abs.*, 1979, **90**, 72 398*s*).

[23] R. O. Fuege, J. L. White, and M. Brown, *J. Amer. Oil Chem. Soc.*, 1978, **55**, 699 (*Chem. Abs.*, 1979, **90**, 39 191*w*).



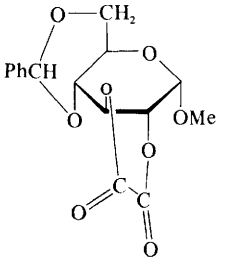
(8) R^1, R^2 = β-D-Glu-*p* or β-gentiobiosyl

Carotenoid glucosyl and gentiobiosyl esters (8) have been prepared by standard methods.[24] 6-D-Glucopyranosyl linolenate, palmitate, oleate, and linoleate have been isolated from *Brassica napus* pollen; their activity as growth promoters was studied.[25] The structure of corynocarpin, isolated from the seeds and plants of *Corynocarpus laevigatus*, has been shown by 220 ^{1}H n.m.r. to be 1,4,6-tri-*O*-(3-nitropropanoyl)-β-D-glucopyranose.[26] The two main components of the 6,6′-diesters of α,α-trehalose isolated from *Corynebacterium diphtheriae* have been characterized by m.s. and chemical degradation and shown to be 6,6′-bis(3-oxo-2-tetradecyl-octadecanoyl) and 6-(3-oxo-2-tetradecyl-octadecanoyl)-6′-(3-hydroxy-2-tetradecyl-octadecanoyl) esters.[27] Two series of long chain esters of α,α-trehalose have been isolated from the paraffin-oxidizing bacterium *Mycobacterium paraffinicum*.[28,29] N.m.r., m.s., and chemical degradation showed one to be 6,6′-di-*O*-acyl-α,α-trehalose where the acyl groups were derived from C_{12}–C_{16} straight-chain fatty acids,[28] while the other was the cord-factor analogue, 6-*O*-mycolyl(C_{32}–C_{44})-α,α-trehalose.[29] Hakamori permethylation of cord-factor and 4-*O*-palmitoyl glucose has been shown to be successful without causing ester migration.[30]

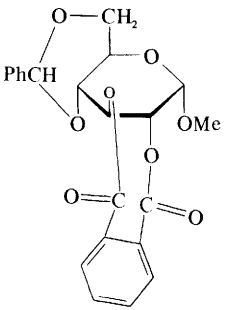
The syntheses of the cyclic esters (9) and (10) derived from oxalic and phthalic acids have been achieved using the acid dichlorides and methyl 4,6-*O*-benzylidene-α-D-glucopyranoside.[31] The compounds 2-, 3-, and 6-amino-6-deoxy-D-glucose have been converted into their *N*-(3-hydroxytetradecanoyl) derivatives by treatment with *N*-hydroxysuccinimide and 3-hydroxytetradecanoic acid. The m.s. of the TMS derivatives provided data useful in locating the position of the *N*-acyl group.[32]

Condensation of 3,4,6-tri-*O*-acetyl-α-D-glucosaminyl chloride with *N*-(benzyloxycarbonyl) derivatives of L-Ser-Gly ethyl ester, L-Ser-Ala methyl ester, L-Ser-Phe methyl ester, and L-Ser-Asp dibenzyl ether gave the β-glycosyl dipeptides

[24] H. Pfander, *Pure Appl. Chem.*, 1979, **51**, 565.
[25] M. D. Grove, G. F. Spencer, P. E. Pfeffer, N. Mandava, J. D. Warthen, and J. F. Worley, *Phytochem.*, 1978, **17**, 1187 (*Chem. Abs.*, 1979, **90**, 39 133*d*).
[26] B. G. Moyer, P. E. Pfeffer, K. M. Valentine, and D. L. Gustine, *Phytochem.*, 1979, **18**, 111 (*Chem. Abs.*, 1979, **91**, 39 739*b*).
[27] G. Puzo, G. Tissié, H. Avrelle, C. Lacave, and J.-C. Promé, *Eur. J. Biochem.*, 1979, **98**, 99.
[28] S. G. Batrakov, O. A. Mukhitdinova, T. V. Koronelli, and L. D. Bergelson, *Bioorg. Khim.*, 1979, **5**, 83.
[29] S. G. Batrakov, O. A. Mukhitdinova, L. D. Bergelson, and T. V. Koronelli, *Bioorg. Khim.*, 1979, **5**, 1495.
[30] M. B. Goren and R. Toubiana, *Biochim. Biophys. Acta*, 1979, **574**, 64, 70.
[31] S. Roseman and K. P. Link, *Carbohydr. Res.*, 1979, **69**, 301.
[32] M. Demary, G. Puzo, and J. Asselineau, *Nouv. J. Chim.*, 1978, **2**, 373 (*Chem. Abs.*, 1978, **89**, 215 684*z*).



(9)



(10)

which were deblocked with hydrogen bromide–acetic acid followed by triethylamine. A further amino-acid residue was added (glycine, alanine, or proline) using standard methods. Many other similar derivatives were made.[33] It has been demonstrated that the amino-acid residues of α-D-glucopyranosyl esters of *N*-acyl-amino-acids undergo migration to O-2 on treatment with diazomethane and acetic anhydride–pyridine, whereas those with the β-configuration do not. The diazomethane appeared to act as a base in the migration.[34]

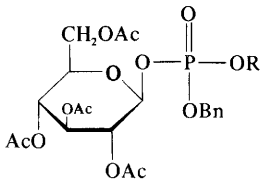
2 Phosphates and Related Esters

Cold plasma reaction (see also Chapter 2, ref. 13) of methane–water mixtures in the presence of apatite yielded some glucose 6-phosphate and ribose 5-phosphate.[35]

Triose, tetrose, and pentose phosphates enriched with ^{13}C have been prepared by the Kiliani–Fischer reaction on the terminal phosphates of the next lower aldose. The mixed nitriles were separated on Dowex 1-X8 resin and reduced with hydrogen over Pd–$BaSO_4$.[36] The synthesis of D-glucose 2-phosphate by phosphorylation of 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride, itself prepared by 2→1 acetyl migration, has been reported. Rates of phosphate hydrolysis in 0.25 M sulphuric acid and in 0.25 M sodium hydroxide were measured for D-glucose monophosphates: in the former the order was 1-phosphate > 2-phosphate > 3-phosphate > 6-phosphate while in the latter it was 3-phosphate > 6-phosphate > 2-phosphate > 1-phosphate.[37]

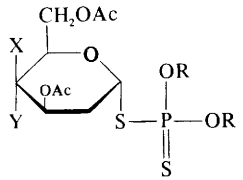
Opening of orthoesters with phosphoric acid and its derivatives has been used to prepare glycosyl phosphates.[14,38,39] Thus 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-orthoacetate reacted with decyl, tridecyl, pentadecyl, and hexadecyl monobenzyl phosphates to give the β-1-phosphate derivatives (11).

[33] H. G. Garg and R. W. Jeanloz, *Carbohydr. Res.*, 1979, **76**, 85.
[34] J. Horvat and D. Keglević, *Carbohydr. Res.*, 1979, **75**, 117.
[35] C. I. Simionescu, S. Dumitriu, V. Bulacovschi, and V. I. Popa, *Cellulose Chem. Technol.*, 1978, **12**, 585 (*Chem. Abs.*, 1979, **90**, 72 394*n*).
[36] A. S. Serianni, J. Pierce, and R. Barker, *Biochemistry*, 1979, **18**, 1192.
[37] H. Suzuki, K. Tokutome, and N. Taguchi, *Waseda Daigaku Rikogaku Kenkyusho Hokoku*, 1977, **79**, 50 (*Chem. Abs.*, 1979, **90**, 23 426*r*).



(11)

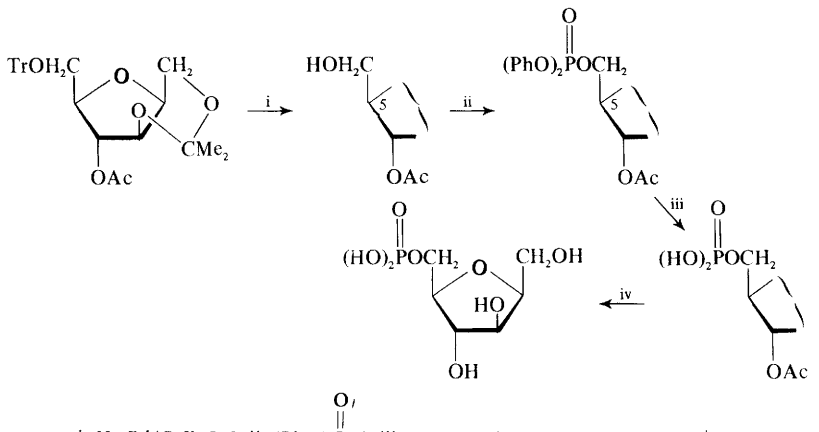
Removal of the benzyl group and conversion to sodium salt gave novel anionic detergents.[38] 3,4,6-Tri-*O*-acetyl β-L-galactose ethyl orthoacetate with anhydrous phosphoric acid in THF and in the presence of collidine, followed by treatment with lithium hydroxide, gave β-L-galactopyranose 1-phosphate, which was converted into GDP-L-galactose to study the enzyme responsible for converting D-mannose into L-galactose in *Chlorella pyrenoidosa*.[39] Phosphorylation of (6) with diphenylphosphoryl chloride gave 41% expected monophosphate without cleavage of the orthoester group, and 11% dicyclohexylidene inositol diphosphate.[14] A number of galactopyranosyl 6-(alkyl phosphites) have been prepared by esterification of 1,2 : 3,4-di-*O*-isopropylidene-α-D-galactopyranose with dialkyl phosphites, and the 3-*O*-ethyl phosphite of 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose was similarly prepared. Palladium complexes of the ethyl compounds were prepared by means of reaction with bis(π-allylpalladium) dichloride.[40] A one-step synthesis of *S*-(2-deoxy-α-D-glycopyranosyl) phosphorodithioates of general formula (12) has been published, involving uncatalysed addition of *O*,*O*-dialkylphosphorodithioic acids to glycals. The reaction was applied to 3,4,6-tri-*O*-acetyl-D-glucal and -galactal. On warming, anomerization to give α,β-anomeric mixtures occurred.[41] 2,5-Anhydro-D-glucitol 6-phosphate has been prepared by the route shown in Scheme 2.[42]



(12) X = H, Y = OAc or X = OAc, Y = H; R = alkyl

6-Phosphatidyl-D-glucose (13) and its thiophosphate analogue have been prepared by reaction of the amidite (14) with 1,2,3,4-tetra-*O*-acetyl-D-glucopyranose followed by oxidation of P^{III} to P^{V} by nitric oxide (or sulphur), deprotection of the glyceryl and phosphate groups, acylation with stearoyl

[38] Yu. L. Sebyakin, L. V. Volkova, V. S. Markin, and R. P. Evstigneeva, *Bioorg. Khim.*, 1979, **5**, 1816.
[39] P. A. Hebda, E. J. Behrman, and G. A. Barber, *Arch. Biochem. Biophys.*, 1979, **195**, 496.
[40] E. E. Nifant'ev and T. S. Kukhareva, *Zh. Obshch. Khim.*, 1979, **49**, 757 (*Chem. Abs.*, 1979, **91**, 108 154*w*).
[41] J. Borowiecka and M. Michalska, *Carbohydr. Res.*, 1979, **68**, C8.
[42] T. A. W. Koerner, R. J. Voll, and E. S. Younathan, *Carbohydr. Res.*, 1979, **70**, 165.



Reagents: i, H_2–Pd/C–EtOH; ii, $(PhO)_2P(O)Cl$; iii, H_2–PtO_2; iv, Dowex 50W-X8 (H^+)

Scheme 2

chloride or anhydride, and finally, selective deacetylation with hydrazine hydrate in methanol (5 h at 20 °C) to give the glyceryl glucos-6-yl phosphate.[43] The same reaction was applied to 1,2,3,6,2′,3′,4′-hepta-*O*-acetyl-maltose.[44] A new synthesis of polyprenyl glycosyl pyrophosphates involves reaction of polyprenyl phosphoimidazolidates (15) with unprotected glycosyl phosphates.[45] Incubation of *Bacillus cereus* membranes with UDP-*N*-acetylglucosamine gave two *N*-acetylglucosaminyl phosphoryl undecaprenols, shown to be the α- and β-anomers by solvolysis of the lipid with 50% phenol at 70 °C.[46]

Methyl 2,3-di-*O*-methyl-α-D-glucopyranoside has been shown to react with phosphites of general formula (R_2PR'), where R = Cl, R′ = OEt or R = R′ = NMe_2 or NEt_2, to give the cyclophosphites (16), which, in turn gave the thiophosphonate on treatment with sulphur.[47] The same group has carried out the reaction of tris(di-ethylamine)phosphine with 2-*O*-methylxylitan to give *endo* and *exo* isomers of the amidocyclophosphite (17) which was converted into various derivatives (Scheme 3).[48,49] Glycosylation of ambident anions from

[43] E. E. Nifant'ev, D. A. Predvoditelev, M. K. Grachev, and V. A. Shin, *Bioorg. Khim.*, 1978, **4**, 1213 (corrected *Chem. Abs.*, 1979, **90**, 187 238*g*).

[44] D. A. Predvoditelev, M. K. Grachev, M. B. Smirnov, and E. E. Nifant'ev, *Bioorg. Khim.*, 1979, **5**, 1509.

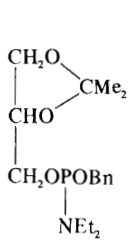
[45] V. N. Shibaev, L. L. Danilov, V. N. Chekunchikov, Yu. Yu. Kusov, and N. K. Kochetkov, *Bioorg. Khim.*, 1979, **5**, 308.

[46] N. Murazumi, S. Yamamori, Y. Araki, and E. Ito, *J. Biol. Chem.*, 1979, **254**, 11 791.

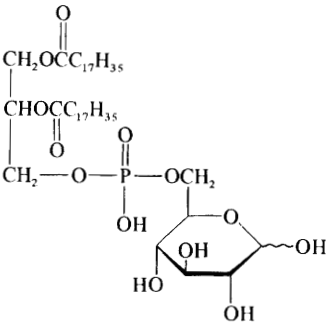
[47] E. E. Nifant'ev, M. P. Koroteev, and N. M. Pugashova, *Zh. Obshch. Khim.*, 1977, **47**, 2633 (*Chem. Abs.*, 1978, **88**, 105 667*t*).

[48] E. E. Nifant'ev, L. T. Elepina, A. A. Borisenko, M. P. Koroteev, L. A. Aslanov, V. M. Ionov, and S. S. Sotman, *Zh. Obshch. Khim.*, 1978, **48**, 2454 (*Chem. Abs.*, 1979, **90**, 152 479*j*).

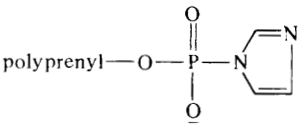
[49] E. E. Nifant'ev, L. T. Elepina, A. A. Borisenko, M. P. Koroteev, L. A. Aslanov, V. M. Ionov, and S. S. Sotman, *Phosphorus Sulphur*, 1979, **5**, 315 (*Chem. Abs.*, 1979, **91**, 123 939*z*).



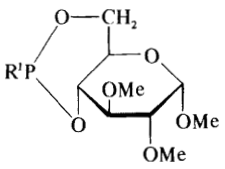
(14)



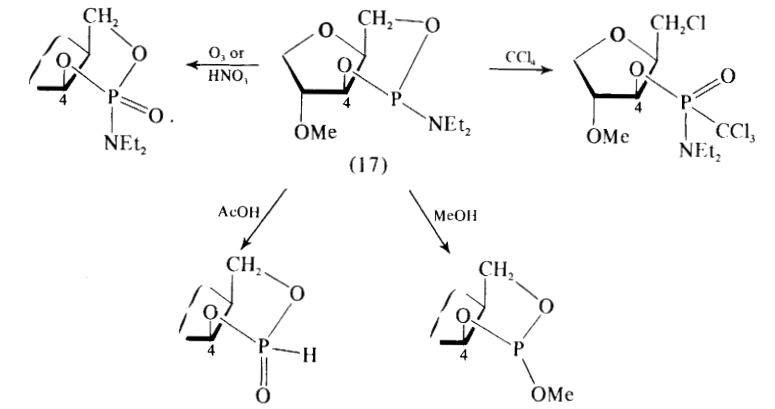
(13)



(15)



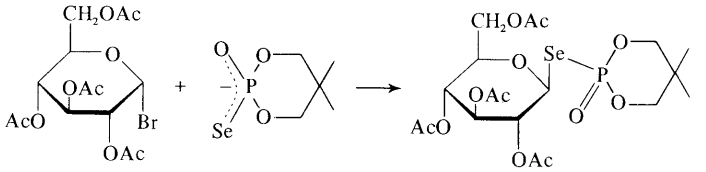
(16)



Scheme 3

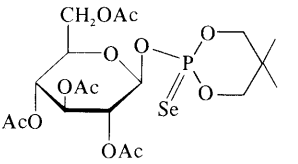
monoselenic acids of phosphorus leads to mixtures of Se-β-D-glycosylphosphoroselenoates and the isomeric *O*-glycosyl derivatives (Scheme 4). As with the corresponding sulphur derivatives (Vol. 12, p. 59) the ratio of the products depends on the counter ion used: NH_4^+ gives predominantly the Se-glycosylphosphate (18) whereas Ag^+ yields mainly the *O*-glycosyl phosphate (19) (Scheme 4), which was also made directly from 2,3,4,6-tetra-*O*-acetyl-β-D-hexopyranose and the seleno-phosphonyl chloridate (20). The β-anomer (18) isomerizes to 80% α-anomer on boiling in xylene for 2 h.[50]

[50] M. Michalska, J. Michalski, and I. Orlich-Krezel, *Pol. J. Chem.*, 1979, **53**, 253.



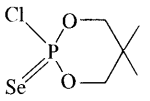
(18)

+



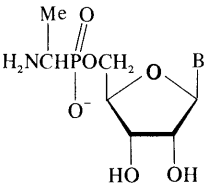
(19)

Scheme 4

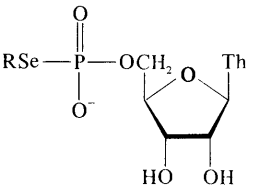


(20)

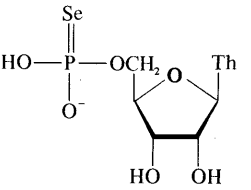
Two publications on the higher ketose mono- and bis-phosphates in rat-liver extracts have described their detection and estimation by colorimetry and enzymic assay,[51] and the synthesis of octulose 1,8-bisphosphates using muscle aldolase.[52] A stereospecific synthesis of adenosine 3′,5′-cyclic phosphothioate has appeared.[53] Standard condensation methods have been used to synthesize the 5′-*O*-(1-aminoethane-phosphonyl) nucleosides (21) and (22). The compounds were shown to be inert to the action of alkaline phosphatase and are poor substrates for 5′-nucleotidase.[54] The selenophosphates (23) and (24) have been prepared.[55] Phosphorylation of nucleosides using dibenzyl hydrogen phosphate,



(21) B = adenin-9-yl
(22) B = uracil-1-yl



(23) R = Et, C_6H_5



(24)

Th = thymin-1-yl

[51] F. Paoletti, J. F. Williams, and B. L. Horecker, *Arch. Biochem. Biophys.*, 1979, **198**, 620.
[52] F. Paoletti, J. F. Williams, and B. L. Horecker, *Arch. Biochem. Biophys.*, 1979, **198**, 614.
[53] J. Baraniak, K. Lesiak, and W. J. Stec, *Pol. J. Chem.*, 1979, **53**, 1387.
[54] K. Lesiak, W. J. Stec, and W. S. Zielínski, *Pol. J. Chem.*, 1979, **53**, 327.
[55] M. Sekin and T. Hata, *Chem. Lett.*, 1979, 801.

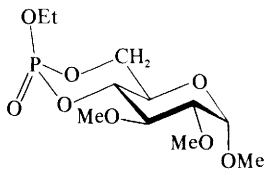
diethyl azodicarboxylate, and triphenyl phosphine produces largely the 5′-esters when the base is a pyrimidine and N^3,5′-cyclic derivatives when the base is a purine.[56] Phosphate reactions used in nucleotide synthesis are covered in Chapter 19.

The structure of the phosphate salt of ascorbic acid obtained by direct phosphorylation has been established by independent synthesis.[57]

An enzyme from mung bean (*Phaseolus aurens*) has been shown to convert L-*myo*-inositol 1-phosphate into ribulose 5-phosphate.[58]

The kinetics of the hydrolysis of D-glucosyl, D-mannosyl, D-galactosyl, and L-fucosyl α- and β-phosphates between pH 1–6 have been determined. For the mono-anionic species the β-anomers were hydrolysed 3–7 times faster than the α-anomers.[59] Extended Hückel calculations have shown that the difference in enthalpy between cyclic AMP and acyclic related phosphate esters is due to larger net exothermic solvation enthalpy in the former, ascribed to extra stabilization of the products of hydrolysis, due to their ability to form more stable hydrogen bonds with water.[60]

Cyclic glucose 4,6-(ethylphosphates), *e.g.*, (25), undergo ring-opening with dilute sodium methoxide to give the 4-(ethyl methylphosphate) as the kinetic product. On standing, the product gives the more thermodynamically stable 6-ester with retention of configuration at phosphorus. The results are consistent with the intermediacy of trigonal bipyramidyl intermediates which break down by apical departure of the leaving group before pseudorotation.[61] A study of various carbohydrates with bacteriophage φX 174 has shown that some reducing sugar phosphates which undergo autoxidation more rapidly than others are also more potent in inactivating the phage. The authors suggest that oxygen-derived free radicals cause the damage to the phage and that DNA is the target molecule for the inactivation reaction.[62]



(25)

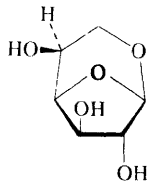
[56] J. Kimura, Y. Fujisawa, T. Yoshizawa, K. Fukuda, and O. Mitsunobu, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1191.
[57] J. Jernow, J. Blount, E. Oliveto, A. Perrotta, P. Rosen, and V. Toome, *Tetrahedron*, 1979, 1483.
[58] B. P. De and B. B. Biswas, *J. Biol. Chem.*, 1979, **254**, 8717.
[59] J. V. O'Connor and R. Barker, *Carbohydr. Res.*, 1979, **73**, 227.
[60] M. M. E. Scheffers Sap and H. M. Buck, *Rec. Trav. Chim. Pays-Bas*, 1978, **97**, 252 (*Chem. Abs.*, 1979, **90**, 23 596*w*).
[61] J. M. Harrison and T. D. Inch, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2855.
[62] N. Kashimura, J. Morita, and T. Komano, *Carbohydr. Res.*, 1979, **70**, C3.

3 Sulphonates

A preparation of 2-*O*-toluene-*p*-sulphonyl-L-arabinose required for synthesizing β-L-ribonucleosides has been published.[63]

Phase-transfer catalysis has been used to prepare toluene-*p*-sulphonates. When sulphonation at the anomeric position of 2,3,5,6-di-*O*-cyclohexylidene-D-mannopyranose was attempted using toluene-*p*-sulphonyl chloride–benzene–butyltriethylammonium chloride, the α-mannosyl chloride and the α,α-linked mannodisaccharide were obtained in addition to the desired product.[64] Selective sulphonation of benzyl (or methyl) 4-*O*-benzyl-α-L-rhamnopyranoside with toluene-*p*-sulphonyl chloride–aqueous sodium hydroxide–dichloromethane gave 65% 2-*O*-toluene-*p*-sulphonate.[65]

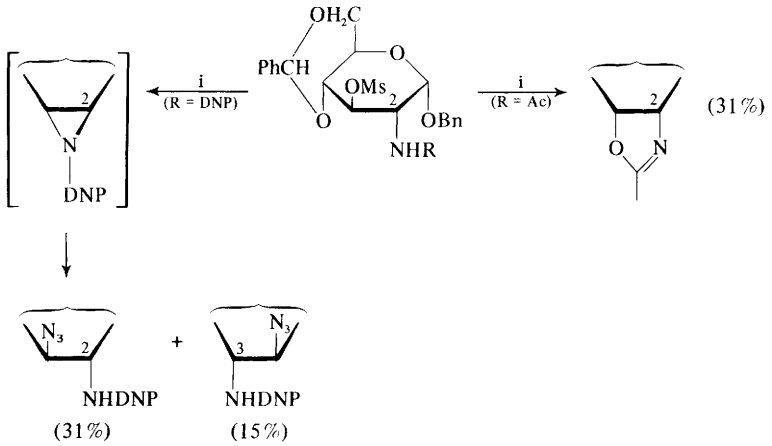
Monotosylation of 1,6-anhydro β-L-idofuranose (26) using 1.1 mol. equiv. of reagent gave 77% 5-, 6% 2-, 5% 2,5-di, and 5% 3,5-di-*O*-tosylates.[66] Selective tosylation of 1,2-*O*-isopropylidene-α-D-glucofuranose gave 80% 6-ester and 11% 3,6-diester whereas the selectivity is much lower with the corresponding allofuranose, the products being around 45% 6-ester, 25% 3,6-diester, and 5% 3-ester.[67]



(26)

Trifluoromethanesulphonates of several pentoses have been prepared and the resulting products subjected to azide displacement.[68] Nucleophilic displacement of 3-mesylates of substituted 2-aminosugars is severely restricted by neighbouring group participation. The reactions proceeded *via* cyclic intermediates as shown in Scheme 5.[69] Attempted deoxygenation at C-3 by photolysis of 3-sulphonyl esters of methyl 4,6-*O*-cyclohexylidene-2-deoxy-2-methoxycarbonylamino-α-D-glucopyranoside resulted instead in efficient S–O bond cleavage to give the free alcohol.[4]

[63] H. Antonin, *Nucleic Acid Chem.*, 1978, **1**, 155.
[64] Yu. A. Zhdanov, Yu. E. Alekseev, I. Siilis, T. P. Sudareva, and G. V. Bogdanova, *Dokl. Akad. Nauk, SSSR*, 1979, **245**, 846 (*Chem. Abs.*, 1979, **91**, 123 938*y*).
[65] V. Pozsgay, *Carbohydr. Res.*, 1979, **69**, 284.
[66] P. Köll and J. Schulz, *Carbohydr. Res.*, 1979, **68**, 365.
[67] K. Kefurt, K. Čapek, Z. Kefurtová, and J. Jary, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2526.
[68] R. Kimmich, H. Bauer, N. Vethaviyasar, and W. Voelter, *Z. Naturforsch., Teil B, Anorg. Chem., Org. Chem.*, 1979, **34**, 734 (*Chem. Abs.*, 1979, **91**, 91 857*c*).
[69] W. Meyer zu Reckendorf and H.-J. Lenzen, *Tetrahedron Lett.*, 1979, 3657.

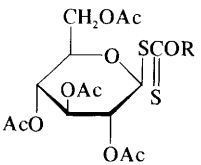


Reagents: i, NaN_3–DMF

Scheme 5

4 Other Esters

Glucopyranosyl xanthates of general formula (27) have been prepared by reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride with potassium alkyl xanthates; o.r.d., c.d., u.v., and n.m.r. spectra of the products were examined and the effect of solvents on the Cotton effect curve was studied.[70] The thermal decomposition of the dixanthates (28) and (29), prepared



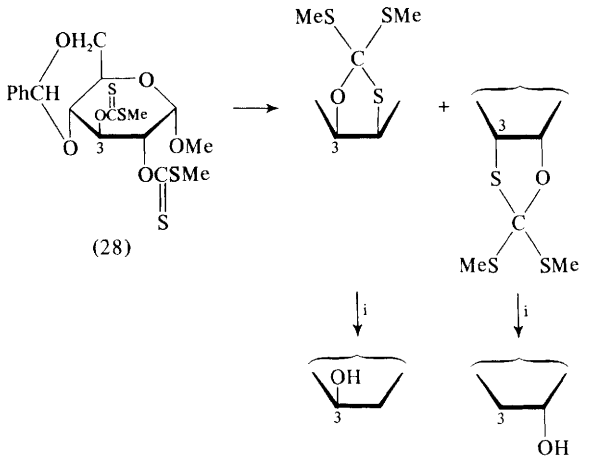
(27) R = Alkyl

from the diol and CS_2–MeI, gave rise to *O,S*,-linked cyclic trithio-orthocarbonates, useful for the preparation of deoxy-sugars (Schemes 6 and 7).[71] Reactions between the dixanthate (30) and monoxanthate (31) and glycine ethyl ester each give good yields of the *N*-xanthosyl glycine (32), which are models for enzyme immobilization. The product is stable over seven days in aqueous ethanol at pH values between 3.6 and 9.0.[72] The primary hydroxy-

[70] K. Satsumabayashi, Y. Nomoto, S. Satsumabayashi, and K. Numanami, *Nippon Shika Daigaku Kiyo, Ippan Kyoiku-kei*, 1979, **8**, 99 (*Chem. Abs.*, 1979, **91**, 91 864c).

[71] A. Faure, B. Krycza, and G. Descotes, *Carbohydr. Res.*, 1979, **74**, 127.

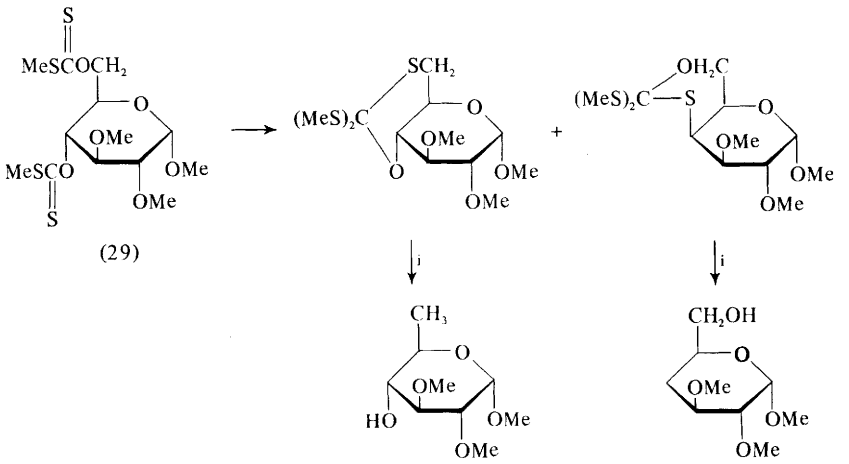
[72] V. C. Borlaza, N. W. H. Cheetham, and P. T. Southwell-Keely, *Carbohydr. Res.*, 1979, **68**, 141.



(28)

Reagents: i, H_2–Raney Ni–EtOH

Scheme 6



(29)

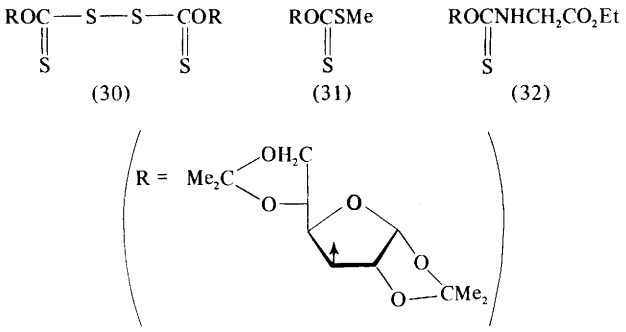
Reagents: i, H_2–Raney Ni–EtOH

Scheme 7

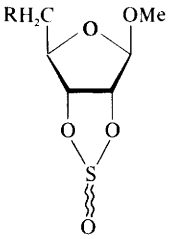
groups of cyclic and acyclic carbohydrates react with aryl isocyanates to give arylcarbamoyl derivatives.[73] An analogous reaction occurs with aryl thiocyanates.[74] Treatment of methyl β-D-ribofuranoside with thionyl chloride in acetonitrile gave the diastereoisomeric 2,3-cyclic sulphites (33), which were

[73] H. Zinner and T. Langer, *J. Prakt. Chem.*, 1978, **320**, 789 (*Chem. Abs.*, 1979, **90**, 39 138*j*).

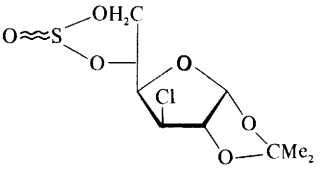
[74] H. Zinner and T. Langer, *J. Prakt. Chem.*, 1978, **320**, 795 (*Chem. Abs.*, 1979, **90**, 39 139*k*).



(30) (31) (32)



(33) R = OH
(34) R = Cl



(35)

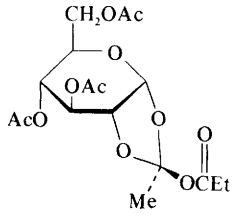
stable and isolable. When HMPT was used as solvent the 5-chloro-5-deoxy cyclic sulphites (34) were produced. Benzyl β-D-ribofuranoside and 1,4-anhydro-DL-ribitol gave similar results. 1,2-*O*-Isopropylidene-α-D-glucofuranose gave the 3-chloro-3-deoxy-5,6-cyclic sulphite (35) which slowly decomposed at room temperature.[75]

Sulphation of lactose with pyridine–SO_3 complex in pyridine resulted in formation of the galactose 6-sulphate. Other oligosaccharides were also studied.[76] The glycolipids, seminolipid and cerebroside sulphate, which are sulphated on the 3-position of galactose, were not oxidized by galactose oxidase.[77]

Acetobromoglucose reacts with silver propionate in THF to give, *via* the 1,2-acetoxonium ion, a mixture of the acyl orthoester (36), the first of its type reported, and the 2,3,4,6-tetra-*O*-acetyl-1-*O*-propionyl-β-D-glucopyranose.[78]

A kinetic study of the acid-catalysed hydrolysis of the ester group in alkyl β-D-glucofuranoside 5,6-carbonates, although giving activation parameters showing a strained transition state ($\Delta S^{\ddagger}$ −17 to −19 e.u.), could not discriminate between the A-1 or A-2 mechanisms.[79] Using the m.s. spectral fragmentations of isomeric 4,4′-bi-2-phenyl-1,3,2-dioxaborolanes and 2,7-diphenyl-1,3,6,8-tetraoxa-2,7-diborabicyclo[4.4.0]decanes as models, the bisboronates of erythritol, L-threitol, the 1-deoxy-D-pentitols, 1,6-dideoxy-D-galactitol and -L-mannitol were

[75] Y. Wang and H. P. C. Hogenkamp, *Carbohydr. Res.*, 1979, **76**, 131.
[76] N. Mian, C. E. Anderson, and P. W. Kent, *Biochem. J.*, 1979, **181**, 387.
[77] C. A. Lingwood, *Can. J. Biochem.*, 1979, **57**, 1138.
[78] G. Wulff, U. Schröder, and W. Schmidt, *Angew. Chem. Internat. Ed.*, 1979, **18**, 309.
[79] J. N. BeMiller and D. J. Nalin, *Carbohydr. Res.*, 1979, **70**, 319.



(36)

shown to be mixtures of structural isomers.[80] Polystyreneboronic acid has been used as an insoluble protecting group for specific diols of a range of glycosides allowing 'one-pot' syntheses of esters formed by substitution of the unprotected hydroxy-groups. High yields were obtained. Specific esterification at O-3 in methyl 6-*O*-trityl-α-D-glucopyranoside was possible since a 2,4-boronate was formed.[81]

Photolysis of primary nitrate esters gave quantitative yields of the free hydroxy compound. Secondary nitrates gave yields depending on stereochemistry. Thus 1,2 : 5,6-di-*O*-isopropylidene-3-nitro-α-D-glucopyranose gave 92% yield of the 3-OH compound whereas the corresponding allose derivative gave quantitative conversion.[82] The crystal structures of D-galactitol diarsenite and D-mannitol diarsenite have been determined. In both cases, the two arsenic atoms are attached to O-1, O-2, O-4 and to O-3, O-5, O-6 respectively with As–O bond lengths of 1.72–1.87 Å.[83]

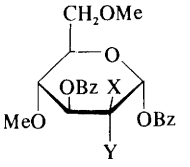
Cyclic phosphite esters of alditols are referred to in Chapter 17. Regioselective partial deacylation of acylated nucleosides is mentioned in Chapter 19.

[80] C. J. Griffiths, I. R. McKinley, and H. Weigel, *Carbohydr. Res.*, 1979, **72**, 35.
[81] J. M. J. Fréchet, L. J. Nuyens, and E. Seymour, *J. Am. Chem. Soc.*, 1979, **101**, 432.
[82] R. W. Binkley and D. J. Koholic, *J. Org. Chem.*, 1979, **44**, 2047.
[83] R. E. Ballard, A. H. Haines, A. A. Khandar Shahabad, and M. Elder, *Carbohydr. Res.*, 1979, **68**, C1.

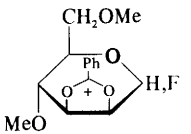
7

Halogeno-sugars

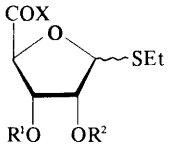
Anhydrous hydrogen fluoride reacted with the tri-*O*-benzoyl-glucose (1) and -mannose (2) derivatives to give the expected glycosyl fluoride initially. On prolonging the reaction both gave the same mannopyranosyl intermediate (3), which on hydrolysis yielded a mixture of 2- and 3-*O*-benzoyl-α- and -β-D-mannopyranosyl fluoride.[1] The preparation of ^{18}F-β-D-glucopyranosyl fluoride from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and Ag^{18}F followed by deacetylation has been reported.[2] Silver fluoride has also been used to displace the halogen of hepta-*O*-acetyl-α-maltosyl bromide. The deacetylated product, α-maltosyl fluoride, was a good substrate for sweet potato β-amylase, as was the corresponding β-fluoride; the products were hydrogen fluoride and β-maltose in each case.[3]



(1) X = H, Y = OBz
(2) X = OBz, Y = H



(3)

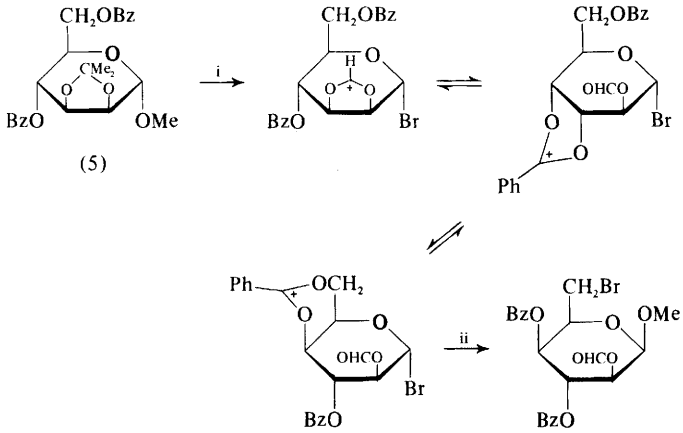


(4) R^1, R^2 = Bz, PhCH=, Me_2C=, or Bn; X = NHMe, NMe_2, or OMe

Treatment of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose with anhydrous hydrogen chloride in dichloromethane gave pure crystalline 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl chloride.[4] Chlorine and bromine react with the thioglycosides of riburonic acid derivatives of general formula (4) to give the corresponding β-riburonosyl halides (see also Chapter 15).[5] The methyl glycosides of per-*O*-*p*-

[1] C. Pedersen and S. Refn, *Acta Chem. Scand., Ser. B*, 1978, **32**, 687.
[2] A. E. Lemire and M. F. Reed, *J. Labelled Compd. Radiopharm.*, 1978, **15**, (Suppl. Vol.), 105 (*Chem. Abs.*, 1979, **91**, 57 314*p*).
[3] E. J. Hehre, C. F. Brewer, and D. S. Genghof, *J. Biol. Chem.*, 1979, **254**, 5942.
[4] R. A. Earl and L. B. Townsend, in 'Nucleic Acid Chemistry', ed. L. B. Townsend and R. S. Tipson, Academic Press, New York, 1978, Vol. 1, p. 203.
[5] K. H. Jung and R. R. Schmidt, *Justus Liebigs Ann. Chem.*, 1979, 1426.

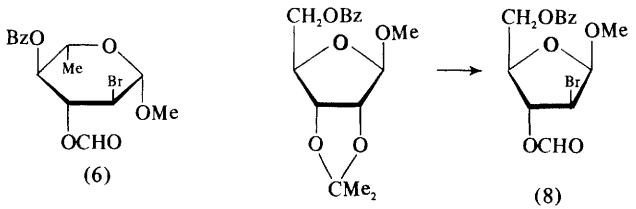
nitrobenzoylated D-ribofuranose, D-xylofuranose, and 3-deoxy-D-*threo*-pentofuranose produce the corresponding β-furanosyl bromides on treatment with acetyl bromide and hydrobromic acid in dichloromethane.[6] The use of dibromomethyl methyl ether for preparing bromo-sugars has been further explored (see also Vol. 7, p. 57 and Vol. 9, p. 54); acetalated glycosides gave formylated bromo-sugars presumed to arise *via* the glycosyl bromide, followed by cleavage of the isopropylidene acetal and formylation to give the formoxonium ion, which then either combines with bromide or rearranges to other acyloxonium ions leading to rearranged bromo-sugars, as illustrated in Scheme 1 for the mannopyranosyl derivative (5). The L-rhamnose analogue of (5) gave the 2-bromo-sugar (6), and the ribo-furanose (7) gave mainly the bromo derivative (8) together with small amounts of rearranged 5-bromo-sugars.[7]



(5)

Reagents: i, $MeOCHBr_2$–$ZnBr_2$; ii, MeOH–Ag_2CO_3

Scheme 1

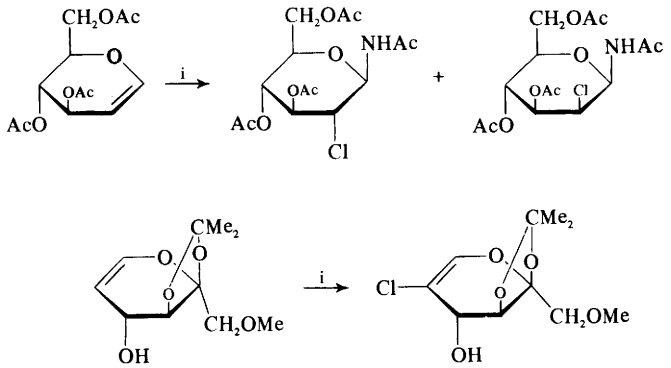


(6) (7) (8)

Addition of trifluoromethyl oxyfluoride to 3,4-di-*O*-acetyl-L-rhamnal led to a synthesis of 2-deoxy-2-fluoro-L-glucose and -L-rhamnose,[8] whilst a similar reaction with 3,4-di-*O*-acetyl-L-fucal was used to prepare 2-deoxy-2-fluoro-L-

[6] H. S. El Khadem, in ref. 4, p. 169.
[7] K. Bock, C. Pedersen, and J. Thiem, *Carbohydr. Res.*, 1979, **73**, 85.
[8] G. C. Butchard and P. W. Kent, *Tetrahedron*, 1979, **35**, 2439.

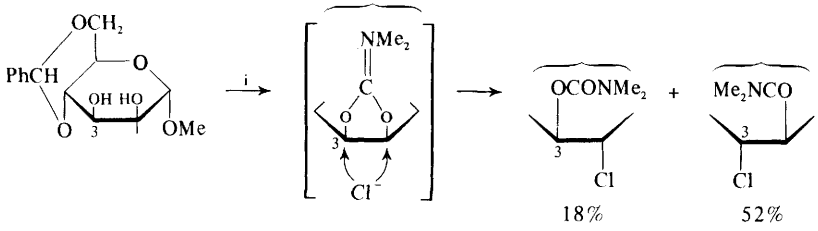
fucose.[9,10] Glycosides of 2-deoxy-2-fluoro-D-glucose are mentioned in Chapter 3. A novel method of replacing tosylate groups by fluoride without causing elimination has been devised: an anion-exchange (Amberlite IRA900 or Amberlyst A-26) resin in a fluoride form, thoroughly dried, is used with a benzene solution of the tosylate at reflux. The reaction was applied to 1,2 : 5,6-di-*O*-isopropylidene-3-*O*-tosyl-α-D-allofuranose to yield 72% of the corresponding 3-deoxy-3-fluoro-glucose derivative, and to 1,2 : 3,4-di-*O*-isopropylidene-6-*O*-tosyl-α-D-galactopyranose giving the expected 6-deoxy-6-fluoro-galactose derivative in 75% yield.[11] Sulphonate displacement of a more conventional kind using tetra-n-butylammonium fluoride in acetonitrile on methyl 2,3,6-tri-*O*-benzyl-4-*O*-mesyl-α-D-galactopyranoside led to a synthesis of 4-fluoro-glucose.[12] The same reaction has been applied to perbenzylated 4,6-di-*O*-mesyl- and 4-*O*-mesyl-6-*O*-trityl-α,α-trehalose to give the 4,6-dideoxy-4,6-difluoro- and 4-deoxy-4-fluoro-α-D-galactosyl-α-D-glucose derivatives which were then debenzylated.[13] 6,6-Difluoro-L-fucose has been synthesized by reaction of diethylammoniumsulphur trifluoride with 1,2 : 3,4-di-*O*-isopropylidene-L-galactodialdose followed by removal of the acetal blocking groups. The paper also contains syntheses by standard methods of 6-deoxy-6-halo-L-galactoses.[14] Solutions of glycals containing vinyl ether units and tetraethylammonium chloride in acetonitrile gave low yields of chloro-deoxy sugars when subjected to anodic addition (*e.g.*, Scheme 2).[15] The same group has synthesized chloro-deoxy sugars by reaction of suitably blocked glycosides containing a *cis*-vicinal diol system with



Reagents: i, NEt_4Cl–MeCN

Scheme 2

[9] C. G. Butchard and P. W. Kent, *Tetrahedron*, 1979, **35**, 2551.
[10] D. J. Winterbourne, C. G. Butchard, and P. W. Kent, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 989.
[11] S. Colonna, A. Re, G. Gelbard, and E. Cesarotti, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2248.
[12] D. P. Lopes and N. F. Taylor, *Carbohydr. Res.*, 1979, **73**, 125.
[13] A. F. Hadfield, L. Hough, and A. C. Richardson, *Carbohydr. Res.*, 1979, **71**, 95.
[14] J. A. May and A. C. Sartorelli, *J. Med. Chem.*, 1979, **22**, 971.
[15] A. Klemer and G. Nicolaus, *Chem. Ber.*, 1979, **112**, 3063.



Reagent: i, $Cl_2C{=}\overset{+}{N}Me_2\bar{Cl}$

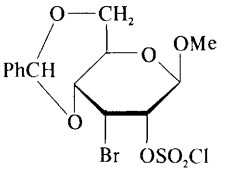
Scheme 3

dichloromethylene dimethylammonium chloride (*e.g.*, Scheme 3).[16] 4-Chloro-L-glucose has been prepared by the action of sulphuryl chloride on methyl 2,3-di-*O*-benzoyl-α-L-fucoside.[17] A study of the reaction of raffinose with sulphuryl chloride in pyridine–chloroform mixtures has been carried out; after dechlorosulphation with alkaline methanolic sodium iodide, 6′-chloro-6′-deoxy- and 6′,6″-dichloro-6′,6″-dideoxy-raffinose, 6-*O*-(4,6-dichloro-4,6-dideoxy-α-D-glucopyranosyl)-α-D-glucopyranosyl 6-chloro-6-deoxy-β-D-fructofuranoside, and methyl 6-chloro-6-deoxy-α- and β-D-fructofuranosides were obtained. Good yields of the corresponding deoxy compounds were obtained by catalytic hydrogenolysis of the chloro-compounds.[18] Reference to the use of halo-derivatives of cellobiose to prepare deoxy-disaccharides is made in Chapter 3, and the use of 5-chloro-5-deoxy-glucose derivatives as intermediates in the synthesis of 5-amino-5-deoxy-sugars is mentioned in Chapter 8.

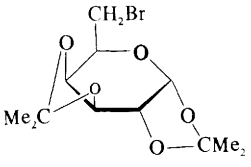
The reaction of carbohydrates with hydrogen bromide in acetic acid has been further studied (see Vol. 10, p. 49; Vol. 11, p. 66). Reaction proceeds *via* acetoxonium ions formed from *vic*-diol units; hexoses reacted as furanoses to give 6-bromo-6-deoxy-sugars, whereas pentoses only gave glycosyl bromides;[19] aldonic acids, lectones, and ascorbic acid reacted similarly.[20] The 1,4- and 1,5-anhydro-alditols containing *cis*-diol units also gave bromo-deoxy-analogues.[19,21] Displacement of chloro-sulphate groups by bromide ion using tetraethylammonium bromide led to the bromo-sugars (9) and (10) in moderate to good yields.[22]

A novel reagent system for converting an hydroxy-group into an iodo group with inversion of configuration used triphenylphosphine–iodine–imidazole in toluene at elevated temperature. Triphenylphosphine-2,4,5-tri-iodoimidazole was also used. Among the examples were the conversion of methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside into the corresponding 2-deoxy-2-iodo-α-D-gluco-

[16] A. Klemer, R. Lemmes, and K. Cimander, *Carbohydr. Res.*, 1979, **68**, 375.
[17] A. F. Hadfield and A. C. Sartorelli, *Carbohydr. Res.*, 1979, **72**, 235.
[18] L. Hough, A. C. Richardson, and M. A. Salam, *Carbohydr. Res.*, 1979, **71**, 85.
[19] C. Pedersen, K. Bock, and I. Lundt, *Pure Appl. Chem.*, 1978, **50**, 1385 (*Chem. Abs.*, 1979, **91**, 175 621*j*).
[20] K. Bock, I. Lundt, and C. Pedersen, *Carbohydr. Res.*, 1979, **68**, 313.
[21] K. Bock, P. Gammeltoft, and C. Pedersen, *Acta Chem. Scand., Ser. B*, 1979, **33**, 429.
[22] D. R. Bundle, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2751.



(9)



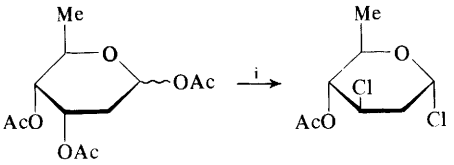
(10)

pyranoside in 82% yield, $^{-N}$ of 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose into the corresponding 3-deoxy-3-iodo-D-allofuranose in 78% yield.[23] Displacements of triphenylphosphonium ions by iodide ion have also been achieved by another group using methyl iodide–diethyl azodicarboxylate–triphenylphosphine. Thus, 1,2 : 5,6-di-*O*-isopropylidene-α-D-gluco- and -allofuranose gave the corresponding 3-deoxy-3-iodo-α-D-allo- and -glucofuranose in 90 and 78% yield respectively.[24]

Trimethyl phosphate with bromine or chlorine converts benzylidene acetals into halo benzoates similarly to *N*-bromosuccinimide, although the reaction has not yet been applied to carbohydrates.[25]

Bromo-derivatives of aldonic and ascorbic acids are noted in Chapter 15. Nucleosides containing chloro-sugars and fluoro-sugars are mentioned in Chapter 19.

Treatment of the acetylated dideoxy sugar (11) with hydrogen chloride in ether unexpectedly gave the 3-chloro-trideoxy derivative (12) (Scheme 4).[26]



(11) (12)

Reagents: i, HCl–Et_2O

Scheme 4

[23] P. J. Garegg and B. Samuelsson, *J. Chem. Soc., Chem. Commun.*, 1979, 978.
[24] H. Kunz and P. Schmidt, *Tetrahedron Lett.*, 1979, 2123.
[25] S. D. Venkataramu, J. H. Cleveland, and D. E. Pearson, *J. Org. Chem.*, 1979, 44, 3082.
[26] E.-F. Fuchs, D. Horton, W. Weckerle, and E. Winter-Mihaly, *J. Med. Chem.*, 1979, 22, 406.

8
Amino-sugars

D-Acosamine (2,3,6-dideoxy-3-amino-D-*arabino*-hexose) has been isolated from hydrolysis of the antibiotic sporoviridin,[1] which contains at least two acosamine moieties in addition to viosamine (4-amino-4,6-dideoxy-D-glucose).[2] 2-Acetamido-glucal has been isolated from the urine of patients with sialuria[3] and 1,5-dideoxy-1,5-imino-D-mannitol is present in the legumes *Lonchocarpus sericens.*[4]

1 Synthesis

Application of the Staudinger reaction to azido-sugars constitutes a convenient means of reducing them to amino-sugars. The azide is first treated with triphenylphosphine and the resulting phosphinimine ($RN{=}PPh_3$), which usually crystallizes out, is readily hydrolysed to the amine in the presence of base.[5] Trifluoromethylsulphonyl (triflyl) esters of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose have been subjected to ammonolysis, but only the primary group of the latter underwent displacement. However, the secondary triflyl group of the former did undergo displacement when sodamide was used as the nucleophilic reagent.[6]

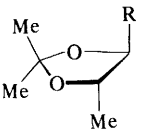
There has been substantial activity in the synthesis of 3-amino-2,3,6-trideoxyhexoses and their di-*N*-methyl derivatives as a result of their wide occurrence in antibiotics. In a paper of substantial proportions, Bartner *et al.*[7] have synthesized by standard reactions all four possible methyl 2,3,6-dideoxy-3-dimethylamino-α-D-hexopyranosides (and some β-anomers) as well as the corresponding L-*ribo* isomer (L-megosaminide). Syntheses have been reported for D-ristosamine (3-amino-2,3,6-trideoxy-D-*ribo*-hexose) from methyl 2-deoxy-D-*arabino*-hexopyranoside[8] and of the isomeric 3-amino-2,3,6-trideoxy-L-*xylo*-hexose (1) from methyl 2,6-dideoxy-L-*arabino*-hexopyranoside,[9] using standard sequences involving azide displacement of sulphonate esters for introducing the nitrogen.

A very elegant synthesis of (1) and its D-enantiomer has been described start-

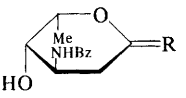
[1] K.-I. Harada, S. Ito, and M. Suzuki, *Carbohydr. Res.*, 1979, **75**, C17.
[2] K.-I. Harada, S. Ito, and M. Suzuki, *Tetrahedron Lett.*, 1979, 3965.
[3] J. P. Kamerling, G. Strecker, J.-P. Farriaux, L. Dorland, J. Haverkamp, and J. F. G. Vliegenthart, *Biochim. Biophys. Acta*, 1979, **583**, 403.
[4] L. E. Fellows, E. A. Bell, D. G. Lynn, F. Pilkiewicz, I. Miura, and K. Nakanishi, *J. Chem. Soc., Chem Commun.*, 1979, 977.
[5] J. Kovacs, I. Pinter, F. Szego, J. Toth, and A. Messmer, *Acta Chem. Acad. Sci. Hung.*, 1979, **101**, 7.
[6] T. W. Flechtner, *Carbohydr. Res.*, 1979, **77**, 262.
[7] P. Bartner, D. L. Boxler, R. Brambilla, A. K. Mallams, J. B. Morton, P. Reichert, F. D. Sancilio, H. Surprenant, G. Tomalesky, G. Lukacs, A. Olesker, T. T. Thang, L. Valente, and S. Omura, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1600.
[8] I. Pelyvas, F. Sztaricskai, L. Szilagyi, T. Bognar, and J. Tamas, *Carbohydr. Res.*, 1979, **68**, 321.
[9] T.-M. Cheung, D. Horton, and H. Weckerle, *Carbohydr. Res.*, 1979, **74**, 93.

ing from non-carbohydrate materials. The key intermediates, 4-deoxy-2,3-*O*-isopropylidene-L-threitol (2) and its D-form, were prepared from L-tartaric acid and L-threonine respectively.[10] Oxidation of (2) to the aldehyde (3) and application of the Wittig reaction afforded the unsaturated hexonic acid (4) as an *E,Z* mixture. Sequential ammonolysis, acid hydrolysis, and *N*-benzoylation gave 3-benzamido-2,3,6-trideoxy-L-*xylo*-hexono-1,5-lactone (5) which was reduced to the free sugar (1) with di-isobutylaluminium hydride. The D-enantiomer was similarly obtained as its *N*-acetyl derivative.

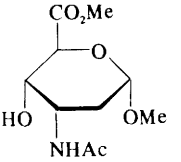
The syntheses of glycosides and esters of daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose) suitable for use in the synthesis of analogues of daunomycin and daunosamine-containing oligosaccharides have been reported.[11] Methyl (methyl 3-acetamido-2,3-dideoxy-α-D-*ribo*-hexopyranosid)uronate (6) has been described in which the carboxylate grouping was established from the corresponding 6-azide by sequential photolysis, mild hydrolysis, and oxidation of the 6-*aldehydo* group.[12]



(2) R = CH_2OH
(3) R = CHO
(4) R = $CH{=}CHCO_2Et$



(1) R = H,OH
(5) R = O



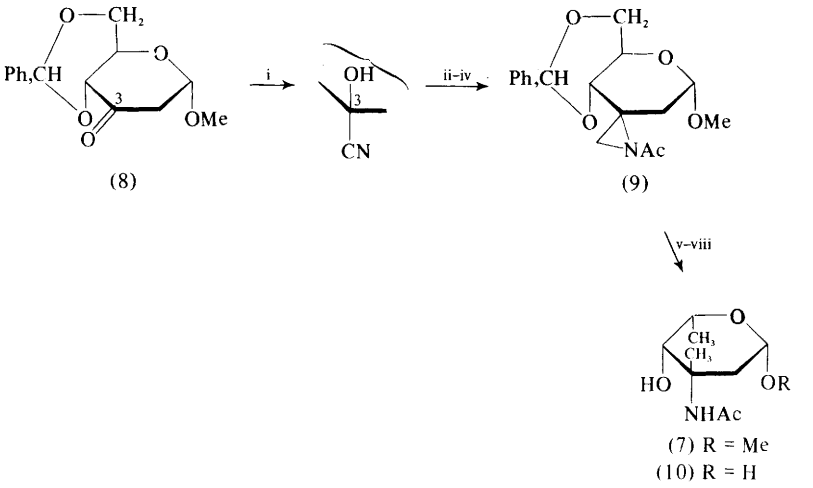
(6)

A methyl glycoside of vancosamine (3-amino-2,3,6-trideoxy-3-*C*-methyl-L-*lyxo*-hexose) (7) has been synthesized from the 3-ulose (8) (Scheme 1). Reductive ring-opening of the spiro-epimine (9) gave the 3-amino-3-*C*-methyl derivative which was converted to (7) by inversion of configuration at C-5 *via* the 5-ene.[13]

The synthesis of the following DL-forms of amino-sugars occurring in antibiotics have been described: DL-vancosamine (10),[14] DL-desosamine,[15] DL-daunosamine,[16] and DL-megosamine.[17] Standard conversions on non-carbohydrate source materials were used.

Oxyamination of olefinic sugars using the Sharpless reaction ($TsNHCl–OsO_4$) has been widely studied as a route to amino-sugars. Compounds of the general

[10] G. Fronza, C. Fuganti, P. Grasselli, and G. Marinoni, *Tetrahedron Lett.*, 1979, 3883.
[11] H. S. El Khadem, A. Liav, and D. L. Swartz, *Carbohydr. Res.*, 1979, **74**, 345; H. S. El Khadem and A. Liav, *Carbohydr. Res.*, 1979, **74**, 199.
[12] D. Horton, W. Weckerle, and B. Winter, *Carbohydr. Res.*, 1979, **70**, 59.
[13] T. T. Thang, F. Winternitz, A. Olesker, A. Lagrange, and G. Lukas, *J. Chem. Soc., Chem. Commun.*, 1979, 153.
[14] I. Dyong and H. Friege, *Chem. Ber.*, 1979, **112**, 3273.
[15] T. Kinoshita, Y. Kawashima, K. Hayashi, and T. Miwa, *J. Chem. Soc., Chem. Commun.*, 1979, 766.
[16] I. Iwataki, Y. Nakamura, K. Takahashi, and T. Matsumoto, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2731.
[17] I. Dyong and H. Bendlin, *Chem. Ber.*, 1979, **112**, 717.



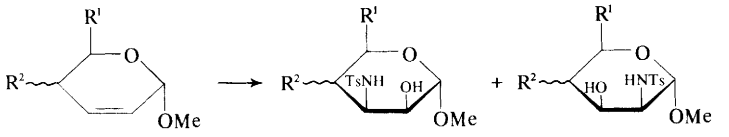
(8) (9)

(7) R = Me
(10) R = H

Reagents: i, KCN; ii, MsCl–py; iii, $LiAlH_4$ or RMgX; iv, Ac_2O; v, Ni–H_2; vi, NBS; vii, AgF; viii, OH^-

Scheme 1

type (11) undergo stereoselective oxyamination on the upper face to give (12) (major product) and (13) together with varying amounts of the 2,3-*cis*-diol (3–48%).[18,19] Application of the reaction to tri-*O*-acetyl-D-glucal and -D-galactal afforded mostly *N*-tosylglycosylamines with the *gluco*- and *galacto*-configurations respectively.[19] The ratio of products obtained from 2-*O*-substituted benzyl 3,4-dideoxy-DL-glycero-pent-3-enopyranosides varies according to the substituent. An acetyl group favours the 3-*N*-tosylamine, whereas a 2-*O*-benzyl substituent steers the tosylamino group to C-4.[20]



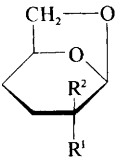
(11) (12) (13)

R^1 = Me,CH_2OR,CH_2NHR, or COR
R^2 = H,OH, or OAc

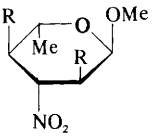
The two methyl pyranosides of 2-acetamido-2,3,4-trideoxy-D-*erythro*-hexose have been prepared as intermediates in the synthesis of purpurosamine C. The crucial intermediate in this synthesis was 1,6-anhydro-3,4-dideoxy-2-*O*-tosyl-β-D-*threo*-hexopyranose (14) which underwent displacement of the sulphonyloxy

[18] A. Banaszek and A. Zamojski, *Pol. J. Chem.*, 1979, **53**, 287, 2029.
[19] I. Dyong, G. Schulte, Q. Lam-Chi, and H. Friege, *Carbohydr. Res.*, 1979, **68**, 257.
[20] I. Dyong and N. Jersch, *Chem. Ber.*, 1979, **112**, 1849; I. Dyong, N. Jersch, and Q. Lam-Chi, *Chem. Ber.*, 1979, **112**, 1859.

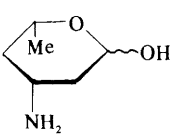
substituent by azide to give (15).[21] 3-Amino-2,3,4,6-tetradeoxy-α-L-*threo*-hexose (16) has been made from the 3-nitropyranoside *via* the dimesylate (17) and reduction ($NaBH_4$) to (18) from which (19) was obtained.[22] All four isomers of (20) have been prepared from the corresponding nitro derivatives[23] and 4-acetamido-4,6-dideoxy-L-glucose has been synthesized by standard procedures.[24] 3-Amino-3-deoxy-α,α-trehalose has been synthesized from the corresponding 3-*O*-triflate (*allo* configuration) by azide displacement, which was reported to be almost instantaneous at room temperature. By contrast, the corresponding mesylate afforded substantial amounts of the 3-ene.[25] The branched chain amino-sugar 2-acetamidomethyl-2-deoxy-D-galactose has been prepared from 2-cyano-2-deoxy-D-galactal.[26]



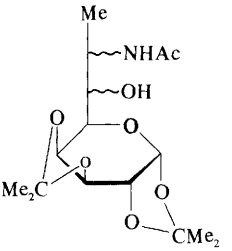
(14) R^2 = Ts, R^1 = H
(15) R^2 = H, R^1 = N_3



(16) R = OH
(17) R = OMs
(18) R = H



(19)

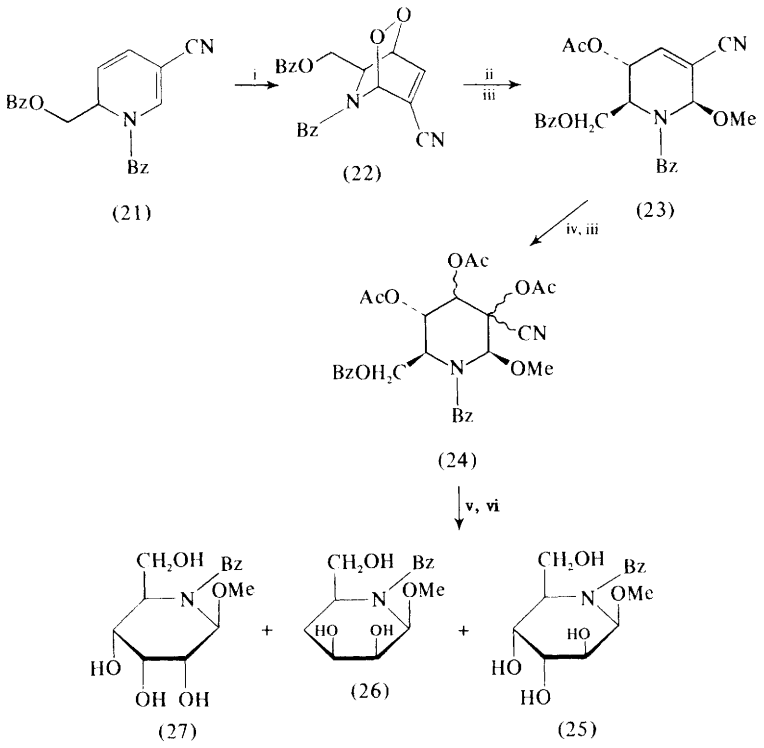


(20)

Two separate syntheses have been reported for *N*-acetyl-lactosamine, one *via* a 1,6 : 2,3-dianhydride derivative of lactose[27] and the other by ascent of the series from *N*-benzyl-3-*O*-β-D-galactopyranosyl-D-arabinosylamine,[28] and the 3-*O*-, 4-*O*- and 6-*O*-α-D-mannopyranosyl derivatives of *N*-acetyl-glucosamine have been reported.[29]

[21] J. S. Brimacombe, F. Hunedy, A. M. Mather, and L. C. N. Tucker, *Carbohydr. Res.*, 1979, **68**, 231.
[22] H. H. Baer and H. R. Hanna, *Carbohydr. Res.*, 1979, **77**, C12.
[23] J. C. Boeyens, M. J. Nolte, and G. R. Woolard, *Carbohydr. Res.*, 1979, **70**, 103.
[24] A. F. Hadfield and A. C. Sartorelli, *Carbohydr. Res.*, 1979, **72**, 235.
[25] H. H. Baer and A. J. Bell, *Carbohydr. Res.*, 1979, **75**, 175.
[26] R. H. Hall, K. Bischofberger, A. J. Brink, O. G. de Villiers, and A. Jordaan, *J. Chem. Soc., Perkin 1*, 1979, 781.
[27] T. Takamura, T. Chiba, and S. Tejima, *Chem. Pharm. Bull.*, 1979, **27**, 721.
[28] R. T. Lee and Y. C. Lee, *Carbohydr. Res.*, 1979, **77**, 270.
[29] K. L. Matta, R. H. Shah, and O. P. Bahl, *Carbohydr. Res.*, 1979, **77**, 255.

An interesting synthesis of 5-amino-5-deoxy-DL-piperidinoses from 1-acyl-1,2-dihydro-pyridines (21) has been reported (Scheme 2). The photochemical oxidation of (21) afforded the bicyclic peroxide (22) which on reduction gave the tetrahydropyridine (23). Hydroxylation of (23) afforded the cyanohydrin (24), which on hydrolysis and reduction afforded the methyl piperidinoses (25)–(27).[30] (See also Vol. 12, p. 71.)



All compounds are racemates

Reagents: i, $h\nu$–O_2; ii, Me_2S–MeOH; iii, Ac_2O–py; iv, OsO_4; v, NaOMe; vi, $NaBH_4$

Scheme 2

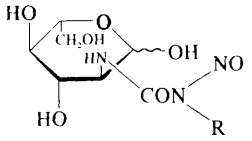
A convenient means of preparing L-glucosamine has been published which proceeds by ascent of the series from L-arabinose ($BnNH_2$–HCN). The required L-glucosamine is separated from the L-*manno*-isomer by conversion to its crystalline Schiff base, formed from 4-methoxybenzaldehyde.[31]

[30] M. Natsume, M. Wada, and M. Ogawa, *Chem. Pharm. Bull.*, 1978, **26**, 3364.

[31] J. P. Johnston, G. S. McCaleb, T. Anderson, and D. S. Murinson, *J. Med. Chem.*, 1979, **22**, 597.

2 Reactions

Several *N*-nitroso derivatives of amino-sugars have been reported. For example, the L-enantiomers of streptozoticin and chlorozoticin, (28) and (29) respectively, have been prepared. The ureido-substituent group which is responsible for the anti-tumour activity of chlorozoticin (29), has been attached to the amino-groups of maltosylamine, cellobiosylamine, and lactosylamine and the products possessed marked anti-tumour activity.[32] Additionally, *N*-nitroso derivatives of aldose–amino-acid adducts formed by the Maillard reaction, have been synthesized, and are believed to be present in foodstuffs which contain nitrite as preservative.[33]



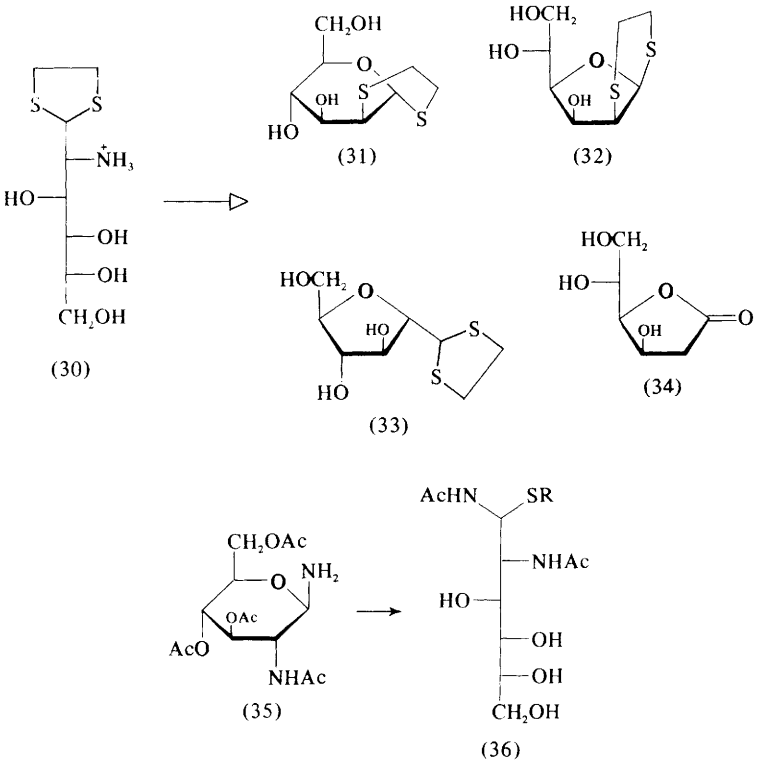
(28) R = Me
(29) R = CH_2CH_2Cl

Further work has been reported in the area of muramyl peptides as a result of their immunoadjuvant activity[34] and the 6-*O*-mycoloyl derivative of *N*-acetyl-muramyl-L-alanyl-D-isoglutamine is reported to possess anti-tumour activity.[35]

Deamination of the ethylene dithioacetal of 2-amino-2-deoxy-D-glucose (30) has been examined in detail. Four products (31)–(34) were obtained in 40, 7.7, 22, and 3% yields respectively when the reaction was conducted at pH 5.6. At pH's <1 using N_2O_3 only the 2-deoxylactone (34) was formed. Products (31) and (32) are formed by migration of 'S' to the electrophilic C-2, followed by attack at C-1 by either HO-5 or HO-4 respectively. The formation of (33) is accounted for simply by attack of HO-5 on C-2, and the formation of (34) occurred by a hydride shift from C-1 to C-2, followed by ring closure by HO-4 and loss of the dithioacetal function.[36] Reaction of the *O*-acetylated glucosyl-amine (35) with thiols yielded the hemithioacetate (36).[37]

Various *N*-substituted derivatives of amino-sugars have been reported. For example, *N*-(6′-bromoacetamidocapryl) and *N*-(*N*′-bromoacetylglycyl) derivatives of 2-amino-2-deoxy-D-glucose have been prepared for coupling to rat hepatic glucokinase,[38] and spin labels have been attached to 2-amino-2-deoxy-D-

[32] T. Suami, T. Machinami, and T. Hisamatsu, *J. Med. Chem.*, 1979, **22**, 247.
[33] K. Heyns, S. Röper, H. Röper, and B. Meyer, *Angew. Chem. Int. Ed. Engl.*, 1979, **18**, 878.
[34] S. Kusumoto, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1177; A. Hasegawa, Y. Kaneda, M. Amano, M. Kiso, and I. Azuma, *Agric. Biol. Chem.*, 1978, **42**, 2187; P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Tetrahedron Lett.*, 1979, 4013.
[35] T. Shiba, S. Okada, S. Kusumoto, I. Azume, and Y. Yamamura, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 3307.
[36] P. Angibeaud, C. Bosso, J. Defaye, D. Horton, C. Cohen-Addad, and M. Thomas, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1583.
[37] B. Paul and W. Korytnyk, *Carbohydr. Res.*, 1979, **72**, 247.
[38] B. A. Connolly and I. P. Trayer, *Eur. J. Biochem.*, 1979, **99**, 299.



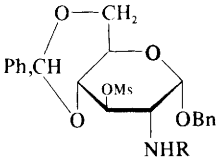
glucose through nitrogen.[39] The *N*-sulphonation of amino-sugars with the pyridine–sulphur trioxide reagent proceeds most efficiently at pH 8.5–9.0[40] and reaction of *O*-blocked amino-sugars with *p*-nitrobenzene diazonium tetrafluoroborate gave triazenyl sugars (RNHN=NAr).[41] It has been shown by ^{1}H n.m.r. and ^{13}C n.m.r. that ethyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside exists in acetone solution at the Schiff base ($R—N=CMe_2$) rather than the previously supposed hemiorthoamide.[42]

Neighbouring group participation severely restricts S_N2 displacements at C-3 of 2-acylamido-2-deoxy-glucopyranosides, so that attempted displacement of the 3-mesylate (37) led mainly to the oxazoline (38). In contrast, the corresponding *N*-2,4-dinitrophenyl derivative (39) reacted with the azide anion *via* 'N-participation' to give the epimine (40), which reacted further with azide to give the 3-azido-*gluco*- and the 2-azido-*altro*-isomers in a ratio of about 2:1 [*i.e.*, diequatorial ring-opening of (40) predominates].[43] The epimine (41) was also the

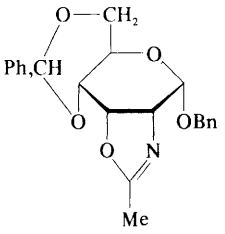
[39] M. J. Adam and L. D. Hall, *Carbohydr. Res.*, 1979, **68**, C17; L. Evelyn and L. D. Hall, *Carbohydr. Res.*, 1979, **70**, C1.
[40] Y. Inoue and K. Nogasawa, *Carbohydr. Res.*, 1979, **69**, 297.
[41] J. M. J. Tronchet and F. Rachidzadeh, *Helv. Chim. Acta*, 1979, **62**, 971.
[42] B. Capon, C. Labbe, and D. S. Rycroft, *Can. J. Chem.*, 1979, **57**, 2978.
[43] W. Meyer zu Reckendorf and H. J. Lenzen, *Tetrahedron Lett.*, 1979, 3657.

major product when (42) was treated with sodium iodide in DMF, but the yield was poor. However, the corresponding *N*-methyl derivative of (42) reacted by 'O-participation' to give the cyclic carbamate (43).[44]

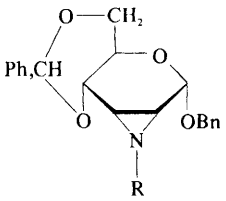
2-Amino-2-deoxy-D-glucose and -D-galactose participate as the amine component in Amadori rearrangements. Thus the former reacts with D-glucuronic acid to give the adduct (44). This and related adducts are hydrolysed to their original components at 70 °C and pH 6.5.[45]



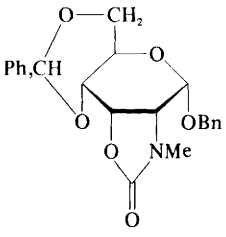
(37) R = Ac
(39) R = $C_6H_3(NO_2)_2$
(42) R = CO_2Bn



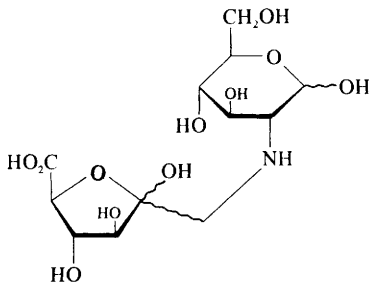
(38)



(40) R = $C_6H_3(NO_2)_2$
(41) R = CO_2Bn



(43)



(44)

Acetolysis of fully acetylated methyl pyranosides of 4-amino-4-deoxy-D-glucose and -D-galactose results in partial rearrangement to 'N-in-ring' furanoses which, it has been shown, arise directly from the glycosides and not from initially formed acetylated pyranoses.[46]

The synthesis of a 4-methylumbelliferyl glycoside of *N*-acetyl-α-D-neuraminic acid has been reported for use as a substrate for the fluorometric detection and estimation of α-neuraminidase.[47]

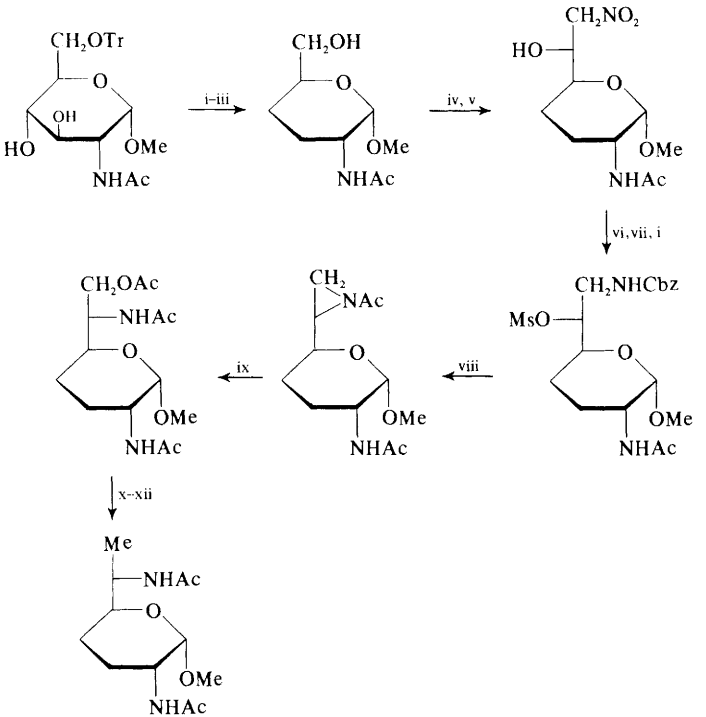
Metal complexes of 2-deoxy-2-methylamino-D-glucose and other amino-hexoses have been studied and stability constants derived.[48]

[44] H. Sano, S. Iimura, T. Tsuchiya, and S. Umezawa, *Bull. Soc. Chem. Jpn.*, 1978, **51**, 3661.
[45] A. Klemer and W. Funcke, *Justus Liebigs Ann. Chem.*, 1979, 1682.
[46] F. W. Lichtenthaler and G. Bambach, *Carbohydr. Res.*, 1979, **68**, 305.
[47] T. G. Warner and J. S. O'Brien, *Biochemistry*, 1979, **18**, 2783.
[48] M. Miyazaki, S. Nishimura, A. Yoshida, and N. Okubo, *Chem. Pharm. Bull.*, 1979, **27**, 532.

3 Diamino-sugars

The synthesis of further analogues of prumycin [4-*N*-(D-alanyl)-2,4-diamino-2,4-dideoxy-L-arabinose] has been reported, namely those with the L-*xylo* and L-*ribo* configurations.[49] The critical intermediates in these syntheses are the corresponding benzyl 2-benzyloxycarbonylamino-4-azido-2,4-dideoxypentopyranosides which are available from benzyl 2-*O*-benzyl-3,4-di-*O*-mesyl-α-D-xylopyranoside by displacement at C-4 by azide followed by formation of the 2,3-anhydride and subsequent ammonolysis and *N*-acylation. Inversion of configuration at C-3 (L-*xylo* → L-*ribo*) may then be accomplished in the usual way. Precursors of the D-*lyxo*-, L-*lyxo*-, and D-*ribo*-analogues of prumycin have also been described.[50]

2-Amino-2-deoxy-D-glucose has been used for the synthesis of a glycoside of 6-epipurpurosamine (2,6-diamino-2,3,4,6,7-pentadeoxy-L-*lyxo*-heptose) which is a component of the antibiotic fortimicin A (Scheme 3).[51] A nine-stage synthesis of methyl α-DL-purpurosaminide B has also been recorded which employed



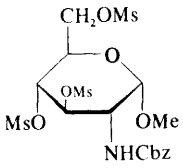
Reagents: i, MsCl–py; ii, NaI–Zn–HOAc; iii, Pd–H_2; iv, DMSO–DCC–pyH^+TfO^-; v, $MeNO_2$–MeO^-; vi, H_2–Ni; vii, CbzCl–NaOH; viii, Me_2CHONa; ix, HOAc; x, NH_3–MeOH; xi, CCl_4–Ph_3P; xii, Bu_3SnH

Scheme 3

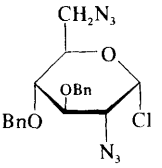
[49] H. Hashimoto, F. Chiba, K. Araki, and J. Yoshimura, *Carbohydr. Res.*, 1979, **72**, 261.
[50] H. Hashimoto, K. Araki, and J. Yoshimura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 3051.
[51] T. Suami, Y. Honda, and T. Kato, *Chem. Lett.*, 1978, 1125.

6-acetyl-2-methoxy-5,6-dihydro-2*H*-pyran as starting material.[52] The methyl α-pyranoside of purpurosamine C (2-amino-6-methylamino-2,3,4,6-tetradeoxy-D-*erythro*-hexose) has been prepared from methyl 2-benzyloxycarbamido-2-deoxy-3,4,6-tri-*O*-mesyl-α-D-glucopyranoside (45) by selective displacement at C-6 by azide and application of the Tipson–Cohen procedure for the establishment of the 3,4-dideoxy-grouping.[53] Synthesis of 3,6-diamino-3,6-dideoxy-D-galactose hydrochloride[54] and of derivatives of 4,6-diamino-4,6-dideoxy-D-gulose[55] have been reported. The synthesis of 2,6′-diamino-2,6′-dideoxy- and 2,6-diamino-2,6-dideoxylactose have also been described.[56]

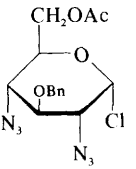
Paulsen's group have employed 2,6-diazido-3,4-di-*O*-benzyl-2,6-dideoxy-α-D-glucopyranosyl chloride (46) and 6-*O*-acetyl-2,4-diazido-3-*O*-benzyl-2,4-dideoxy-α-D-glucopyranosyl chloride (47) for the preparation of 1,3-α-linked disaccharides containing three or four amino-groups.[57] Cyclohepta-amylose has been converted into deca- and undeca-amino derivatives, which was accomplished by selective mesylation with 2.15 equivalents of mesyl chloride per hexose moiety, followed by reaction with sodium azide which gave a cycloamylose composed of 6-azido-glucose units containing further mesyl groups at the 2- and 3-positions. Subsequent treatment of this mixture of products with base afforded two cycloamyloses. One comprised three 6-azido-2,3-anhydrohexose and four 6-azido-glucose moieties. The other contained four of the former and three of the latter. Reaction of each of these with azide anion, followed by reduction, afforded the aforementioned amino-derivatives, which possessed marked anti-microbial activity.[58]



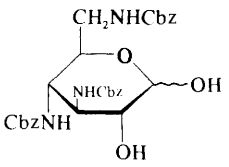
(45)



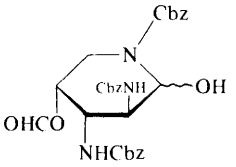
(46)



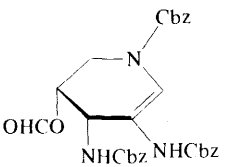
(47)



(49)



(48)



(50)

[52] M. Chmielewski, A. Konowal, and A. Zamojski, *Carbohydr. Res.*, 1979, **70**, 275.
[53] I. Watanabe, T. Deushi, T. Yamaguchi, K. Kamiya, M. Nakayama, and T. Mori, *J. Antibiot.*, 1979, **32**, 1066.
[54] Y. Kondo, M. Harai, and S. Hirano, *Carbohydr. Res.*, 1979, **70**, 155.
[55] Y. Ali and M. Hussain, *Carbohydr. Res.*, 1979, **69**, 311.
[56] I. Jezo and J. Zemek, *Chem. Zvesti*, 1979, **33**, 533.
[57] H. Paulsen, O. Lockhoff, B. Schroder, and W. Stenzel, *Carbohydr. Res.*, 1979, **68**, 239.
[58] K. Tsujihara and H. Kurita, *Chem. Lett.*, 1978, 1333.

2,3,5-Trisbenzylcarbamido-2,3,5-trideoxy-4-*O*-formyl-D-*arabino*-pyranose (48) has been made by periodate oxidation of 3,4,6-trisbenzyloxy-carbamido-3,4,6-trideoxy-D-glucose (49). Attempted conversion into the lactam using the Pfitzner–Moffatt reaction afforded the corresponding glycal (50).[59]

[59] M. Kinoshita, S. Aburaki, Y. Kawada, T. Yamasaki, Y. Suzuki, and Y. Niimura, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 3261.

9

Miscellaneous Nitrogen Derivatives

1 Glycosamines

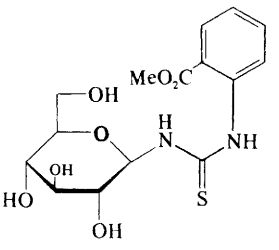
β-Maltosylamine has been prepared crystalline by prolonged exposure of maltose to methanolic ammonia containing ammonium chloride, and it was shown to possess specific inhibitory activity towards sweet potato β-amylose.[1] Furanoid glycosylamines have been similarly prepared from 5-*O*-trityl-D-ribose, -D-arabinose, -D-xylose, and -2-deoxy-D-ribose and are potentially useful in the synthesis of nucleosides.[2]

The pyranoid *N*-*p*-nitrophenyl-glycosylamines derived from either D-ribose or D-lyxose and their *O*-acetylated derivatives have been examined using ^{1}H n.m.r. spectroscopy. The α-anomers, in which the aglycone is axial in the $^4C_1$ conformation, exist either substantially or totally in the $^1C_4$ conformation.[3] A series of glycosylamines derived from the common aldoses and *o*- and *m*-aminobenzaldoximes have been prepared by a fusion procedure. Whilst the *meta*-derivatives were colourless, the *ortho*-isomers were bright yellow. The reason for this would appear to be the more extended resonance possible in the *ortho*-isomer, although the authors suggest it might be attributed to the existence of the nitroso-tautomer.[4] Reaction of D-glucose, D-mannose, and cellobiose with 1,2-diaminoethane afforded bisglycosylamines ($GlyNHCH_2CH_2NHGly$).[5] The synthesis of several glycopeptides containing 2-acetamido-1-*N*-(aspart-4-oyl)-2-deoxy-β-D-glucosylamine units linked to amino-acid sequences corresponding to those of bovine ribonuclease (residues 32–34, 33–35, 33–37, and 33–38) has been reported.[6] Several papers have dealt with the non-enzymic incorporation of hexose units into peptides by *in vivo* incubation. These reactions proceed *via* the formation of a glycosylamine with the ε-amino-groups of either lysine or valine followed by an Amadori rearrangement.[7]

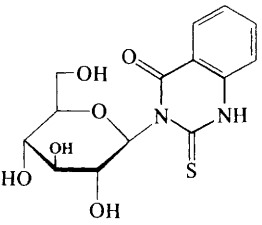
A series of *N*-acyl and related glycosylamines have been prepared from glycosyl isothiocyanates (GlyNCS). For example, simple *N*-acylglycosylamines arise by treatment of the isothiocyanate with the appropriate acid in the

[1] D. E. Walker and B. Axelrod, *Arch. Biochem. Biophys.*, 1979, **195**, 392.
[2] B. L. Kam and N. J. Oppenheimer, *Carbohydr. Res.*, 1979, **77**, 273.
[3] D. Piskorska and J. Sokolowski, *Pol. J. Chem.*, 1979, **53**, 1001; S. Sokolowski and E. Paszkiewicz, *Pol. J. Chem.*, 1979, **53**, 305.
[4] J. Sykulski and J. Czyzewska, *Acta Pol. Pharm.*, 1978, **35**, 169 (*Chem. Abs.*, 1979, **90**, 39 181*t*).
[5] J. M. McLeod, *Carbohydr. Res.*, 1979, **75**, 71.
[6] H. G. Garg and R. W. Jeanloz, *Carbohydr. Res.*, 1979, **70**, 47.
[7] J. F. Day, S. R. Thorpe, and J. W. Baynes, *J. Biol. Chem.*, 1979, **254**, 595; J. F. Day, R. W. Thornburg, S. R. Thorpe, and J. W. Baynes, *J. Biol. Chem.*, 1979, **254**, 9394; H. F. Bunn, R. Shapiro, M. McManus, L. Garrick, M. J. McDonald, P. M. Gallop, and K. H. Gabbay, *J. Biol. Chem.*, 1979, **254**, 3892; R. Dolhoffer and O. W. Wieland, *FEBS Lett.*, 1979, **100**, 133; **103**, 282; H. S. Lee, L. C. Sen, A. J. Clifford, J. R. Whitaker, and R. E. Feeney, *J. Agric. Food Chem.*, 1979, **27**, 1094.

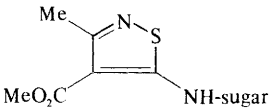
presence of triethylamine,[8] although some di-*N*-glycosyl-ureas (GlyNHCONHGly) and -thioureas were obtained as side products. The addition of a variety of amines to the isothiocyanates afforded the corresponding *N*-glycosylthioureas (GlyNHCSNHR).[9] In the case of the *N*-glycosylthioureas derived from ethyl *o*-aminobenzoate [*e.g.* (1)], subsequent ring close occurred on treatment with zinc chloride giving the thioquinazoline (2).[10] The glycosylisothiocyanates also reacted with ethyl 3-aminocrotonate, giving the corresponding isothiazolines [*e.g.* (3)],[11] and with *o*-phenylenediamine they gave a 1:1 adduct (4) which on treatment with methyl iodide and triethylamine underwent a cyclodesulphurization to give the benzimidazole derivative (5). When 2,3-diaminopyridine was used, the thiourea (6) was obtained which also underwent ring closure to give (7).[12] Similarly, reaction of glycosyl isothiocyanates with hydrazides and hydrazines has been employed in the synthesis of similar heterocyclic *N*-glycosides.[13]



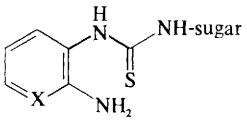
(1)



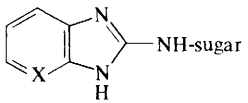
(2)



(3)



(4) X = CH
(6) X = N



(5) X = CH
(7) X = N

[8] S. E. Zurabyan, R. G. Macharadze, and A. Yu. Khorlin, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1979, 877 (*Chem. Abs.*, 1979, **91**, 91 887*n*).

[9] I. Singh, Y. Kishimoto, R. R. Vunnam, and N. S. Radin, *J. Biol. Chem.*, 1979, **254**, 3840; H. Takahashi, N. Nimura, and H. Ogura, *Chem. Pharm. Bull.*, 1969, **27**, 1130; (with R. Takeda) 1137.

[10] H. Takahashi, N. Nimura, and H. Ogura, *Chem. Pharm. Bull.*, 1979, **27**, 1143.

[11] H. Takahashi, N. Nimura, and H. Ogura, *Chem. Pharm. Bull.*, 1979, **27**, 1147.

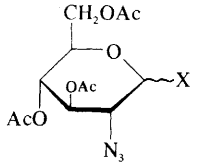
[12] H. Takahashi, N. Nimura, N. Obata, H. Sakai, and H. Ogura, *Chem. Pharm. Bull.*, 1979, **27**, 1153.

[13] H. Ogura, H. Takahashi, and M. Sakaguchi, *Nucleic Acids Res., Spec. Publ.*, 1978, **5** (Symp. Nucleic Acids Chem., 6th) 251 (*Chem. Abs.*, 1979, **90**, 138 131*h*); M. Wojtowicz and W. Wieniawski, *Acta Pol. Pharm.*, 1978, **35**, 37 (*Chem. Abs.*, 1979, **90**, 152 534*y*).

2 Azido-sugars

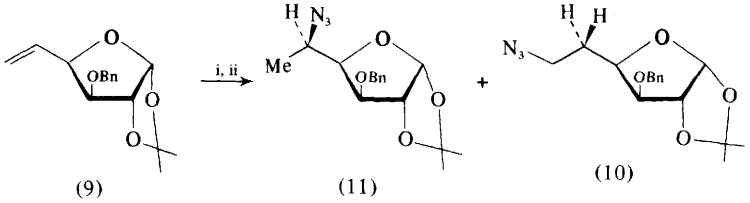
A new synthesis of glycosyl azides has been reported which involves sequential treatment of an aldose, in which all hydroxy-groups other than that at C-1 are blocked (acetals or ethers), with HMPT and mesityleneoxytris(dimethylamino)-phosphonium azide. The glycosyl azides are usually formed in overall yields of 65–80% yields and the 1,2-*cis*-isomers are formed exclusively. The method has been applied successfully to 2,3 : 5,6-di-*O*-isopropylidene-D-mannofuranose and its *allo*-isomer, 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranose, 2,3 : 4,6-di-*O*-benzylidene-D-mannopyranose, and 2,3,5-tri-*O*-benzyl-D-arabinofuranose. The products are useful for the synthesis of nucleoside analogues.[14]

A search for an improved synthesis of α-D-galactosaminides has prompted studies on the synthesis of 2-azido-2-deoxy-D-galactose derivatives, such as (8), by addition of the pseudohalogens N_3Cl, N_3Br, and N_3ONO_2 to tri-*O*-acetyl-D-galactal. In all cases the *galacto*-isomer predominated.[15]



(8) X = Cl, Br, or ONO_2

Application of the azidomercuration–demercuration procedure to the 5-ene (9) gave the 6- and 5-azides (10) and (11) in the ratio 1:4 (Scheme 1).[16] The synthesis of azido-sugars *via* 2,3-anhydro-furanosides has been reported. Methyl 2,3-anhydro-α-D-lyxofuranoside (12) gave the 3-azide (13) in 95% yield, and its 5-*O*-tosyl ester (14) gave the 3,6-diazide (15) in 84% yield. In contrast, the 2,3-anhydro-riboside (16) gave a mixture of the 2- and 3-azides (17) and (18) in the ratio 3:2. However, substitution at C-5 of (16) resulted in steric hindrance of the approach to C-3 and consequently the 5-tosylate (19) afforded the 2,6-

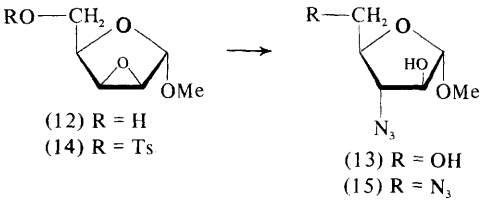


(9) (11) (10)

Reagents: i, $Hg(OCOCF_3)_2$, NaN_3; ii, $NaBH_4$

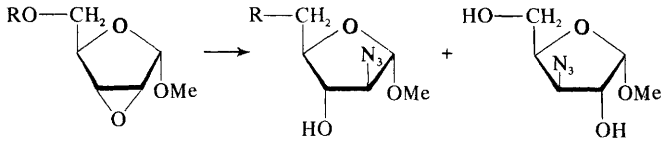
Scheme 1

[14] F. Chretien, B. Castro, and B. Gross, *Synthesis*, 1979, 937.
[15] R. U. Lemieux and R. M. Ratcliffe, *Can. J. Chem.*, 1979, **57**, 1244; N. V. Bovin, S. E. Zurabyan, and A. Yu. Khorlin, *Bioorg. Khim.*, 1979, **5**, 1257.
[16] S. Czernecki, C. Georgoulis, and C. Provelenghiou, *Tetrahedron Lett.*, 1979, 4841.



(12) R = H
(14) R = Ts

(13) R = OH
(15) R = N_3



(16) R = H
(19) R = Ts
(21) R = Bz

(17) R = OH
(20) R = N_3
(22) R = OBz

(18)

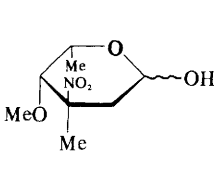
diazide (20) in 84% yield and the 5-*O*-benzoate (21) gave the 2-azide (22) in 85% yield.[17] The synthesis of 2-azido-2-deoxy-D-arabinose by periodate oxidation of 3-azido-6-*O*-benzoyl-3-deoxy-D-glucose has been reported.[18]

3 Nitro-sugars

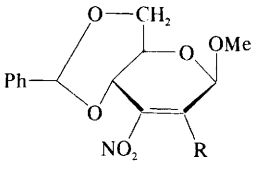
A review has been published on the influence of configuration and conformation on the reactivity of nitro-derivatives of carbohydrates.[19] A new naturally occurring nitro-sugar, L-rubranitrose (3-*C*-methyl-4-*O*-methyl-3-nitro-2,3,6-trideoxy-L-*xylo*-hexose) (23) has been isolated from the hydrolysate of the antibiotic rubradirin.[20]

Pyrazolones [*e.g.* (24)] are derived from 3-nitro-2-enes [*e.g.* (25)] by reaction with diazomethane. In the case of (25) and its α-anomer the preferred attack by reagent is on the α-face of the molecule to give, for example, (24). However, the C-4 epimers gave products resulting from addition to the β-face of the molecule suggesting that the 4-substituent plays a major directive role. The pyrazolones on heating eliminated nitrogen to give either the 2-*C*-ethyl-2-ene (26) [from (24)] or the cyclopropane derivative (27) (from the β-*threo*-isomer).[21] Reaction of the 3-nitro-2-ene [(25) α-anomer] with the *S*-ylide $Me_2SCHCOPh$ afforded, as previously noted, the isoxazoline *N*-oxide (28), but this has now been shown to react further to give the open-chain derivative (29).[22]

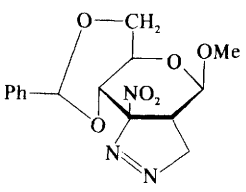
[17] F. M. Unger, R. Christian, and P. Waldstätten, *Carbohydr. Res.*, 1979, **69**, 71.
[18] M. Bobek, *Carbohydr. Res.*, 1979, **70**, 263.
[19] H. Baer, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, **6**, 51.
[20] S. A. Mizsak, H. Hoeksema, and L. M. Pschigoda, *J. Antibiot.*, 1979, **32**, 771.
[21] H. H. Baer, F. Linhart, and H. R. Hanna, *Can. J. Chem.*, 1978, **56**, 3087.
[22] T. Sakakibara and R. Sudoh, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 3401.



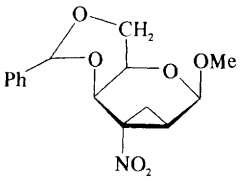
(23)



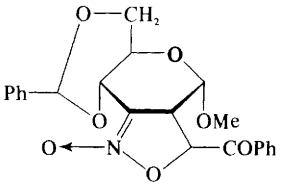
(25) R = H
(26) R = Me



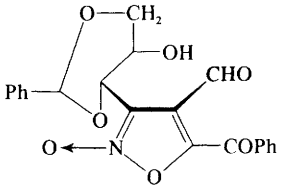
(24)



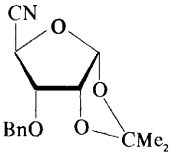
(27)



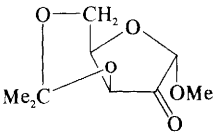
(28)



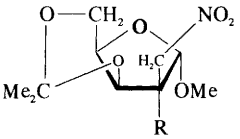
(29)



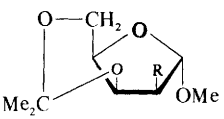
(30)



(31)



(32) R = OH
(33) R = H



(34) R = CBr_2NO_2
(35) R = CN

4 Cyano- and Isocyano-sugars

Where etherified glycosyl halides react with mercuric cyanide to give the glycosyl cyanides, they give the corresponding glycosyl isocyanides when silver cyanide is used. Mild acid hydrolysis of these isocyanides afforded the corresponding *N*-formylglycosylamine from which the isocyanide may be regenerated by reaction with phosphoryl chloride.[23] The furanurononitrile (30) and several related nitriles have been synthesized from the corresponding pentadialdo-1,4-furanoses by dehydration of the derived oximes.[24] The 2-cyano-2-deoxy-furanoside (35) has been synthesized from the 2-ulose (31) by condensation

[23] P. Boullanger, D. Marmet, and G. Descotes, *Tetrahedron*, 1979, 163.
[24] A. J. Brink, O. G. de Villiers, and A. Jordaan, *S. Afr. J. Chem.*, 1978, **31**, 59 (*Chem. Abs.*, 1979, **91**, 57 362).

with nitromethane to give the adduct (32) followed by *O*-acetylation and reduction with sodium borohydride to give the 2-deoxy-2-*C*-nitromethylfuranose (33). Conversion of (33) into a dibromo derivative (34) and reaction with triphenylphosphine gave (35).[24] The authors of this paper note that the Cotton effects displayed by (32) and (33) do not obey the rule previously proposed for acyclic nitromethyl derivatives. A new twist to the chain elongation method using cyanide has been described[25] involving reaction of acetylated diethyl dithioacetals with iodine cyanide, which affords 2-deoxy-2-ethylthio-aldononitriles, which on reductive desulphurization affords 2-deoxy-aldonitriles. Several 2-alkylamino-2-deoxy-aldononitriles have been prepared by reaction of the appropriate *N*-alkylglycosylamine with hydrogen cyanide. The reaction appears to be highly stereospecific and affords the D-*glycero*-L-*gluco*-, D-*glycero*-D-*ido*-, and D-*glycero*-D-*galacto*-heptononitriles in yields of 75–89% starting with *N*-ethyl-β-D-galactopyranosylamine and the D-*gluco* and D-*manno*-analogues respectively. Similar yields are also achieved by reaction of the appropriate hexose with a mixture of the amine and hydrogen cyanide.[26]

5 Hydrazones, Osazones, and Related Derivatives

The structure of dehydro-L-ascorbic acid bisphenylhydrazide has been shown to be (36) by i.r. and u.v. studies.[27] The rate of acid-catalysed elimination of *p*-nitroaniline from *p*-nitrophenylhydrazones in aqueous and non-aqueous media has been studied in connection with osazone formation. The rate of reaction is proportional to the concentration of acid and the *p*-nitro-group tends to retard the reaction. Higher yields of osazones are obtained in non-aqueous media.[28]

The structures of hydrazones derived from D-glucose, D-mannose, D-arabinose, D-ribose, sodium D-glucuronate, D-glucofuranurono-3,6-lactone, and D-glyceraldehyde with isonicotinyl hydrazide and other hydrazides and hydrazines have been examined in solution by ^{13}C- and ^{1}H-n.m.r., and the rate of mutarotation and interconversion with acyclic forms has been correlated with the basicity of the hydrazide or hydrazine used.[29] The *p*-acetamidobenzoylhydrazones of D-mannose, D-arabinose, D-xylose, D-galactose, and lactose have been described. The *galacto*-hydrazone undergoes cyclization on boiling with acetic anhydride to give (37).[30] Similarly, hydrazones derived from phenylacetylhydrazide are described and similarly undergo cyclization into products analogous to (37).[31]

[25] P. Herczegh, R. Bognar, and E. Timar, *Org. Prep. Proceed. Int.*, 1978, **10**, 211 (*Chem. Abs.*, 1978, **89**, 215 676*y*).

[26] M. Gomez Guillen, J. A. Galbis Perez, J. I. Remon Alvarez-Arenas, and J. L. Jimenez Requejo, *An. Quim.*, 1978, **74**, 651 (*Chem. Abs.*, 1979, **90**, 23 464*b*); M. Gomez Guillen, J. A. Galbis Perez, P. Areces Bravo, J. I. Fernandez Garcia-Hierro, M. Avalos-Gonzalez, and J. M. Ramirez Fernandez, *An. Quim.*, 1978, **74**, 633 (*Chem. Abs.*, 1979, **90**, 72 406*t*).

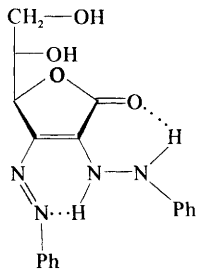
[27] G. A. F. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1979, 603.

[28] L. Rateb, S. Delgado, and H. Simon, *Indian J. Chem., Sect. B.*, 1978, **16B**, 469 (*Chem. Abs.*, 1979, **90**, 55 175*z*).

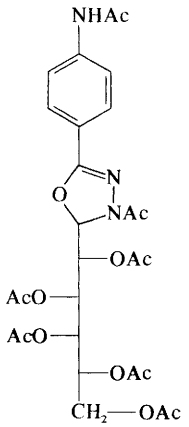
[29] Y. Takeda, *Carbohydr. Res.*, 1979, **77**, 9.

[30] E. S. H. El Ashry, R. Soliman, and K. Mackawy, *Carbohydr. Res.*, 1979, **72**, 305.

[31] M. M. Abdel Rahman, E. S. H. El Ashry, A. A. Abdallah, and N. Rashed, *Carbohydr. Res.*, 1979, **73**, 103.

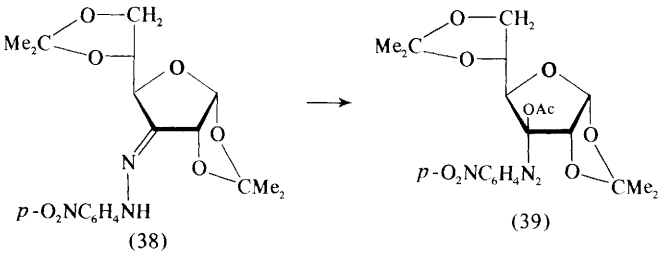


(36)



(37)

Oxidation of the *p*-nitrophenylhydrazine (38) derived from 1,2 : 5,6-di-*O*-isopropylidene-α-D-*ribo*-hex-3-ulose with lead tetra-acetate gave the 3-*C*-(*p*-nitrophenyldiazo)glucofuranose (39) exclusively, with none of the *allo*-isomer. A number of other hydrazones were similarly oxidized, the reactions in most cases showing a high degree of stereoselectivity.[32]



(38) (39)

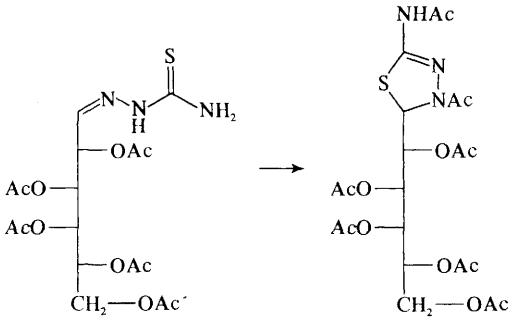
The *O*-methyloximes of several aldoses have been examined in solution by ^{13}C-n.m.r. and by g.l.c. and the authors conclude that the *O*-methyl oximes exist as acyclic modifications. Those derived from simple aldoses existed as the *anti*-isomer, whereas those derived from 2-deoxyaldoses existed as *anti*- and *syn*-mixtures.[33] The oxime of D-glucose, however, exists as a mixture of the *syn*- and *anti*-forms and the two pyranose forms. All four isomers have been separated by t.l.c. and identified spectroscopically.[34]

[32] J. M. J. Tronchet, F. Rachidzadeh, J. Tronchet, and C. Cottet, *Carbohydr. Res.*, 1979, **69**, 287.

[33] W. Funke and C. von Sonntag, *Carbohydr. Res.*, 1979, **69**, 247.

[34] M. Hranisavljevic-Jakovljevic, J. Miljkovic-Stojanovic, V. Dzaja-Erceg, and R. Dimitrijevic, *Glas. Hem. Drus. Beograd.*, 1978, **43**, 487 (*Chem. Abs.*, 1979, **90**, 152 489*h*).

Acetylation of D-galactose semicarbazone gave the penta-*O*-acetate, but prolongation of the reaction led to cleavage of the N–C(S) bond to give the *N*,*N*-diacetylhydrazone penta-*O*-acetate from which penta-*O*-acetyl-*aldehydo*-D-galactose could be obtained by reaction with nitrous acid. Reaction of the thiosemicarbazone penta-*O*-acetate (40) with acetic anhydride–zinc chloride afforded the cyclic derivative (41), a thioheterocyclic analogue of (37).[35]



(40) (41)

6 Heterocyclic Derivatives

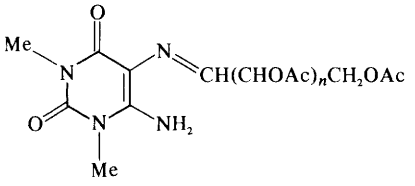
Several nitrogen-containing heterocyclic derivatives with alditol and other carbohydrate side chains have been reported in connection with the synthesis of analogues of nucleosides. For example, the Schiff base (42) formed by condensation of aldoses with 5,6-diamino-1,3-dimethyluracil, cyclizes in the presence of mercuric chloride to give the theophylline derivative (43).[36] Similarly, sugar aldehydes such as (44) undergo condensation with *O*-phenylenediamine followed by air oxidation to the 3-substituted benzimidazole (45). However, the α,β-unsaturated branched-chain aldehyde (46) undergoes 1,3-addition of the diamine to give the benzodiazepine (47) rather than the benzimidazole. An explanation for this behaviour was provided by the ^{13}C n.m.r. parameters which revealed an extraordinary low electron density on C-3, whereas the α,β-unsaturated aldehyde (48), which reacts with *O*-phenylenediamine normally, possessed a considerably higher electron density on C-3.[37]

Horton's group have extended their studies on acyclic nucleoside analogues by condensing 1-bromo-1-*S*-ethyl-1-thioalditols with the appropriate nucleoside bases to give the derivatives (49) and (50). The 6-mercaptopurine derivative (51) was made from (49) by a standard reaction. In all cases except one, a single

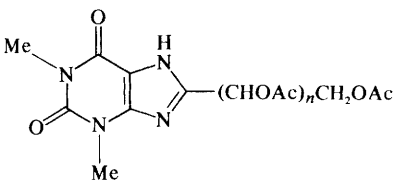
[35] L. Somogyi, *Carbohydr. Res.*, 1979, **75**, 325.

[36] H. Ogura, M. Sakaguchi, T. Okamoto, K. Gonda, and S. Koga, *Heterocycles*, 1979, **12**, 359 (*Chem. Abs.*, 1979, **91**, 39 771*f*); M. Sakaguchi, Y. Miyata, H. Ogura, K. Gonda, S. Koga, and T. Okamoto, *Chem. Pharm. Bull.*, 1979, **27**, 1094.

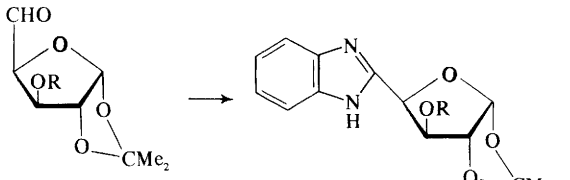
[37] J. M. J. Tronchet and B. Gentile, *Helv. Chim. Acta*, 1979, **62**, 1298.



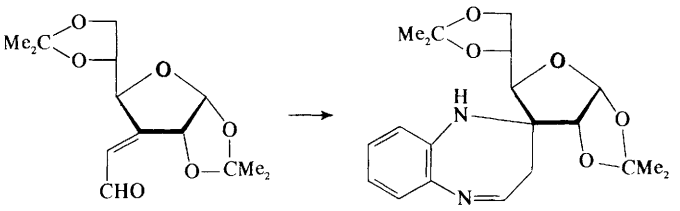
(42)



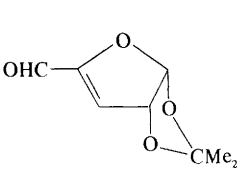
(43)



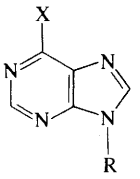
(44) (45)



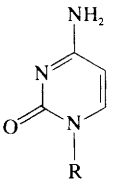
(46) (47)



(48)

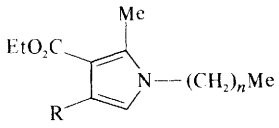


(49) X = Cl
(51) X = SH
R = CH(SEt)(CHOH)$_n$CH$_2$OH

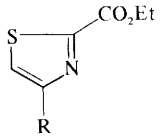


(50)

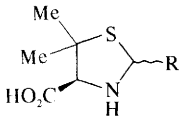
1′-epimer was encountered.[38] Condensation of various 1-alkylamino-1-deoxy-D-*arabino*-hexuloses with ethyl acetoacetate afforded a series of pyrrole derivatives with a D-*arabino*-hexityl side chain at C-3 [*e.g.* (52)][39] and condensation of 1-bromo-1-deoxy-D-*ribo*-hexulose with $HSC(=NH)CO_2Et$ afforded the thiazole (53).[40] Cyclization of D-penicillamine with 2,3,4,5-tetra-*O*-acetyl-*aldehydo*-L-arabinose afforded, after *O*-deacetylation the thiazolidine (54) as a mixture of two diastereoisomers.[41] The tetrazoles (55) are prepared by condensation of the appropriate benzoylated aldononitrile with ammonium azide, followed by *O*-debenzoylation.[42]



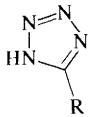
(52)



(53)



(54)



(55)

R = $(CHOH)_nCH_2OH$

A series of 5-amino-3-glycosylisoxazolines [*e.g.* (56)] has been synthesized from the corresponding *aldehydo*-sugar derivatives by reaction with the Wittig reagent $Ph_3PC(Br)CN$, followed by condensation with hydroxylamine. The corresponding 3-amino-5-glycosylisoxazoles were obtained when hydroxyurea was used instead of hydroxylamine.[43] The uridine derivative (57) was obtained from condensation of 2,3-*O*-isopropylidene-D-glyceraldehyde with ethyl acetoacetate and urea (Biguinelli reaction). The related compound (58) was obtained by base-catalysed condensation of ethyl 2-deoxy-4,5-*O*-isopropylidene-D-*glycero*-

[38] D. C. Baker and D. Horton, *Carbohydr. Res.*, 1979, **69**, 117; D. C. Baker, K. Bliesznerand D. Horton, *Nucleic Acid Chem.*, 1978, **2**, 627 (*Chem. Abs.*, 1979, **90**, 72 416*w*); D. Horton and S. S. Kokrady, *Nucleic Acid Chem.*, 1978, **1**, 267 (*Chem. Abs.*, 1979, **90**, 39 173*s*).

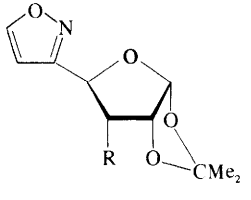
[39] F. Garcia-Gonzalez, J. Fernandez-Bolanos, G. Martin Jimenez de la Plata, N. Lopez Partida, and I. Robina Ramirez, *An. Quim.*, 1978, **74**, 1281 (*Chem. Abs.*, 1979, **91**, 108 169*e*).

[40] T. Vanek, J. Farkas, and J. Gut, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1339.

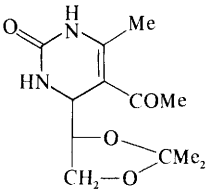
[41] R. Bognar, Z. Gyorgydeak, L. Szilagyi, P. Sandor, and L. Radics, *Justus Liebigs Ann. Chem.*, 1979, 701.

[42] O. G. Marzoa, I. M. E. Thiel, and J. O. Deferrari, *Carbohydr. Res.*, 1979, **73**, 323.

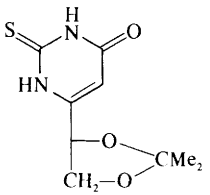
[43] J. M. J. Tronchet, O. R. Martin, A. Grouiller, and N. Sarda, *Helv. Chim. Acta*, 1979, **62**, 833.



(56)



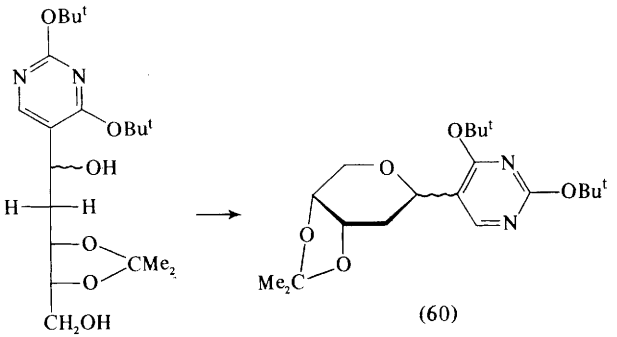
(57)



(58)

pent-3-ulosonate with thiourea. Several other related uridines were similarly prepared.[44]

Several adenines substituted at the 9-position with 2,3,4-trihydroxybutyl groups have been synthesized using the sodium salt of adenine as a nucleophilic species and a suitable sulphonate ester of an alditol.[45] Condensation of the 5-lithiated derivative of 2,4-di-*O*-t-butyluracil with 3,4-*O*-isopropylidene-2-deoxy-D-ribose afforded (59) which on selective tosylation at the primary hydroxy-group and treatment with base gave the 5-(2-deoxy-3,4-*O*-isopropylidene-D-*erythro*-pentopyranosyl)-2,4-di-*O*-t-butyluracil (60).[46] The synthesis of 3,6-anhydro-1,2-dideoxy-1-(uracil-1-yl)-D-*arabino*-hexitol (61) and related compounds have been carried out by alkylation of the appropriate uracil with 3,6-anhydro-2-deoxy-4,5-*O*-isopropylidene-1-*O*-tosyl-D-*arabino*-hexitol.[47] Reaction of sugar lactones with lithiated heterocycles has yielded a series of *C*-nucleoside lactols [*e.g.* (62)], which undergo reduction with borohydride to give the corre-



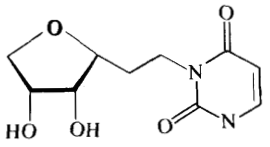
(59) (60)

[44] F. J. Lopez Aparicio and F. J. Lopez Herrera, *Carbohydr. Res.*, 1979, **69**, 243; (with M. Valpuesta Fernandez) 235.

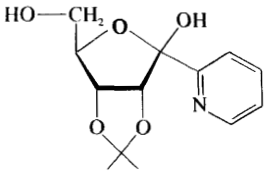
[45] A. Holy, *Collect. Czech Chem. Commun.*, 1979, **44**, 593; *Nucleic Acids Res., Spec. Publ.*, 1978, **4**, (Symp. Chem. Nucleic Acid, 4th) 13 (*Chem. Abs.*, 1979, **90**, 104 264*p*).

[46] D. M. Brown, S. D. Bridges, R. C. Ogden, and R. P. L. Conrad, in ref. 45, p. 121 (*Chem. Abs.*, 1979, **90**, 87 809*a*).

[47] V. Cavrini, L. Garuti, G. Giovanninetti, M. Amarosa, A. Mannini Palenzona, and J. Defaye, *Farmaco Ed. Sci.*, 1979, **34**, 635 (*Chem. Abs.*, 1979, **91**, 175 655*y*).



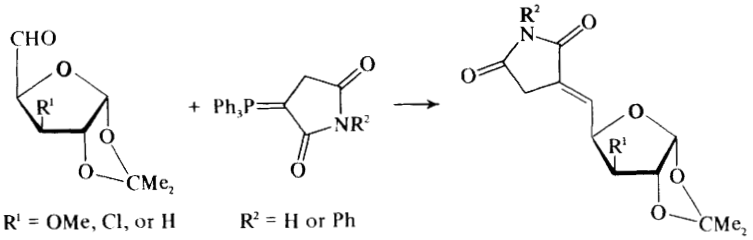
(61)



(62)

sponding *C*-heterocyclic alditol derivative.[48] The reaction of 2-deoxy-D-ribose with purines and pyrimidines in aqueous solution gives 2,3-dideoxy-3-(1′-pyrimidino)-pentose and 2,3-dideoxy-3-(9′-purino)-pentose as major products. It was suggested that 2-deoxyribose first undergoes dehydration to give 2,3-dideoxy-D-*glycero*-pent-2-enose followed by a Michael-type addition to C-3 of the base.[49]

A number of analogues of showdomycin have been prepared from aldehyde sugars by the Wittig reaction illustrated for xylodialdose derivatives in Scheme 2.[50]



R^1 = OMe, Cl, or H R^2 = H or Ph

Scheme 2

[48] H. Ogura, K. Furuhata, and H. Takahashi, *Nucleic Acids Res., Spec. Publ.*, 1977, **3**, 23 (*Chem. Abs.*, 1978, **89**, 75 429*t*).

[49] G. L. Neisestuen, *Biochemistry*, 1979, **18**, 2843.

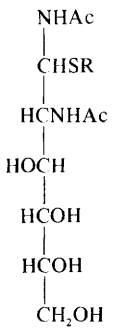
[50] J. M. J. Tronchet and B. Gentile, *Helv. Chim. Acta*, 1979, **62**, 977.

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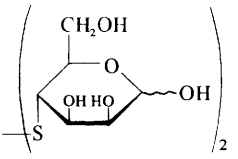
Thio- and Phosphoro-sugars

Reviews on the preparation, properties, and reactions of carbohydrates containing sulphur[1] and on chiral sulphur in carbohydrates[2] have appeared.

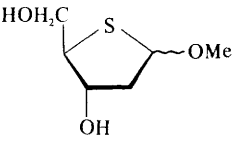
2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine with, respectively, ethanethiol and benzylthiol gave the ring-opened products (1) and (2).[3]



(1) R = Et
(2) R = $PhCH_2$

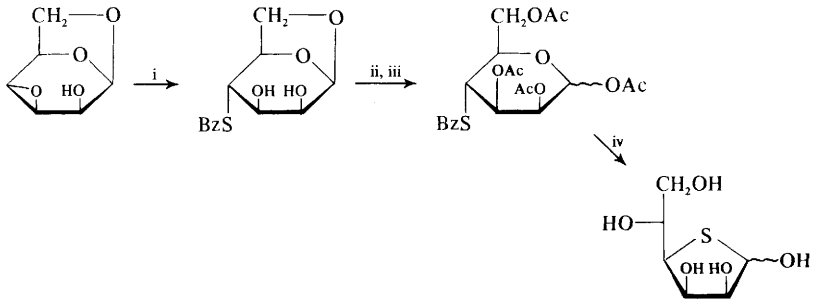


(4)



(5)

4-Thiomannose (3) has been synthesized from 1,6 : 3,4-dianhydro-β-D-talopyranose in four steps (Scheme 1). When methanolic sodium methoxide was used in the absence of oxygen the thio-sugar (3) was converted into the



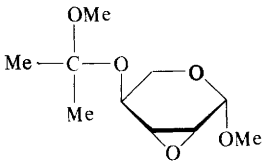
(3)

Reagents: i, pyridinium thiolbenzoate–DMF; ii, Ac_2O–py; iii, H_2SO_4–Ac_2O–HOAc; iv, MeOH–NaOMe

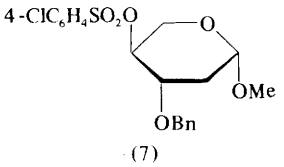
Scheme 1

[1] J. R. Daniel, R. L. Whistler, and R. A. Zingaro, *Phosphorus, Sulphur*, 1979, **7**, 31 (*Chem. Abs.*, 1979, **91**, 141 112*d*).
[2] R. L. Whistler and K. K. De, *J. Carbohydr., Nucleosides Nucleotides*, 1979, **6**, 199.
[3] B. Paul and W. Korytnyk, *Carbohydr. Res.*, 1979, **72**, 247.

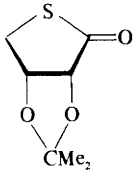
disulphide (4).[4] The methyl glycosides of 2-deoxy-4-thio-*erythro*-pentose (5) have been synthesized in nine steps from methyl 2-*O*-benzyl-3-*O*-tosyl-β-L-arabinopyranoside. Key steps in the synthesis involved cleavage of the anhydrolyxoside (6) with lithium aluminium hydride and displacement of the sulphonate ester group from the deoxyglycoside (7) with thioacetate ion.[5] Evidence has been presented that aldonolactones undergo alkyl oxygen fission with thionucleophiles. Thus 2,3-*O*-isopropylidene-D-erythrono-1,4-lactone gives the 1,4-thiolactone (8) on treatment with potassium thioacetate in DMF followed



(6)

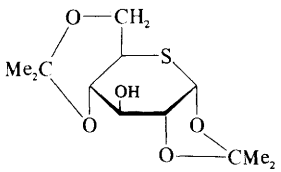


(7)



(8)

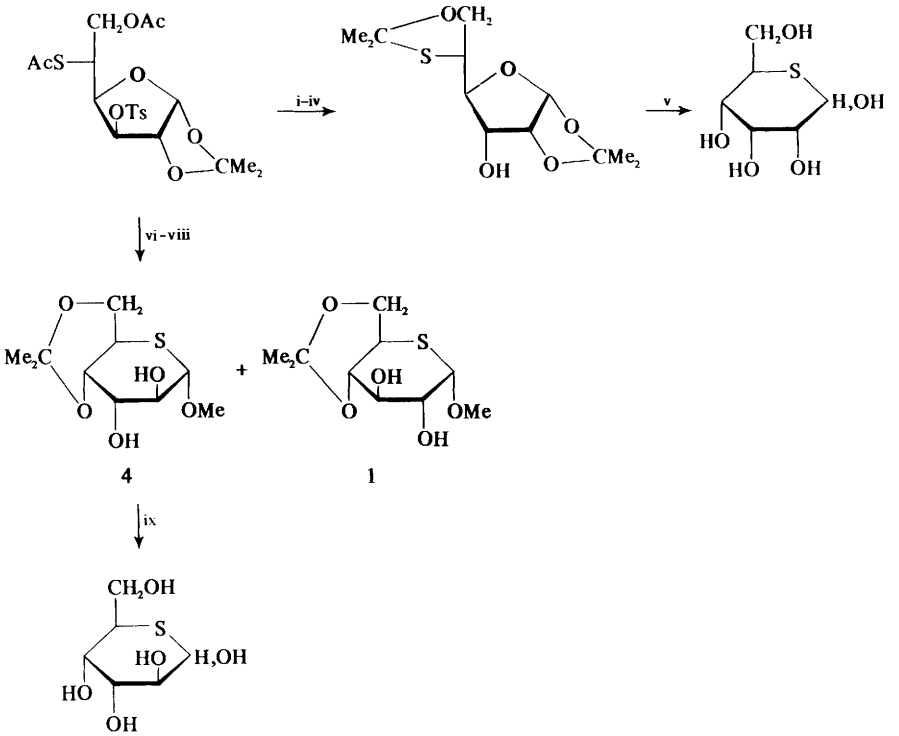
by distillation.[6] Reaction of 5-thio-D-glucose with acetone in the presence of acid gave 1,2 : 5,6-di-*O*-isopropylidene-5-thio-α-D-glucofuranose, whereas a mixture of acetone and dimethoxypropane gave 2,3 : 4,6-di-*O*-isopropylidene-5-thio-α-D-glucopyranose, the structure of which was confirmed by *X*-ray crystallography. On treatment with acid and acetone the latter isomerized to the 1,2 : 5,6-diacetal. 5-Thio-3-*O*-tosyl-α,β-D-glucopyranose with acetone–dimethoxypropane followed by sodium methoxide gave the diacetal (9), which also rearranged to



(9)

the 1,2 : 5,6-diacetal on treatment with acid and acetone.[7] Syntheses of 5-thio-D-allose and -D-altrose,[8] and of 5-thio-D-galactose and its anomeric methyl glycosides[9] have been achieved using the routes shown in Schemes 2 and 3. Also prepared was 2,3,4,6-tetra-*O*-acetyl-5-thio-α-D-galactopyranosyl bromide.[9]

[4] R. H. Shah, J. L. Bose, and O. P. Bahl, *Carbohydr. Res.*, 1979, **77**, 107.
[5] Y.-L. Fu and M. Bobek, *Nucleic Acid Chem.*, 1978, **1**, 183 (*Chem. Abs.*, 1979, **90**, 23 593*t*).
[6] J. N. Dominguez and L. N. Owen, *Carbohydr. Res.*, 1979, **75**, 101.
[7] W. Clegg, N. A. Hughes, and N. Al-Masoudi, *J. Chem. Soc., Chem. Commun.*, 1979, 320.
[8] N. A. Hughes, *J. Chem. Soc., Chem. Commun.*, 1979, 319.
[9] J. E. N. Shin and A. S. Perlin, *Carbohydr. Res.*, 1979, **76**, 165.



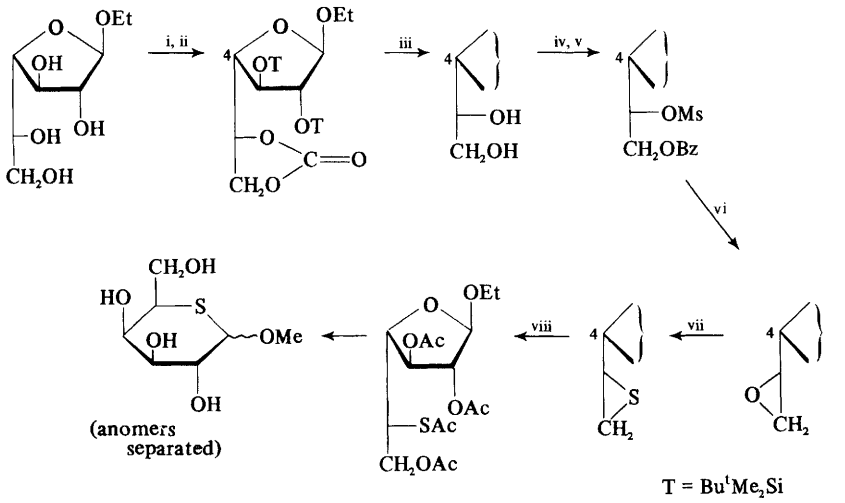
Reagents: i, MeOH–TsOH–$Me_2C(OMe)_2$; ii, MeOH–OH^-; iii, Ac_2O–DMSO; iv, $NaBH_4$; v, H^+; vi, MeOH–HCl; vii, Me_2CO–H^+; viii, OH^-; ix, H_3O^+

Scheme 2

A study on the anomeric ratio and preponderance of sulphur in the sugar ring in 5- and 6-thio-D-fructose has been carried out using ^{13}C and ^1H n.m.r. in D_2O and proton n.m.r. of hydroxy-groups in DMSO. The results show that the anomeric ratio is about 9:1 in favour of the β-anomers for the sulphur ring forms, and that the conformations are the same as for the oxygen analogues.[10] 6-Thio-D-fructose has also been studied to determine ring-opening reaction rates for the pyranose form and the positions of equilibria in the reactions involved.[11]

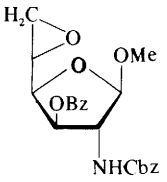
Some derivatives of 2-amino-2-deoxy-5-thio-sugars have been synthesized *via* the key intermediate (10) prepared by treatment of the 5,6-anhydro-sugar (11) with thiourea.[12] Intramolecular cyclizations of *O*-tosyl derivatives of D-ribose, D-arabinose, and D-glucose dithioacetals have been used to obtain 4-thiofuranoses and 5-thiopyranoses illustrated for an idopyranose derivative in Scheme 4. New acetonides of D-ribose diethyl dithioacetal and D-xylose diethyl dithioacetal,

[10] M. Chmielewski and R. L. Whistler, *Carbohydr. Res.*, 1979, **69**, 259.
[11] C. E. Grimshaw, R. L. Whistler, and W. W. Cleland, *J. Am. Chem. Soc.*, 1979, **101**, 1521.
[12] A. Hasegawa, E. Tanahashi, S. Murakami, and M. Koso, *Gifu Daigaku Nogakubu, Kenkyu Hokoku*, 1978, 133 (*Chem. Abs.*, 1979, **91**, 91 892*k*).

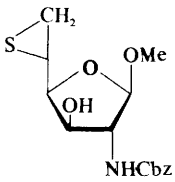


Reagents: i, CH_2O, CH_2O, C=O; ii, Bu^tMe_2SiCl; iii, BaOMe; iv, BzCl; v, MsCl; vi, NaOMe; vii, NH_2CNH_2; vii, NaOAc

Scheme 3



(10)



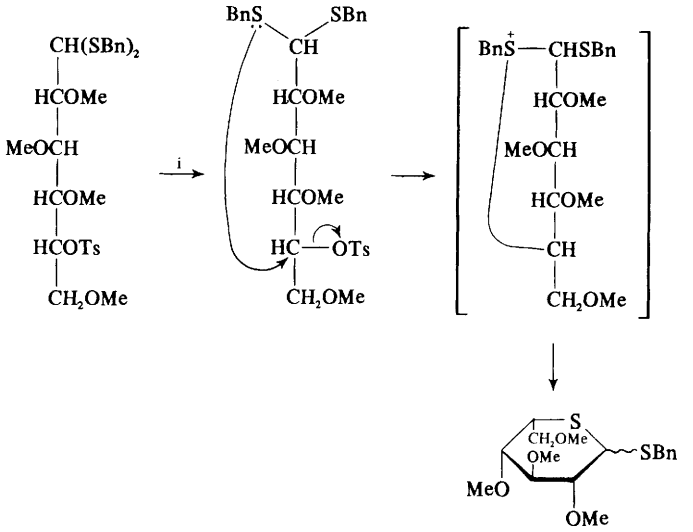
(11)

namely the respective 2,4 : 3,5-di-*O*-isopropylidene derivatives were obtained as the second product of prolonged treatment of the parent dithioacetal with acetone in the presence of copper(II) sulphate.[13]

Ring expansion products of deamination reactions of amino-sugar cyclic diethioacetals are mentioned in Chapter 8.

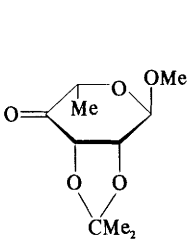
Phosphinates and phosphonates have been added to the carbonyl groups of the L-oxosugar (12) to give the α-hydroxyphosphinates (13) to (15). The

[13] K. Blumberg, A. Fuccello, and T. Van Es, *Carbohydr. Res.*, 1979, **70**, 217.

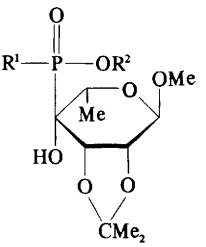


Reagents: i, NaI–Me_2CO

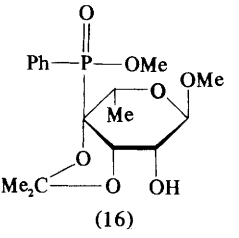
Scheme 4



(12)



(13) R^1 = Ph, R^2 = Me
(14) R^1 = OMe, R^2 = Me
(15) R^1 = OEt, R^2 = Et

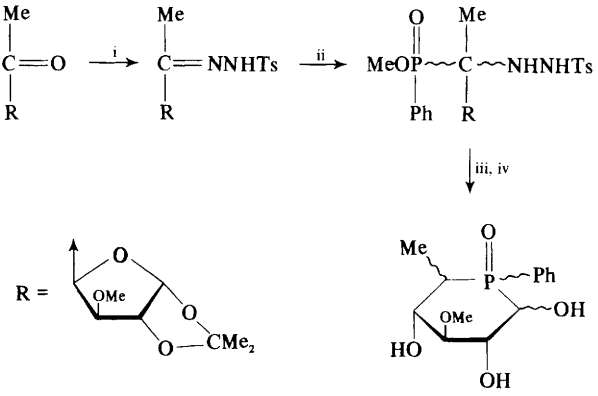


(16)

product (13) was deacetalated by 4 M-HCl in methanol. Re-isopropylidenation gave (13) and the 3,4-*O*-isopropylidene compound (16).[14] The phosphorus-ring sugar (17) has been synthesized by the route shown in Scheme 5.[15]

Ethanethio-derivatives of *C*-nucleosides (and anhydro-nucleoside analogues containing selenium bridges) are described in Chapter 19.

[14] P. T. Long, M. Yamashita, and S. Inokawa, *Carbohydr. Res.*, 1979, **76**, C4.
[15] M. Yamashita, Y. Nakatsukasa, H. Yoshida, T. Ogata, S. Inokawa, K. Hirotsu, and J. Clardy, *Carbohydr. Res.*, 1979, **70**, 247.



(17)

Reagents: i, $TsNHNH_2$–MeOH; ii, $HP(O)(OMe)Ph$; iii, $NaBH_4$; iv, HCl

Scheme 5

11

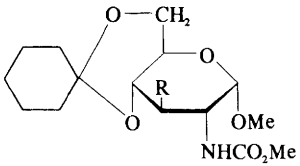
Deoxy-sugars

The *X*-ray crystal structure of the ionophorous antibiotic carriomycin shows that it contains a single carbohydrate component, 2,3,6-trideoxy-4-*O*-methyl-D-*erythro*-hexose,[1] and variose, a component of variamycin, has been shown to be D-cymarose (2,6-dideoxy-3-*O*-methyl-D-*ribo*-hexose) rather than the previously supposed 4-*O*-methyl ether. This confusion arose because the methyl glycoside formed by methanolysis of the antibiotic was erroneously assumed to be a pyranoside, and this led to the incorrect assignment of the n.m.r. spectrum.[2]

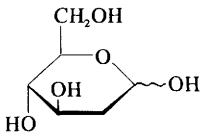
Several new procedures for the introduction of deoxy-groups into sugar moieties have been described. Triflates (trifluoromethanesulphonates) undergo conversion to deoxy-derivatives on reduction with sodium in liquid ammonia, or better, on photolysis. For example, methyl 4,6-*O*-cyclohexylidene-2-deoxy-2-methoxycarbamido-α-D-glucopyranoside 3-triflate (1) afforded the 3-deoxy-derivative (2) in ~80% yield by either procedure, whereas 1,2 : 5,6-di-*O*-isopropylidene-3-*O*-triflyl-α-D-glucofuranose gave the 3-deoxy-derivative under conditions of photolysis only. In the case of methyl 6-deoxy-6-toluene-*p*-sulphonamido-2-*O*-triflyl-α-D-glucopyranoside, reduction with sodium in liquid ammonia resulted in ring contraction giving methyl 5-amino-2,5-dideoxy-2-*C*-hydroxymethyl-α-D-ribofuranoside in 45% yield. The required 2-deoxy-pyranoside was isolated in only 9% yield.[3] In aqueous HMPT the 3-*O*-acetate (4) was converted into the 3-deoxy-pyranoside (2) by photolysis in good yield, whereas the corresponding tosylate (3) was merely converted to the corresponding alcohol (5).[4] Binkley has reported further examples of the photolytic reductive deiodination of iodo-compounds, and in most cases the yields were excellent.[5]

A new method for the direct conversion ROH → RH involves reduction of the oxyphosphonium salts [$ROP^{+}(NMe_2)_3Cl^{-}$] with lithium triethylborohydride. The salts are easily made by treatment of the alcohol with hexamethylphosphorous triamide in carbon tetrachloride, which functions as $ClP^{+}(NMe_2)_3CCl_3^{-}$. The reaction is highly selective for primary hydroxy-groups which may be selectively deoxygenated in the presence of secondary hydroxy-groups.[6] The reductive deoxygenation of alcohols by treatment of the derived thiocarbamate ($ROCSNEt_2$) with potassium solubilized by 18-crown-6 in t-butylamine has been applied to 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose,[7] but the yield of

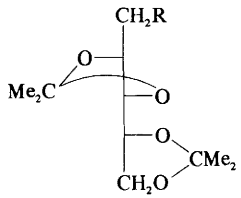
[1] H. Nakayama, N. Otake, H. Miyamae, S. Sato, and U. Saito, *J. Chem. Soc., Perkin Trans. 2*, 1979, 293.
[2] J. S. Brimacombe, Z. Al-Hasan, and A. S. Mengech, *Carbohydr. Res.*, 1979, **72**, C6.
[3] T. Tsuchiya, F. Nakamura, and S. Umezawa, *Tetrahedron Lett.*, 1979, 2805.
[4] T. Kishi, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 3015.
[5] R. W. Binkley and D. G. Hehemann, *Carbohydr. Res.*, 1979, **74**, 337.
[6] P. Simon, J. C. Ziegler, and B. Gross, *Synthesis*, 1979, 951.
[7] A. G. M. Barrett, P. A. Prokopiou, and D. H. R. Barton, *J. Chem. Soc., Chem. Commun.*, 1979, 1175.



(1) R = OSO_2CF_3
(2) R = H
(3) R = OTs
(4) R = OAc
(5) R = OH



(6)



(7) R = OH
(8) R = CN
(9) R = OSO_2CF_3

3-deoxy sugar was poor. The stereochemistry of the deoxygenation reaction based on the reduction of *S*-methyldithiocarbonates with tributylstannane has been examined using several 1,2 : 5,6-di-*O*-isopropylidene-D-hexose 3-xanthates and deuterated reagent. The reaction proceeds *via* a radical intermediate with the hydrogen being introduced from the sterically most accessible face of the molecule. Several 3-deuterio and 3,3-dideuterated derivatives of 3-deoxy compounds were prepared.[8] The conversion C=O → CH_2 has been successfully applied to several aldosulose derivatives by reduction of the derived tosylhydrazone with sodium cyanoborohydride. The derived tosylhydrazine derivative then undergoes decomposition in boiling ethanol containing sodium acetate to give the deoxy-compound.[9]

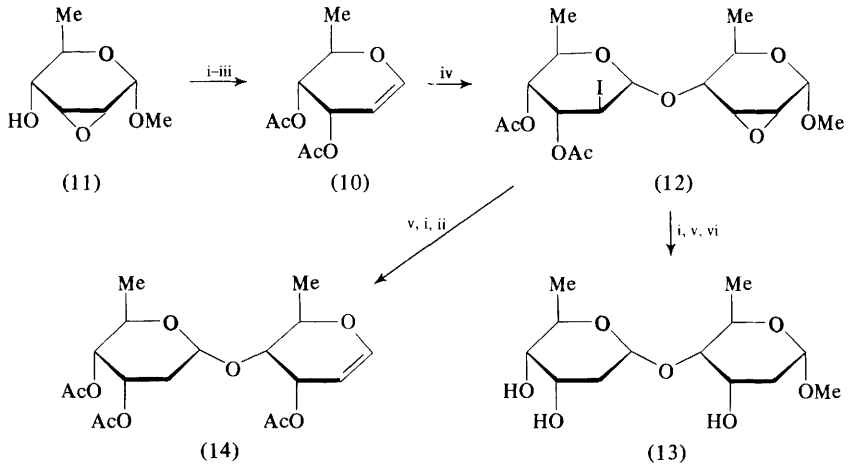
A novel method for the reductive deamination of amino-sugars has been published by Barton's group. The process involves sequential *N*-formylation, dehydration to the isocyano-derivative, and reduction with tributylstannane. Application of the reaction to 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose afforded 2-deoxy-D-glucose tetra-acetate in 72% yield.[10]

A new route to 2-deoxy-D-ribose involves application of a Wittig-based chain extension reaction to 2,4-*O*-ethylidene-D-erythrose, which is conveniently obtained as a dimer from the oxidation of 4,6-*O*-ethylidene-D-glucose by periodate. The erythrose acetal reacts with the Wittig reagent $(MeO)_3P$=CHSPh to give the phenylvinyl sulphide (RCH=CHSPh), hydrolysis of which with mercuric chloride gives the 2-deoxy-pentose (RCH_2CHO) derivative.[11] A synthesis of 2-deoxy-DL-ribose has also been described starting from non-carbohydrate precursors.[12]

A new synthesis of 2-deoxy-D-*arabino*-hexose (6) uses 2,3 : 4,5-di-*O*-isopropylidene-D-arabinitol (7) as starting material. The acetal was converted into the 1-cyano-derivative (8) *via* the 1-*O*-triflyl ester (9). Partial reduction of (8)

[8] J. J. Patroni and R. V. Stick, *Aust. J. Chem.*, 1979, **32**, 411.
[9] V. Nair and A. K. Sinhababu, *J. Org. Chem.*, 1978, **43**, 5013.
[10] D. H. R. Barton, G. Bringmann, G. Lamotte, R. S. H. Motherwell, and W. B. Motherwell, *Tetrahedron Lett.*, 1979, 2291.
[11] J. R. Hauske and H. Rapoport, *J. Org. Chem.*, 1979, **44**, 2472.
[12] M. Chmielewski, *Carbohydr. Res.*, 1979, **68**, 144.

with Raney nickel in formic acid afforded (6).[13] However, the most favoured method for the synthesis of 2-deoxy-aldoses is the hydration of the appropriate glycal, and Zólyomi *et al.* have employed this procedure for the synthesis of 2-[^{3}H]-2-deoxy-D-ribose. Placement of two tritium atoms at C-2 was accomplished by boiling 2-deoxy-D-ribose in tritiated water containing sodium hydroxide.[14]



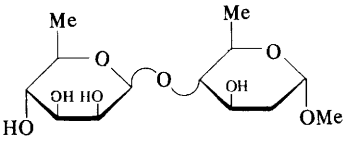
Reagents: i, LiI; ii, MeLi; iii, Ac_2O; iv, (11) + NIS; v, $NiCl_2$–$NaBH_4$; vi, NaOMe

Scheme 1

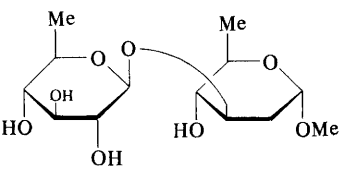
3,4-Di-*O*-acetyl-6-deoxy-D-allal (10) has been used as a starting material in an elegant synthesis of oligosaccharides containing 2-deoxy-α-D-*ribo*-hexose units (Scheme 1). The required glycal (10) may be conveniently synthesized from the 2,3-epoxide (11) by sequential treatment with lithium iodide, butyllithium, and acetic anhydride. The glycal (10) reacts highly selectively with alcohols in the presence of *N*-iodosuccinimide to give the 2-iodo-α-glycoside. Hence reaction of (10) with its precursor (11) gave the α-linked disaccharide (12) in 72% yield. Ring-opening of the epoxide (12) with iodide, followed by reductive dehalogenation afforded methyl 2,6-dideoxy-4-*O*-(2,6-dideoxy-α-D-*ribo*-hexopyranosyl)-α-D-*ribo*-hexopyranoside (13) in good overall yield. Alternatively, the disaccharide (12) may be converted into 4-*O*-(2,6-dideoxy-α-D-*ribo*-hexopyranosyl)-D-allal (14) which serves as a starting material for an extension of the process to tri- and higher-oligosaccharides.[15] 2,6-Dideoxy-L-*arabino*-hexose has been prepared *via* L-rhamnal, and one of the intermediates, methyl 2,6-dideoxy-α-L-*arabino*-hexopyranoside, has been employed in the synthesis of

[13] C. Y. Shiue, R. R. MacGregor, R. E. Lade, C.-N. Wan, and A. A. Wolf, *Carbohydr. Res.*, 1979, **74**, 323.
[14] G. Zólyomi, D. Banfi, and J. Kuszmann, *Acta Chem. Acad. Sci. Hung.*, 1979, **101**, 323.
[15] J. Thiem, P. Ossowski, and J. Schwentner, *Angew Chem. Int. Ed. Engl.*, 1979, **18**, 222.

the L-*lyxo*-analogue by selective benzoylation at HO-3, followed by mesylation and benzoate displacement.[16] Methyl 2,6-Dideoxy-4-*O*-(6-deoxy-β-D-manno-pyranosyl)-D-*arabino*-hexopyranoside (15) and its 3-*O*-(6-deoxy-β-D-gluco-pyranosyl) analogue (16) have been made from the corresponding glycals.[17]



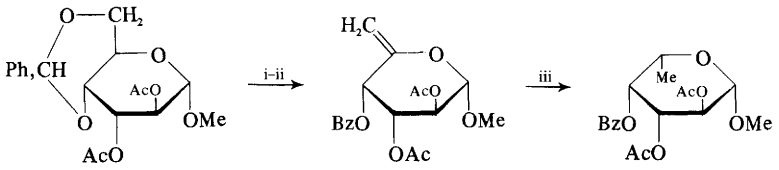
(15)



(16)

The acid-catalysed addition of phenol to 3,4,6-tri-*O*-acetyl-D-galactal afforded phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*lyxo*-hexopyranoside which was nitrated with nitric acid in a mixture of acetic acid and acetic anhydride to give the *p*-nitrophenyl glycoside.[18]

A new synthesis of L-fucose has been reported which uses methyl α-D-gluco-pyranoside as starting material (Scheme 2). The glucoside was converted into the



(17) (18) (19)

Reagents: i, NBS–$BaCO_3$; ii, AgF; iii, Pd–H_2

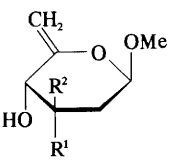
Scheme 2

altropyranoside (17) by known procedures and inversion of configuration at C-5 was accomplished by conversion of the altropyranoside to the 5,6-unsaturated derivative (18), which on hydrogenation afforded the fucoside (19) as the major product. The overall yield of α-L-fucose from the altropyranoside (17) was 19.3%.[19] However, hydrogenation (Pd–$BaSO_4$) of the methyl 2-deoxy-β-D-glyc-5-enosides (20) strongly favoured hydrogen addition from the α-side, giving the 6-deoxy-β-D-isomers almost exclusively.[20]

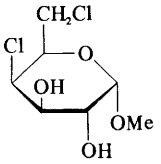
[16] A. F. Hadfield, L. Cunningham, and A. C. Sartorelli, *Carbohydr. Res.*, 1979, **72**, 93.
[17] J. Thiem and A. Sievers, *Chem. Ber.*, 1979, **112**, 1035; J. Thiem and H. Karl, *Chem. Ber.*, 1979, **112**, 1046.
[18] K. J. Dean and C. C. Sweeley, *J. Biol. Chem.*, 1979, **254**, 10 006.
[19] T. Chiba and S. Tejima, *Chem. Pharm. Bull.*, 1979, **27**, 2838.
[20] I. Pelyvas, F. Sztaricskai, L. Szilagyi, R. Bognar, and J. Tamas, *Carbohydr. Res.*, 1979, **76**, 79.

Methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside (21) has been used as starting material for the synthesis of chalcose (22) and several glycosides and disaccharides containing this antibiotic-derived sugar. The 3-*O*-methyl group was introduced either selectively by reaction of (21) with diazomethane, followed by reductive dehalogenation, or by reductive dehalogenation (Bu_3SnH) followed by selective benzoylation of the 2-hydroxy-group and methylation. The glycosyl bromide (23) was used for the synthesis of the β-glycosides, and subsequent reductive dehalogenation afforded the chalcose glycosides.[21] 6′-Deoxyraffinose and 6′,4″,6″-trideoxyraffinose have been prepared by reductive dehalogenation of the corresponding chloro-derivatives using Raney nickel catalyst.[22]

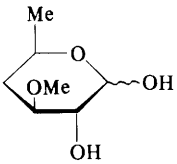
L-Ascarylose (3,6-dideoxy-L-*xylo*-hexose) has been synthesized from L-rhamnono-1,5-lactone. Benzoylation of the lactone in pyridine resulted in elimination of the 3-substituent to give (24). Subsequent hydrogenation of (24) was highly stereoselective, giving the 3,6-dideoxy-lactone (25) in 90% yield from which L-ascarylose and several of its derivatives were prepared.[23]



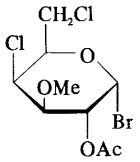
(20) $R^1 = N_3$, NHBz, R^2 = H; R^1 = H, R^2 = OH



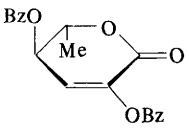
(21)



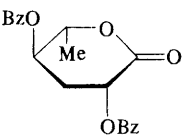
(22)



(23)



(24)

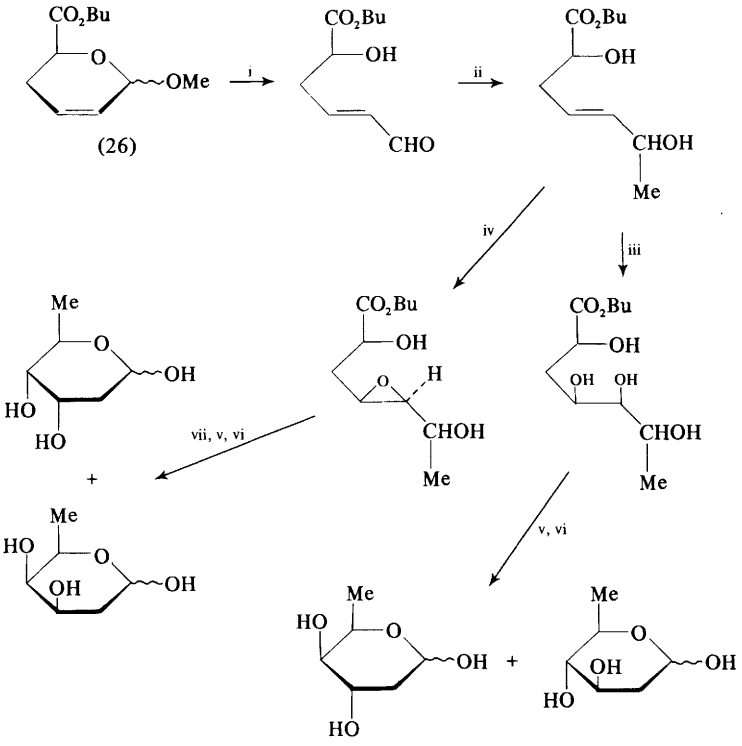


(25)

All four 2,6-dideoxy-DL-hexoses have been elaborated from the product (26) derived from the Diels–Alder reaction between butyl glyoxylate and 1-methoxybut-1,4-diene (Scheme 3).[24]

3,4-Di-*O*-acetyl-L-rhamnal (27) has been rearranged to the 2-ene (28) from which 2,3,6-trideoxy-L-*erythro*- and L-*threo*-hexopyranosides are available.[25]

[21] H. Redlich and W. Roy, *Carbohydr. Res.*, 1979, **68**, 275.
[22] L. Hough, A. C. Richardson, and M. A. Salam, *Carbohydr. Res.*, 1979, **71**, 85.
[23] O. J. Varela, A. F. Cirelli, and R. M. de Lederkremer, *Carbohydr. Res.*, 1979, **70**, 27.
[24] M. Chmielewski, *Tetrahedron*, 1979, **35**, 2067.
[25] H. S. El Khadem and R. C. Cermak, *Carbohydr. Res.*, 1979, **75**, 335.

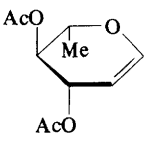


(26)

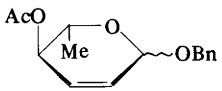
(All compounds racemic)

Reagents: i, H^+; ii, MeMgBr; iii, OsO_4; iv, *m*-$ClC_6H_4CO_3H$; v, $Ba(OAc)_2$–$CaCO_3$; vi, H_2O_2–Fe^{2+}; vii, HOAc

Scheme 3



(27)



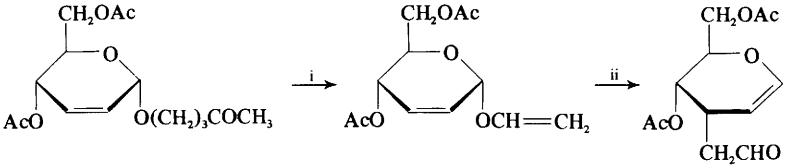
(28)

12

Unsaturated Derivatives

1 Glycals

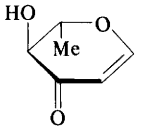
A new route to D-allal involves treatment of methyl 2-deoxy-2-iodo-α-D-altropyranoside with butyl-lithium in THF, the iodo-compound being obtained from methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside.[1] A novel route to a branched-chain glycal derivative is outlined in Scheme 1. Norrish type II degradation of the 4-oxopentyl glycoside, which was prepared from tri-*O*-acetyl-D-glucal, gave the vinyl analogue in 19% yield, and this then underwent the thermal Claisen rearrangement to regenerate a compound with glycal structure.[2]



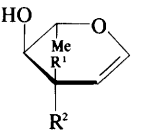
Reagents: i, *hν*; ii, Δ

Scheme 1

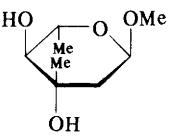
The oxidation of L-rhamnal with manganese dioxide gave the ketone (1) which, on treatment with methyl-lithium afforded L-olivomycal (2) with L-mycaral (3) as minor product. From the former the glycoside (4) and a disaccharide analogue were produced by use of alcohols in the presence of *N*-iodosuccinimide.[3] D-Glucal and ethyl 2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside also underwent allylic oxidation when treated with benzylideneacetophenone in the presence of a triphenylphosphine ruthenium hydride complex. Hydrogen transfer occurs during this procedure so that products of reduction of the double bonds of the starting material and the unsaturated ketone are also formed.[4] The



(1)



(2) R^1 = Me, R^2 = OH
(3) R^1 = OH, R^2 = Me

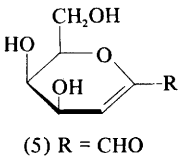


(4)

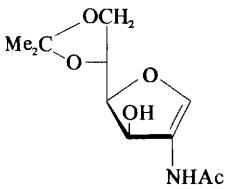
[1] R. D. Guthrie and R. W. Irvine, *Carbohydr. Res.*, 1979, **2**, 285.
[2] L. Cottier, G. Remy, and G. Descotes, *Synthesis*, 1979, 711.
[3] J. Thiem and J. Elvers, *Chem. Ber.*, 1979, **112**, 818.
[4] G. Descotes, J.-C. Martin, D. Sinou, and T.-C. Dung, *Bull. Chem. Soc. Fr., Part II*, 1979, 61.

allylic rearrangement reaction of glycals to give 2,3-unsaturated glycosides, which is referred to in Chapter 3 and later in this Chapter, usually proceeds readily only when ester groups at C-3 and C-4 are *trans*-related. Tin(IV) chloride has, however, now been shown to catalyse the reaction for tri-*O*-acetyl-D-galactal from which good yields of α-unsaturated glycosides can be obtained. The 2-deoxy products of addition of the alcohols to the double bond of the glycal are formed concurrently in small proportions.[5]

In the area of glycal derivatives with substituents on the double bonds, compounds (5) and (6) have been prepared from tetra-*O*-acetyl-β-D-galactopyranosyl cyanide for their study as substrates for β-D-galactosidase.[6] Penta-*O*-acetyl-β-D-glucopyranose on heating under vacuum at high temperature undergoes loss of acetic acid to give 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-1-enitol which, under these conditions, partially rearranges to 1,2,4,6-tetra-*O*-acetyl-3-deoxy-β-D-*erythro*-hex-2-enose.[7] A study has been carried out of the relative propensities of tetra-*O*-acetylhexopyranosyl bromides to undergo nucleophilic substitution and elimination on treatment with sodium *p*-nitrophenate in DMF, and they gave diminishing elimination/substitution ratios in the following order D-*allo*, D-*gulo*, D-*gluco*, D-*galacto*.[8] A direct method of preparation of the furanoid substituted glycal (7) involves treatment of 2-acetamido-2-deoxy-D-



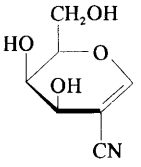
(5) R = CHO
(6) R = CH_2OH



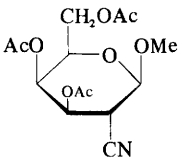
(7)

glucose with 2,2-dimethoxypropane in the presence of toluene-*p*-sulphonic acid. Compound (7) is obtainable in 43% yield together with an equal amount of the 4,6-acetal of the starting material.[9] Treatment of the cyanoglycal (8) with methoxide ion gave, after acetylation, the product (9) of addition in 47% yield together with a small proportion of the β-*talo*-isomer, whereas the 3,4-isopropylidene acetal of compound (8), under these conditions, underwent nucleophilic attack at C-1 and allylic displacement of O-3. Nucleophilic addition then occurred at the 2,3-double bond to give the dimethoxy product (10) and some of the α-anomer. Compound (8) is very unreactive towards electrophilic addition reagents.[10]

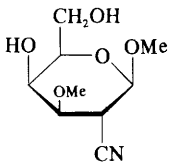
[5] G. Grynkiewicz, W. Priebe, and A. Zamojski, *Carbohydr. Res.*, 1979, **68**, 33.
[6] H.-M. Dettinger, G. Kurz, and J. Lehmann, *Carbohydr. Res.*, 1979, **74**, 301.
[7] P. Köll, E. Steinweg, U. Lackmann, and J. Mezger, *Tetrahedron Lett.*, 1979, 223.
[8] R. H. Shah and O. P. Bahl, *Carbohydr. Res.*, 1979, **74**, 105.
[9] A. Hasegawa and M. Kiso, *Carbohydr. Res.*, 1979, **74**, 341.
[10] R. H. Hall, K. Bischofberger, A. J. Brink, O. G. de Villiers, and A. Jordaan, *J. Chem. Soc., Perkin Trans 1*, 1979, 781.



(8)



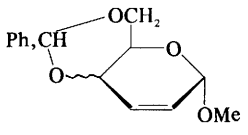
(9)



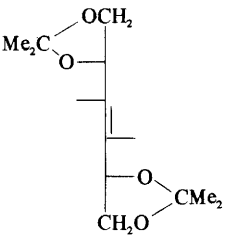
(10)

2 Other Unsaturated Derivatives

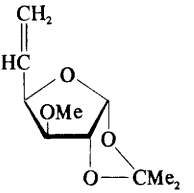
Several unsaturated compounds are mentioned elsewhere in the Report; in particular, unsaturated nucleosides are noted in Chapter 19. A review of enolone derivatives covers their synthesis, reactions of preparative interest and conversion to γ-pyrones.[11] A further general procedure for the conversion of carbohydrate α-diols to alkenes has been developed by Barton and colleagues, and involves the treatment of derived methylxanthate esters with tributyltin hydride. Compounds (11)–(13) were made in yields ranging from 50–80%, the *erythro*-isomer of compound (11) being obtained from both the D-*gluco*- and D-*manno*-precursors.[12] The same two glycoside acetals afford this alkene in good yield when treated with tri-iodoimidazole and triphenylphosphine in refluxing toluene, and this procedure seems to be of general applicability and to be an improvement on the related method which utilizes imidazole and iodine.[13] The method of preparing alkenes by heating trimethylammonium salts derived from *N*,*N*-dimethylformamide acetals of α-diols has been covered as part of a review on the chemistry of such acetals,[14] and electrochemical eliminations applied to nucleosides with halogen atoms at C-2′ and C-3′ have been shown to give mainly 2′,3′-unsaturated analogues. However, in some cases, the solvent and the electrolyte controlled the type of reaction, and furans, products of solvolytic cleavage of the glycosyl bond, and products of reductive dehalogenation were also observed.[15]



(11)



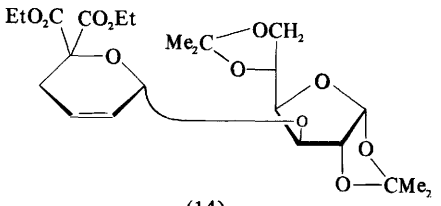
(12)



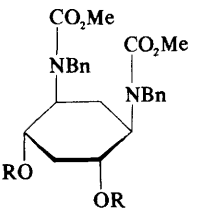
(13)

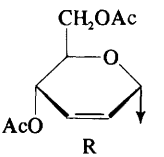
[11] F. W. Lichtenthaler, *Pure Appl. Chem.*, 1978, **50**, 1343 (*Chem. Abs.*, 1979, **91**, 157 984*a*).
[12] A. G. M. Barrett, D. H. R. Barton, and R. Bielski, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2378.
[13] P. J. Garegg and B. Samuelsson, *Synthesis*, 1979, 813.
[14] R. F. Abdulla and R. S. Brinkmeyer, *Tetrahedron*, 1979, 1675.
[15] T. Adachi, T. Iwasaki, I. Inoue, and M. Miyoshi, *J. Org. Chem.*, 1979, **44**, 1404.

2,3-Unsaturated disaccharide derivatives have been made by (4 + 2) cycloadditions applied to monosaccharide butadienyl ethers. For example, 3-*O*-butadienyl-1,2 : 5,6-di-*O*-isopropylidene-D-glucose treated with diethyl mesoxalate [$(EtO_2C)_2CO$] affords compound (14) and the corresponding β-anomer from which hexopyranyl moieties were derived by decarboxylation and reduction.[16] Other aspects of this work are referred to in Chapter 3 and, from the 2,3-unsaturated compounds, 3,4-unsaturated analogues were made by application of intramolecular rearrangement reactions. A more usual method of preparing 2,3-unsaturated disaccharides involves glycosylation with acylated glycals or 2-hydroxyglycals, and Thiem and colleagues have reported further examples.[17] By application of the same approach the amino-glycoside antibiotic analogue (15) has been made and converted into corresponding deoxy, saturated com-



(14)



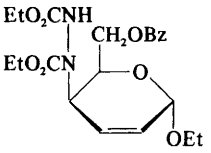


(15)

pounds, some having amino-deoxy groups at the primary centres.[18] Condensed with benzimidazole and benzotriazole, tri-*O*-acetyl-D-glucal affords mixtures of 2′,3′-unsaturated nucleoside analogues and isomeric 3′-substituted glycal derivatives, and conditions were found which favoured each kind of product. While there are precedents for these reactions, the paper also refers to the conversion of the products into furan derivatives having the bases bonded to C-5, which is a novel and certainly unexpected process.[19] Treatment of ethyl 6-*O*-benzoyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside and its 4-epimer with diethyl azodicarboxylate in the presence of triphenylphosphine caused inversion of configuration but no allylic rearrangement and gave the hydrazine derivative (16) and its epimer.[20]

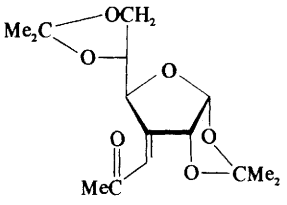
In the series of furanoid 3,4-unsaturated compounds the well known 3-deoxy-1,2 : 5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose is obtained when the trifluoromethanesulphonate of 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucose is treated with strong bases. It was resistant to reaction with ammonia.[21] An alternative, suitable means of preparing such alkenes involves the treatment of tosylhydrazones of keto-compounds with sodium acetate in DMF which is a

[16] S. David, J. Eustache, and A. Lunineau, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1795.
[17] J. Thiem, J. Schwentner, E. Schüttpelz, and J. Kopf, *Chem. Ber.*, 1979, **112**, 1023.
[18] A. Canas-Rodriguez and A. Martinez-Tobed, *Carbohydr. Res.*, 1979, **68**, 43.
[19] F. G. De Las Heras and M. Stud, *An. Quim*, 1979, **75**, 112 (*Chem. Abs.*, 1979, **91**, 141 155*v*).
[20] G. Grynkiewicz, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, 1978, **26**, 537 (*Chem. Abs.*, 1979, **90**, 72 430*w*).
[21] T. W. Flechtner, *Carbohydr. Res.*, 1979, **77**, 262.

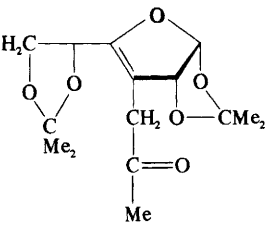


(16)

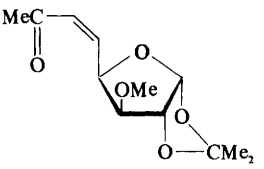
modification of the Bamford–Stevens elimination reaction.[22] Thermal rearrangement of the (*E*)-isomer of compound (17) and its C-4 epimer gives the deconjugated product (18), probably by a [1,5]-sigmatropic shift, and, likewise, the octose derivative (19) isomerizes to give the 4-enose (20), these reactions offering novel routes to unsaturated sugars.[23] Levoglucosenone (21), a 3,4-unsaturated pyranoid product of the pyrolysis of cellulose, in aqueous acid undergoes



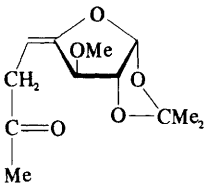
(17)



(18)



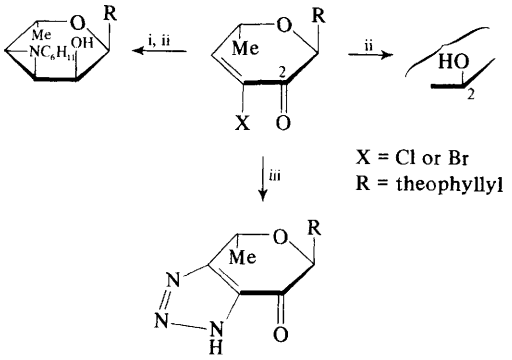
(19)



(20)

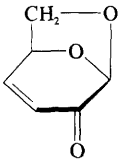
decomposition to hydroxymethylfurfural, but the product of hydration (22) can be isolated. Likewise, methanolysis leads to the methyl ether (23) and then to its dimethyl ketal, and reduction with borohydride of the hydrate (22) gives mainly the D-*arabino*-diol.[24] In the nucleoside series a study has been carried out on the related halogeno enones from which a series of products were obtained as shown in Scheme 2. Oxidation of the hydroxy-group of the 3,4-anhydro-6-deoxy-L-*talo*-analogue and treatment of the ketone with lithium chloride and bromide gave access to the starting enones.[25]

[22] V. Nair and A. K. Sinhababu, *J. Org. Chem.*, 1978, **43**, 5013.
[23] J. M. J. Tronchet, B. Gentile, and T. Nguyen-Xuan, *Helv. Chim. Acta*, 1979, **62**, 110.
[24] F. Shafizadeh, R. H. Furneaux, and T. T. Stevenson, *Carbohydr. Res.*, 1979, **71**, 169.
[25] J. Herscovici and K. Antonakis, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2682.

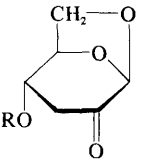


Reagents: i, $C_6H_{11}NH_2$–$MeNO_2$; ii, $NaBH_4$; iii, NaN_3–NH_4Cl

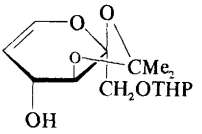
Scheme 2



(21)

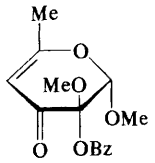


(22) R = H
(23) R = Me

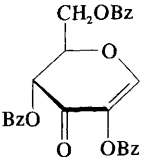


(24)

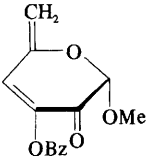
4,5-Unsaturated pyranoid compounds are less common, having 'glycal' vinyl ether character, and one (24) has been reported as the product of treatment of 2,3 : 4,5-di-*O*-isopropylidene-1-*O*-tetrahydropyranyl-β-D-fructopyranose with lithium di-isopropylamide in ether.[26] An enone (25) of this series is formed by treatment of the 'glycal' enone (26) with methanol in DMSO in the presence of potassium carbonate by nucleophilic attack at C-1, migration of the ester group from O-2 to O-3 and two β-eliminations to give the intermediate (27) which affords the product following a second attack by methanol, this time at C-2.[27]



(25)

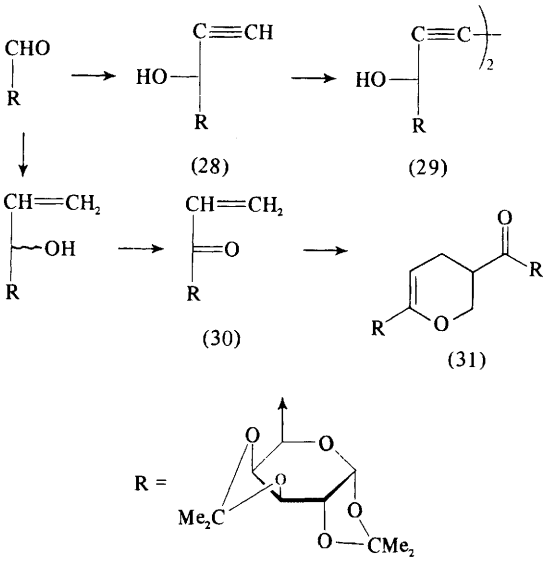


(26)



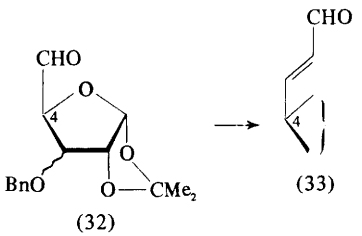
(27)

[26] A. Klemer and D. Balkau, *J. Chem. Res. (S)*, 1978, 303.
[27] F. W. Lichtenthaler, S. Nishiyama, and P. Jarglis, *Angew. Chem. Int. Ed. Engl.*, 1979, **18**, 936.



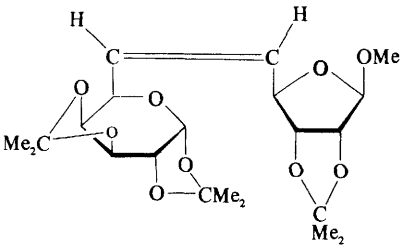
Scheme 3

The terminal acetylenic sugar (28) has been dimerized to give the diyne derivative (29) which could be reduced to corresponding diene and deoxy derivatives of higher sugar dialdoses. Both epimers of (28) and the analogous epimeric vinyl sugar could be oxidized to unsaturated keto sugars; the glycenosulose (30) polymerized on standing, which the reviewer suggests is the Diels–Alder dimer (31) or its regioisomer (Scheme 3) rather than the alkene polymer proposed by the authors. The epimeric pentodialdose derivatives (32) were also converted to the corresponding heptenodialdoses (33) by a Wittig synthesis.[28]



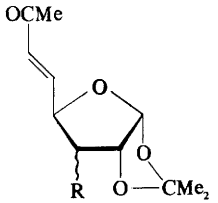
A novel type of C=C linked disaccharide derivative (34) has been produced in good yield by condensation of a phosphorus ylide derived from 6-deoxy-6-iodo-1,2 : 3,4-di-*O*-isopropylidene-α-D-galactopyranose with the aldehyde corresponding to the furanoid moiety. Likewise, an alkene was obtained using this

[28] D. Horton and J.-J. Tsai, *Carbohydr. Res.*, 1979, **75**, 151.

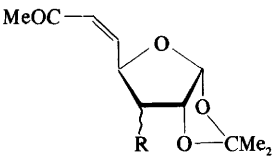


(34)

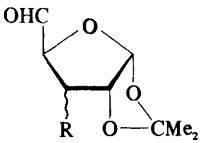
ylide with 2,3 : 4,5-di-*O*-isopropylidene-*aldehydo*-D-arabinose, and, again, only the *Z*-isomer was formed. The isomers with the *E*-stereochemistry were, however, obtainable by photochemical isomerization. This approach therefore provides means of coupling two monosaccharide units and opens the way to interesting, extended-chain compounds.[29] Tronchet and his colleagues have been very active in this area and have produced a wide range of furanoid 5-enose derivatives by condensation between 1,5-dialdofuranoses and Wittig reagents. A study of methyl ketones (35) and (36) produced from aldehydes (37) with *cis*- and *trans*-related groups at C-3 and C-4 showed that *trans*-compounds, or compounds without lone pairs of electrons on the substituent atom at C-3, led to the *E*-isomers (35) exclusively, whereas the *cis*-compounds and those with lone pairs of electrons on the substituent give rise to mixtures of the products (35) and (36). A rationalization of these observations was provided.[30] In the cases of the bromoalkenes (38) the *Z*-isomers were produced exclusively or with only small proportions of the *E*-compounds.[31] Further references to unsaturated compounds of this category and to their addition reactions are given in Chapters 9, 13, and 19.



(35)



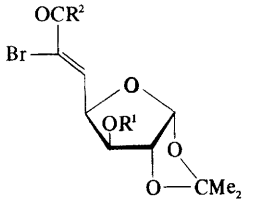
(36)



(37)

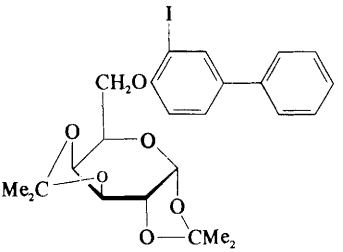
A high yielding synthesis of pyranoid 5-enose derivatives involves the photolysis of iodobiphenyl ethers which are obtained from appropriate sulphonyl esters. For example, compounds (39) gave the corresponding 6-deoxy-5-enose diacetal in 80% yield together with small amounts of the product of reductive de-iodination. However, applied to the ether of 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose, it gave only the product of dehalogenation, so the method

[29] J. A. Secrist and S.-R. Wu, *J. Org. Chem.*, 1979, **44**, 1434.
[30] J. M. J. Tronchet and B. Gentile, *Helv. Chim. Acta*, 1979, **62**, 2091.
[31] J. M. J. Tronchet, O. R. Martin, and J.-B. Zumwald, *Helv. Chim. Acta*, 1979, **62**, 1303.



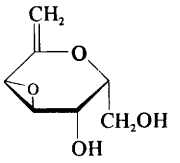
(38)

R^1 = Me or Bn; R^2 = Me, Ph, or OEt

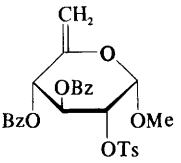


(39)

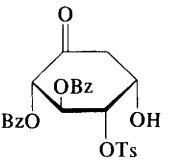
cannot be advocated as generally applicable, but when it is, it has the advantages that the preparations of the ethers and their photolysis are mild procedures.[32] Compound (40), prepared from a 2,6-anhydroheptose derivative which, in turn, was derived from a glycosyl cyanide, inhibits *E. coli* β-galactosidase irreversibly,[33] and the related unsaturated glycoside (41) has been converted in high yield into the inosose derivative (42) by treatment with mercury(II) chloride in aqueous acetone. This represents a new and mild means of obtaining carbocyclic compounds from carbohydrates.[34]



(40)

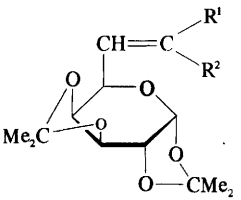


(41)



(42)

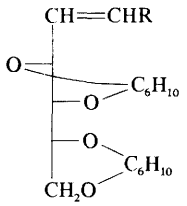
In the area of 6,7-unsaturated pyranoid derivatives compounds (43) (*E,Z* isomeric mixtures) have been made in Tronchet's laboratory and are of potential value for the synthesis of derivatives of lincomycin.[35] The related methyl compound (44) (*Z*-isomer) has been used to obtain compounds with epoxypropyl side-chains related to the antibiotic asperlin.[36]



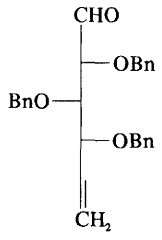
(43) R^1 = CN, COMe, COPh, CO_2Me, CO_2Et, CO_2H, or $CONH_2$
R^2 = H or Br
(44) R^1 = H, R^2 = Me

[32] R. W. Binkley and D. J. Koholic, *J. Org. Chem.*, 1979, **44**, 3357.
[33] J. Lehmann and B. Schwesinger, *Chem. Ber.*, 1978, **111**, 3961.
[34] R. J. Ferrier, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1455.
[35] J. M. J. Tronchet and M. A. M. Massoud, *Helv. Chim. Acta*, 1979, **62**, 1632.
[36] S. Lesage and A. S. Perlin, *Can. J. Chem.*, 1978, **56**, 3117.

Wittig reactions applied to 2,3 : 4,5-di-*O*-cyclohexylidene-D-arabinose using a phase transfer procedure gave aromatic compounds (45) where the Wittig substituents (R) were α-naphthyl, *p*-nitro-β-styryl, and benzimidazolyl.[37] A particularly interesting ring-opening reaction occurred when methyl 2,3,4-tri-*O*-benzyl-6-bromo-6-deoxy-β-D-glucopyranoside was treated with zinc in refluxing ethanol to give the acyclic alkene (46).[38] The conversion of this product into functionalized cyclopentane derivatives is described in Chapter 23.



(45)



(46)

An n.m.r. study of methyl 4,6-*O*-benzylidene-α- and -β-D-*erythro*-hex-2-enopyranoside is mentioned in Chapter 20. Iron carbonyl complexes of sugar enones are described in Chapter 16.

[37] Yu. A. Zhdanov, Yu. E. Alekseev, V. G. Alekseeva, E. L. Korol, V. A. Tyumenev, I. I. Popov, and V. A. Polenov, *Dokl. Akad. Nauk SSSR*, 1979, **244**, 1122 (*Chem. Abs.*, 1979, **90**, 204 382*n*).

[38] B. Bernet and A. Vasella, *Helv. Chim. Acta*, 1979, **62**, 1990.

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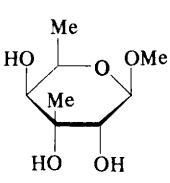
Branched-chain Sugars

1 Natural Products

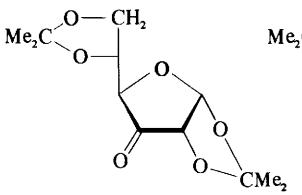
Methanolysis of the anti-tumour antibiotic virenomycin afforded methyl 6-deoxy-3-*C*-methyl-β-D-gulopyranoside (1), the structure of which was indicated by a combination of periodate oxidation and spectroscopic studies.[1]

2 Compounds with an R^1—C—OR^2 Branch

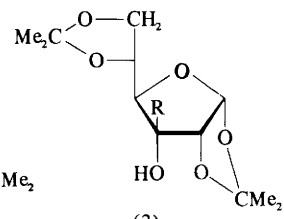
The synthesis of chiral tertiary alcohols by addition of Grignard reagents to glycosulose derivatives has been reviewed.[2] The reaction of 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexos-3-ulose (2) with Grignard reagents affords better yields of the *allo*-adduct (3) when conducted at −30 °C in the presence of anhydrous magnesium bromide.[3]



(1)



(2)



(3)

The stereoselectivity of the reaction between various glycosulosides and diazomethane to give the *spiro*-epoxide has been examined and shown to be strongly influenced by neighbouring oxygen groups, the methylene group of the *spiro*-epoxide preferring a *cis*-relationship to neighbouring oxygens. Thus the 2-uloside (4) gives the *altro*-epoxide (5), whereas (6) favours the *gluco*-isomer (7). When the two oxygen functions at C-1 and C-3 are *trans*-related as in (8), a mixture of *spiro*-epoxides are formed (Scheme 1).[4] This method has been compared to that involving direct epoxidation of the corresponding methylene derivatives derived from the Wittig reaction. Both procedures favour the same *spiro*-epoxide showing that oxidation of the alkene must occur from the side remote from the neighbouring oxygen.[5]

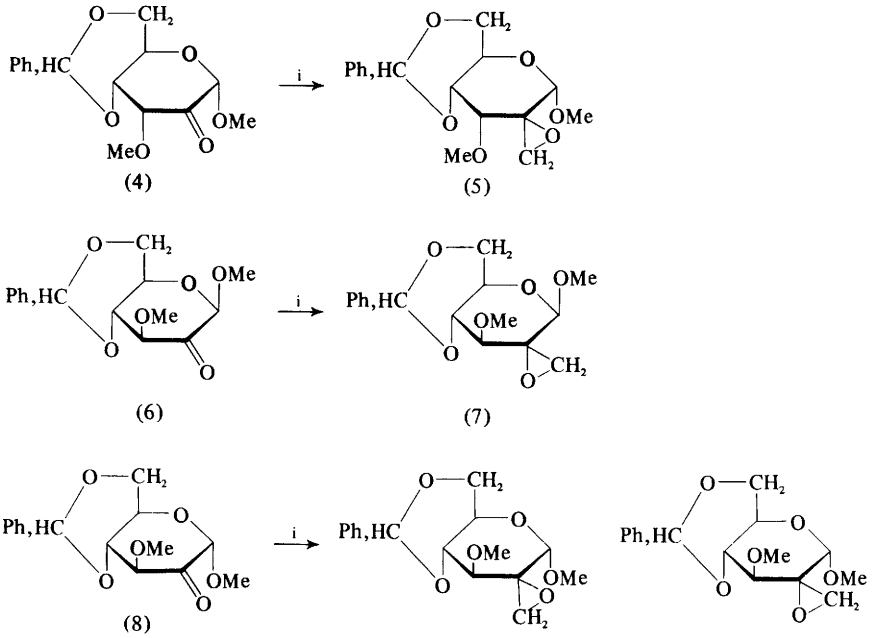
[1] V. V. Kulyaeva, M. K. Kudinova, N. P. Potapova, L. M. Rubasheva, M. G. Brazhnikova, B. V. Rozynov, and A. R. Bekker, *Bioorg. Khim.*, 1978, **4**, 1087 (*Chem. Abs.*, 1979, **90**, 104 282*t*).

[2] J.-C. Fischer and D. Horton, *J. Carbohydr., Nucleosides Nucleotides*, 1979, **6**, 101.

[3] A. Rosenthal and S. M. Mikhailov, *J. Carbohydr., Nucleosides Nucleotides*, 1979, **6**, 237.

[4] K.-I. Sato and J. Yoshimura, *Carbohydr. Res.*, 1979, **73**, 75.

[5] J. Yoshimura, K. Sato, and M. Funabashi, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2630.



Reagent: i, CH_2N_2

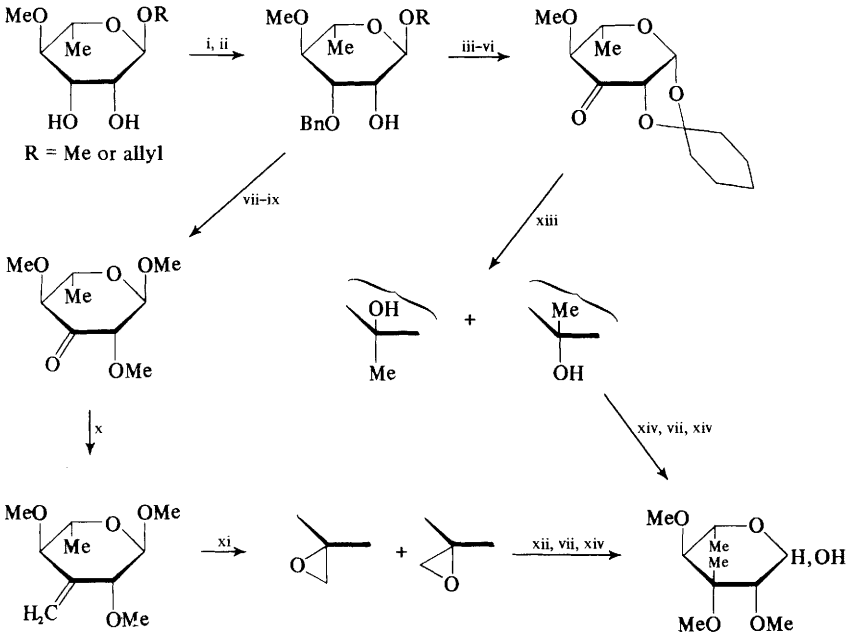
Scheme 1

Two syntheses of L-nogalose (6-deoxy-3-*C*-methyl-2,3,4-tri-*O*-methyl-L-mannose) have been reported which proceed through similar sequences (Scheme 2).[6,7]

The synthesis of evalose (6-deoxy-3-*C*-methyl-D-mannose), which is closely related to nogalose has been achieved by related procedures.[8]

The reaction of the glycos-3-ulose (2) with nitromethane has been examined under a variety of conditions; the 3-*C*-nitromethyl-glucofuranose is obtained in only 29% yield under strongly basic conditions, but the use of solid potassium carbonate in the presence of dibenzo-18-crown-6 as catalyst afforded the allofuranose (3, R = CH_2NO_2) in 43% yield without the resinification apparent under the more basic conditions. The use of a phase transfer catalyst and a two phase system did not have any advantages.[9] The 3-ulose has also been treated with ethyl cyanoacetate to give the *allo*-adduct (3, R = $CH(CN)CO_2Et$) which was hydrogenated to the corresponding amino derivative (3, R = $CH(CH_2NH_2)CO_2Et$).[10]

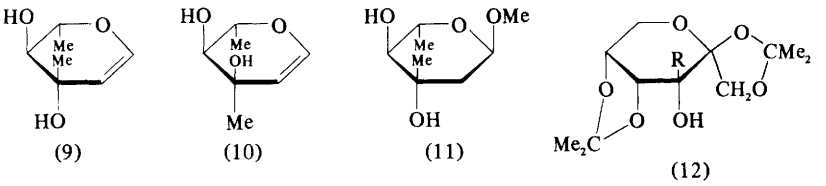
[6] J. Yoshimura, N. Hong, and M. Funabashi, *Chem. Lett.*, 1979, 687.
[7] L. Valente, L. E. S. Barata, A. Olesker, R. Robanal, G. Lukacs, and T. T. Thany, *Tetrahedron Lett.*, 1979, 1153.
[8] J. Yoshimura, N. Hong, and K. Sato, *Chem. Lett.*, 1979, 1263.
[9] Yu. A. Zhdanov, Yu. E. Alekseev, I. Siilis, and S. Kuren, *Dokl. Akad. Nauk, SSSR*, 1978, **238**, 1355 (*Chem. Abs.*, 1978, **89**, 43 993*f*).
[10] A. Rosenthal and R. H. Dodd, *J. Carbohydr., Nucleosides Nucleotides*, 1978, **5**, 545.



Reagents: i, Bu_2SnO; ii, BnBr; iii, $CH_2{=}CH{\cdot}CH_2Br$–NaH; iv, Bu^tOK; v, $HgCl_2$; vi, $(CH_2)_5C(OMe)_2$–TsOH; vii, MeI–NaH; viii, Pd–H_2; ix, RuO_4; x, $Ph_3P{=}CH_2$; xi, *m*-CPBA; xii, $LiAlH_4$; xiii, MeMgI; xiv, H^+

Scheme 2

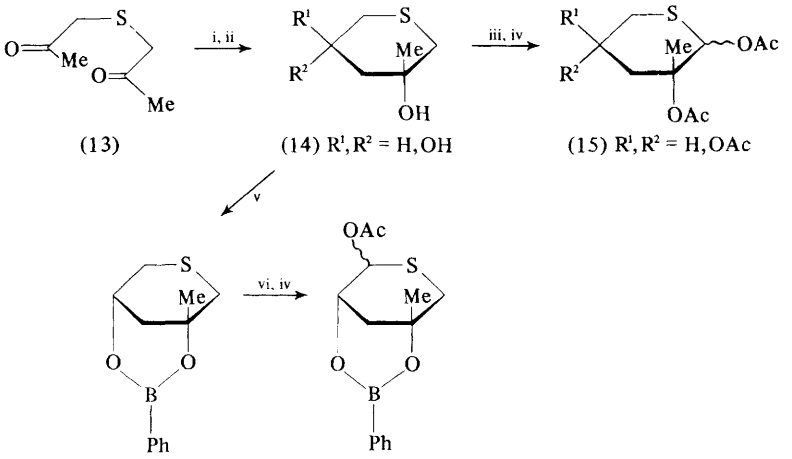
L-Olivomycal (9) and L-mycaral (10) have been prepared from L-rhamnal by oxidation of the 3-hydroxy-group with manganese dioxide followed by condensation with methyl lithium; (9) has been used for the synthesis of methyl α-L-olivomycoside (11) and disaccharides containing olivomycose (see also Chapter 11).[11] The addition of the lithiated derivatives pyridin-2-yl lithium, pyridin-2-ylmethyl lithium, and ethynyl lithium to 1,2:4,5-di-*O*-isopropylidene-β-D-*erythro*-hex-2,3-diulo-2,6-pyranose afforded the appropriate 2-*C*-substituted psicose derivatives (12). However, butyl lithium and Grignard reagents did not afford analogous derivatives but caused decomposition through proton abstraction.[12]



(9) (10) (11) (12)

[11] J. Thiem and J. Elvers, *Chem. Ber.*, 1979, **112**, 818.
[12] P. C. M. Herve du Penhoat and A. S. Perlin, *Carbohydr. Res.*, 1979, **71**, 135.

Racemic branched-chain sugars with 'sulphur in the ring' have been synthesized from diacetonyl sulphide (13) (Scheme 3). The sulphide underwent a base-catalysed intramolecular aldol condensation to give, after borohydride reduction of the remaining carbonyl group, a mixture of *cis*- and *trans*-dihydroxythianes (14). Each isomer of (14) was then subjected to oxidation to the sulphoxide followed by the Pummerer rearrangement to give the corresponding 2-*C*-methyl branched-chain sugars (15) as the major products. Conversion of the *cis*-isomer of (14) to the phenylboronate ester, followed by a similar sequence of reactions, afforded the 4-*C*-methyl derivative (16), which was converted into *O*-glycosides and nucleosides.[13]



(13) (14) R^1,R^2 = H,OH (15) R^1,R^2 = H,OAc

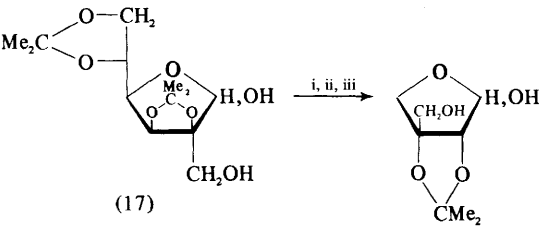
All compounds are racemic (16)

Reagents: i, NaOH; ii, $NaBH_4$; iii, H_2O_2, HOAc; iv, Ac_2O, AcOH; v, $PhB(OH)_2$; vi, *m*-CPBA

Scheme 3

Further details and examples of the introduction of a 2-*C*-hydroxymethyl group by aldol condensation of aldose derivatives with formaldehyde have been published (Vol. 12, p. 114). Condensation of 2,3 : 5,6-di-*O*-isopropylidene-D-mannose with formaldehyde afforded the 2-*C*-hydroxymethyl derivative (17) which was converted into 2,3-*O*-isopropylidene-D-apiose by standard reactions (Scheme 4). The L-enantiomer was similarly prepared from a derivative (18) of hamamelose.[14] The same derivative (18) has also been employed in a stereospecific synthesis of the di-*O*-isopropylidene derivative (19) of L-dendroketose (Scheme 5).[15] Similar methods have been used to synthesize 4-*C*-hydroxymethyl-D-ribose and its derived nucleoside derivatives.[16]

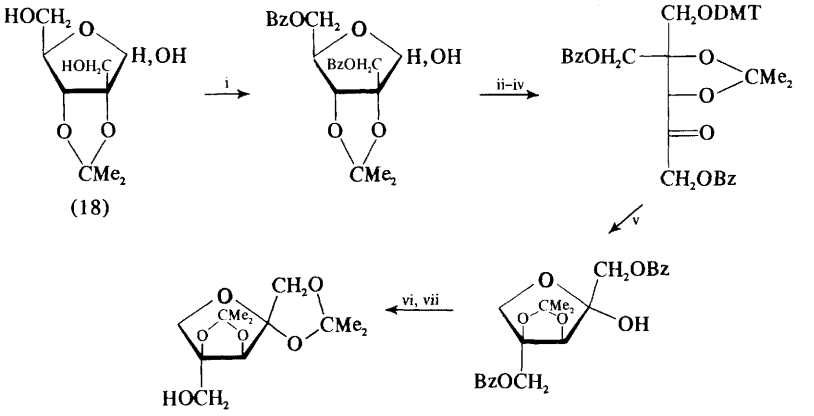
[13] M. Gannon, J. E. McCormick, and R. S. McElhinney, *J. Chem. Res. (S)*, 1979, 52.
[14] P.-T. Ho, *Can. J. Chem.*, 1979, **57**, 381.
[15] P.-T. Ho, *Can. J. Chem.*, 1979, **57**, 384.
[16] R. D. Youssefyeh, J. P. H. Verheyden, and J. G. Moffat, *J. Org. Chem.*, 1979, **44**, 1301.



(17)

Reagents: i, H^+; ii, $NaBH_4$; iii, $NaIO_4$

Scheme 4



(18)

(19)

Reagents: i, BzCl; ii, $NaBH_4$; iii, DMT–Cl; iv, CrO_3; v, H^+; vi, OH^-; vii, Me_2CO, H^+.
DMT = dimethoxytrityl

Scheme 5

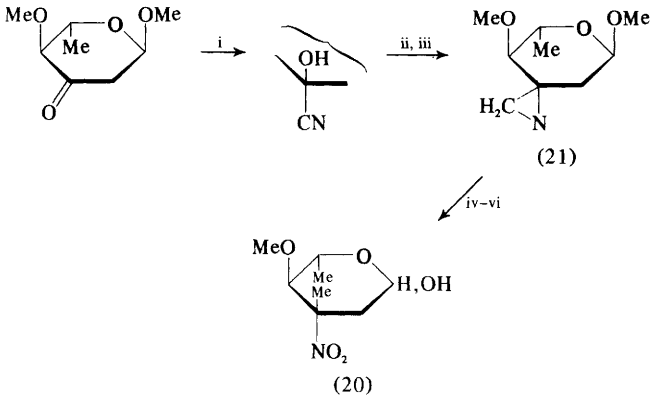
3 Compounds with an R—C—N Branch

The synthesis of evernitrose (2,3,6-trideoxy-3-*C*-methyl-4-*O*-methyl-3-nitro-L-*arabino*-hexose) (20) has been achieved as outlined in Scheme 6. The required stereochemistry and functionality of the branching point was established by way of the spiro-epimine (21) which was reductively ring-opened to the amine, which was then oxidized to the required nitro-derivative.[17]

In related work, Brimacombe's group have described two separate approaches to the synthesis of the amino-precursor of (20) starting from methyl 3-acetamido-2,3,6-trideoxy-3-*C*-methyl-α-L-glucopyranoside, and have also synthesized the corresponding D-enantiomer of the precursor.[18]

[17] J. Yoshimura, M. Matsuzawa, K.-I. Sato, and Y. Nagasawa, *Carbohydr. Res.*, 1979, **76**, 67.

[18] J. S. Brimacombe, A. S. Mengech, and M. S. Sayeed, *Carbohydr. Res.*, 1979, **75**, C5.

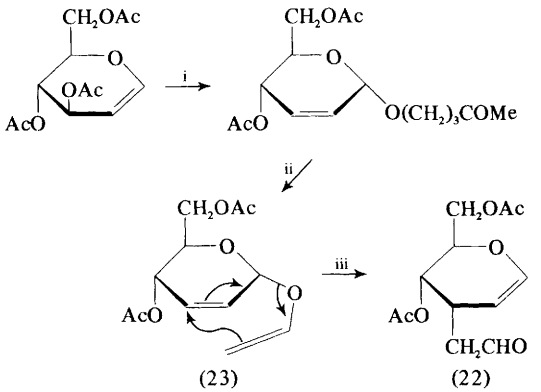


Reagents: i, HCN; ii, MsCl; iii, $LiAlH_4$; iv, Ni, H_2; v, *m*-CPBA; vi, H^+

Scheme 6

4 Compounds with an $R^1—C—R^2$ Branch

Descotes and his group report a facile and rather interesting synthesis of a 3-*C*-branched glycal (22) from glucal itself (Scheme 7), the key steps being the photolytic Norrish Type II cleavage of the pent-4-one-1-yl glycoside to give the vinyl glycoside (23) which underwent thermal rearrangement to give 4,6-di-*O*-acetyl-3-*C*-(2-oxoethyl)-D-allal (22).[19]



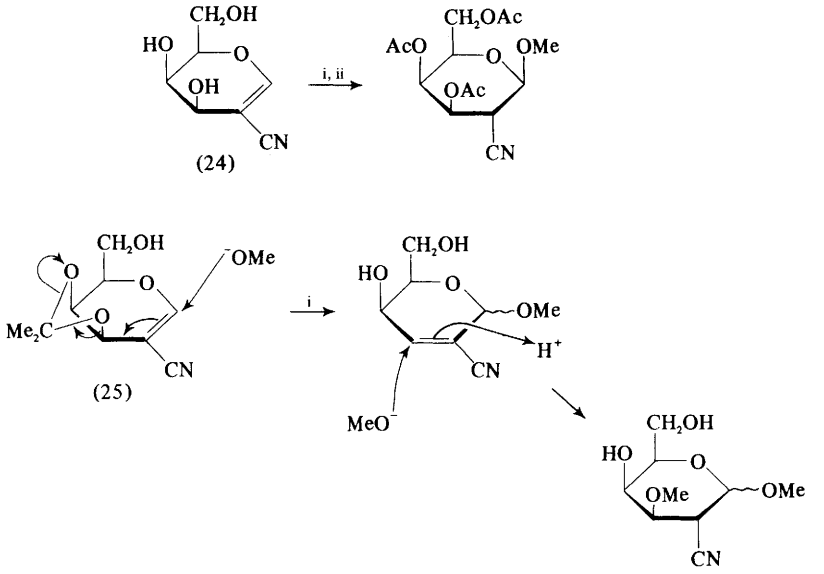
Reagents: i, $HO(CH_2)_3COMe$, BF_3; ii, *hν*; iii, Δ

Scheme 7

The use of 2-*C*-cyano-D-galactal (24) and its 3,4-*O*-isopropylidene derivative (25) for the synthesis of 2-*C*-branched chain sugars is hampered by the lack of reactivity of these glycals towards electrophilic reagents. However, addition of

[19] L. Cottier, G. Remy, and G. Descotes, *Synthesis*, 1979, 711.

methanol to (24) in the presence of base proceeds normally to give 2-cyano-2-deoxygalactoside, which has been reduced to the corresponding amine. The corresponding isopropylidene derivative (25) gave mainly methyl 2-cyano-2-deoxy-3-*O*-methyl-α-D-galactopyranoside by the mechanism shown in Scheme 8.[20]

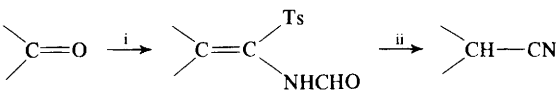


Reagents: i, NaOMe; ii, Ac_2O

Scheme 8

The attempted synthesis of *C*-cyano sugars, by addition of tosylmethylisocyanide to carbonyl compounds (Scheme 9) was unsuccessful. Only lactones reacted with the reagent, and on treatment with sodium ethoxide the adducts afforded the products in which ethanol had added across the double bond rather than the expected cyanide.[20]

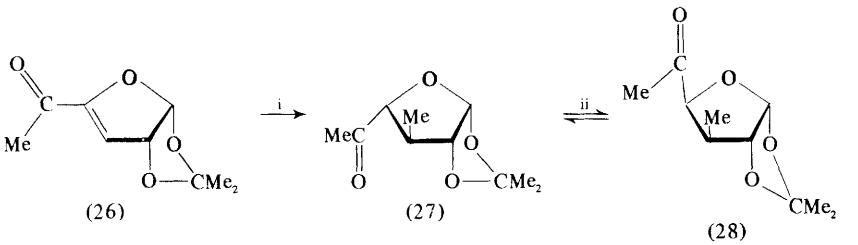
Photolysis of 3-*O*-acetyl-3-*C*-methyl-1,2 : 5,6-*O*-isopropylidene-α-D-allofuranose yielded the corresponding 3-deoxy-3-*C*-methyl-D-allose derivative (see



Reagents: i, K^+ $^-$CH(Ts)NC; ii, base

Scheme 9

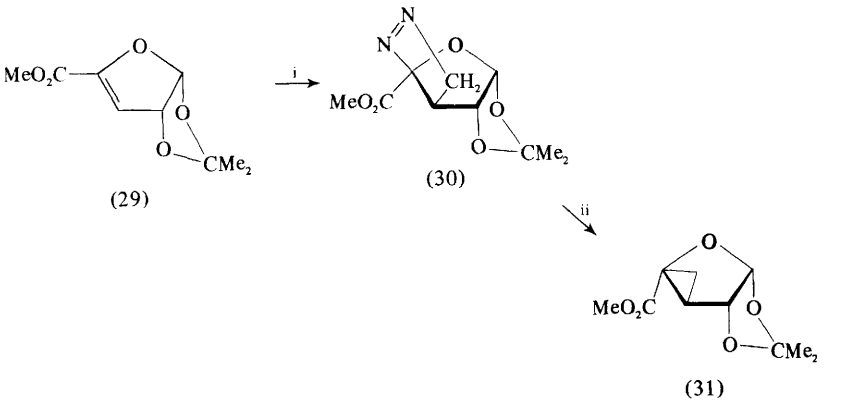
[20] R. H. Hall, K. Bischofberger, A. J. Brink, O. G. de Villiers, and A. Jordaan, *J. Chem. Soc., Perkin Trans. 1*, 1979, 781.



Reagents: i, $LiCuMe_2$; ii, ^-OH

Scheme 10

also Chapter 11).[21] The same group also reported the addition of lithium dimethylcuprate to the unsaturated keto-sugar (26) to afford 3,6-dideoxy-3-*C*-methyl-1,2-*O*-isopropylidene-α-L-*arabino*-hexos-5-ulofuranose (27) which is in the presence of base equilibrated with its 4-epimer (28), giving a ratio of (27) to (28) of 1:1.7 (Scheme 10), and the synthesis of several sugar derivatives containing fused cyclopropane rings, *e.g.*, addition of diazomethane to the α,β unsaturated ester (29) afforded the adduct (30) which underwent photolytic loss of nitrogen to give the cyclopropane derivative (31) (Scheme 11).[21]



Reagents: i, CH_2N_2; ii, *hν*

Scheme 11

A variety of other unsaturated sugars have been converted into cyclopropane derivatives by addition of dihalocarbene generated in the usual way, or by the Simmons–Smith reaction.[22,23]

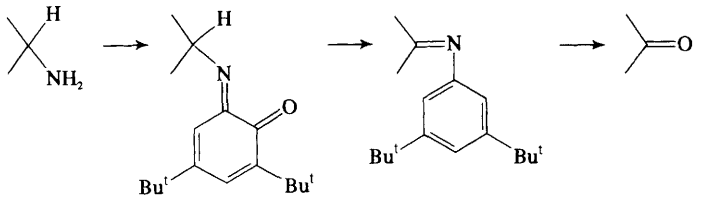
[21] R. Bonjouklian and B. Ganem, *Carbohydr. Res.*, 1979, **76**, 245.
[22] P. Duchaussoy, P. Di Cesare, and B. Gross, *Synthesis*, 1979, 198.
[23] A. Sele, B. Baehler, and J. M. J. Tronchet, *Helv. Chim. Acta*, 1979, **62**, 866.

14

Dicarbonyl Compounds and their Derivatives

This Chapter contains material relevant to dialdoses, aldosuloses, diuloses, and other dicarbonyl compounds and their derivatives. Unsaturated members of the series are referred to in Chapter 12.

The oxidation of aldose phenylosazones to aldos-2-uloses (osones) and the g.l.c. separation of the TMS ethers of the products have been examined in the cases of the D-*threo*-pentose, D-*arabino*-, D-*lyxo*-, L-*xylo*-hexose compounds, and two disaccharide derivatives.[1] A new approach to the synthesis of ketonic carbohydrate derivatives involves the treatment of amino-compounds with sterically hindered quinones to prepare imines which rearrange as shown in Scheme 1 to give products which undergo hydrolysis to ketones. The method gave the 2- and 3-ulosides, respectively, when applied to methyl 2-amino-2-deoxy-α- and β-D-glucopyranoside and methyl 3-amino-3-deoxy-β-D-allofuranoside.[2]



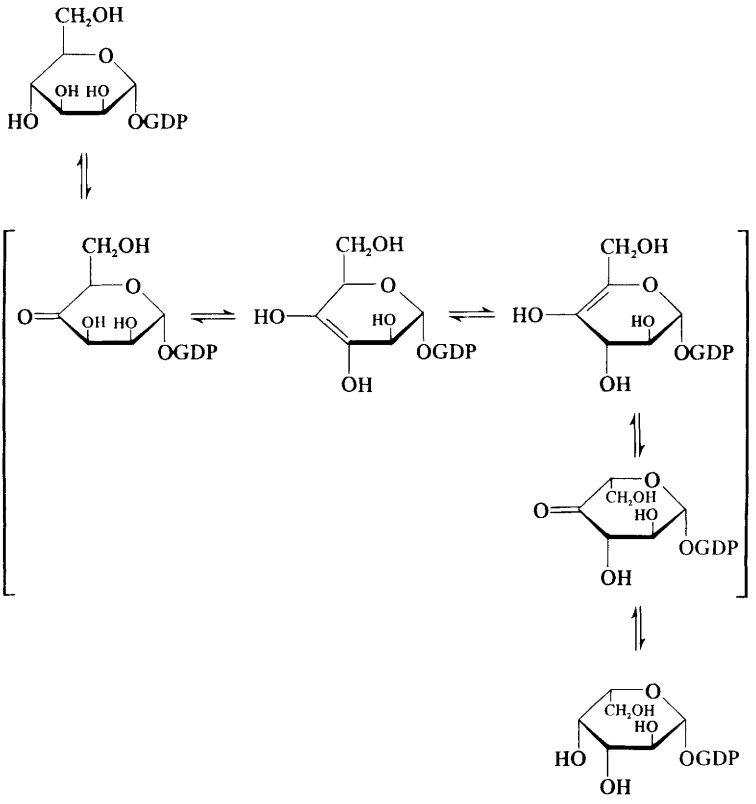
Scheme 1

γ-Radiolysis of sugars also provides dicarbonyl compounds, but much less specifically. From D-fructose the following products have been identified by g.l.c.-m.s.: 6-deoxy-D-*threo*-2,5-hexodiulose, 2-deoxy-D-*threo*-hexos-5-ulose, 5-deoxy-L-*glycero*-2,4-hexodiulose, 4-deoxy-L-*glycero*-2,5-hexodiulose, 1-deoxy-D-*arabino*-hexulose, D-*arabino*-hexosulose, and D-*threo*-2,5-hexodiulose.[3] Bromine water oxidation of α,α-trehalose and β,β-trehalose is also, as anticipated, a non-specific process, and from the former the 2- and 4-keto- and the 2,2′-, 2,4′- and 4,4′-diketo-derivatives were characterized as their *O*-methyloximes. From the latter the 2-, 3-, and 4-ketones were recognized amongst the products.[4] More specific is the oxidation by brominolysis to α- or β-hydroxyketones of dibutylstannylene acetals although, of course, two products can be formed. Nevertheless, good yields were obtained from the dibutylstannylene derivatives of the

[1] A. Soler, P. A. Garcia Ruiz, and I. Cartagena, *An. Univ. Murcia, Cienc.*, 1975 (Pub. 1978), **32**, 21 (*Chem. Abs.*, 1979, **90**, 168 843*j*).
[2] B. Lengstad and J. Lönngren, *Carbohydr. Res.*, 1979, **72**, 312.
[3] Y. Kito, S. Kawakishi, and M. Namiki, *Agric. Biol. Chem.*, 1979, **43**, 713.
[4] E. Scholander, *Carbohydr. Res.*, 1979, **73**, 302.

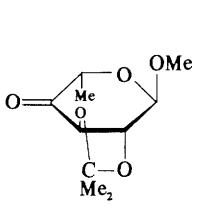
following (specific products are indicated in brackets): benzyl 2,3-di-*O*-benzyl-α-D-glucopyranoside (4-uloside, 87%), benzyl 2,6-di-*O*-benzyl-α-D-glucopyranoside (4-uloside, 75%), benzyl 4,6-*O*-benzylidene-α-D-galactopyranoside (3-uloside, 72%), 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (5-ulose, 48%).[5]

A general method for the synthesis of dialdoses and nucleoside 5′-aldehydes has been described, and their asymmetric reaction with nucleophiles discussed.[6] The reversible interconversion of GDP-D-mannose and -L-galactose which is brought about by a *Chorella* enzyme system was proposed to occur as shown in Scheme 2 following the observation that tritium incorporation from the solvent occurred equally at C-3 and C-5.[7] Epimerizations at positions α to the carbonyl groups can also be photoinduced, and compound (1) has now been isolated in

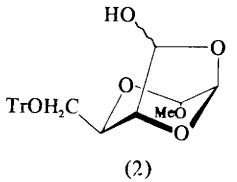


Scheme 2

[5] S. David and A. Thieffrey, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1568.
[6] G. H. Jones, J. G. Moffatt, R. S. Ranganathan, N. P. Damodaran, G. B. Howarth, M. D. Edge, H. Ohrui, and C. M. Gupta, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, **6**, 127.
[7] G. A. Barber, *J. Biol. Chem.*, 1979, **254**, 7600.



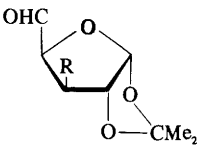
(1)



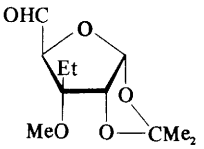
(2)

19% yield following irradiation of its C-3 epimer which is derivable from L-rhamnose. Previously, furanoid products of this process have been recognized.[8]

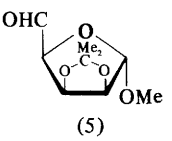
Relative rates of the cleavage of the α-diols of methyl pyranosides of β-L-arabinose, α- and β-D-xylose, α- and β-D-galactose, α- and β-D-glucose, and α-D-mannose have been measured by g.l.c. analysis of the trimethylsilyl ethers of dithioacetals derived from the oxidation products. The observed rates were then correlated with configurational factors associated with the hydroxy-groups and the aglycones.[9] Periodate oxidation of methyl 6-*O*-triphenylmethyl-α-D-glucopyranoside in aqueous THF or DMF occurs to give, in high yield, the product of cleavage of the C-2–C-3 diol which exists in the bicyclic form (2).[10] Periodate cleavage (of the exocyclic diols of corresponding hexose derivatives) has also afforded means of obtaining the aldehydes (3)–(7).[11]



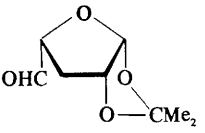
(3) R = H, F, or Cl



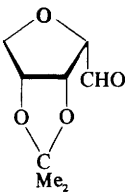
(4)



(5)

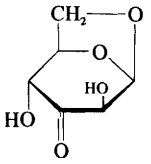


(6)

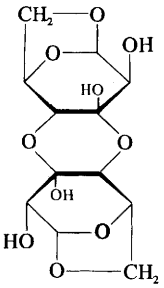


(7)

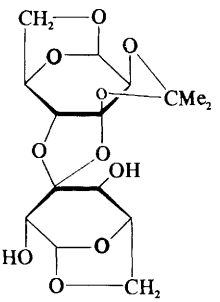
[8] P. M. Collins, F. Farnia, and A. S. Travis, *J. Chem. Res. (S)*, 1979, 266; (*M*, 2955).
[9] S. Honda, H. Hamajima, and K. Kakehi, *Carbohydr. Res.*, 1979, **68**, 77.
[10] L.-C. Li, F.-C. Chien, and P. Chang, *Hua Hsueh Tung Pao*, 1978, 328 (*Chem. Abs.*, 1979, **90**, 204 417*j*).
[11] J. M. J. Tronchet, B. Gentile, A. P. Bonenfant, and O. R. Martin, *Helv. Chim. Acta*, 1979, **62**, 696.



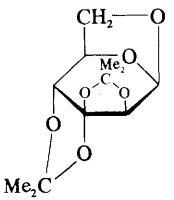
(8)



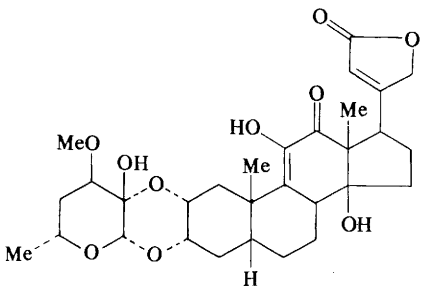
(9)



(10)



(11)

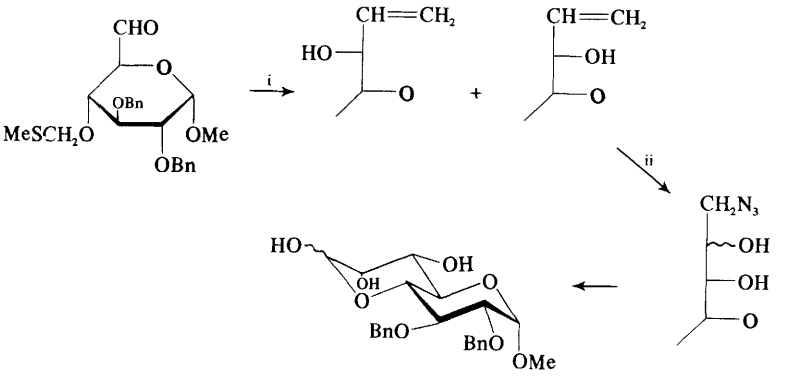


(12)

A further example of the complexity of the structures of derivatives of hydroxycarbonyl carbohydrates is provided by 1,6-anhydro-β-D-*arabino*-hexopyranos-3-ulose (8) which exists as the dimer (9) (see Vol. 5, p. 107), but gives the isopropylidene compounds (10) (with a rearranged structure) as well as the diacetal (11) on acetonation. The *xylo*-isomer behaves similarly. The *X*-ray crystal structure of compound (10) was determined.[12] The *X*-ray structure of affinoside B (12), a cardiac glycoside which contains a 4,6-dideoxy-3-*O*-methyl-β-D-*erythro*-hex-2-uloside moiety, has also been published.[13] Apramycin contains an octose

[12] P. Köll and J. Kopf, *Carbohydr. Res.*, 1979, **68**, 189.
[13] T. Yamauchi, K. Miyahara, F. Abe, and T. Kawasaki, *Chem. Pharm. Bull.*, 1979, **27**, 2463.

moiety, and two related octodioses [(13) and the C-7 epimer] have been prepared as outlined in Scheme 3. They existed in the illustrated bicyclic forms, and on methanolysis gave new glucosidic centres at C-8 with axial aglycone groups.[14]



(13)

Reagents: i, CH_2=CHMgBr; ii, standard methods; iii, *hν*

Scheme 3

[14] H. C. Jarrell and W. A. Szarek, *Can. J. Chem.*, 1979, **57**, 924.

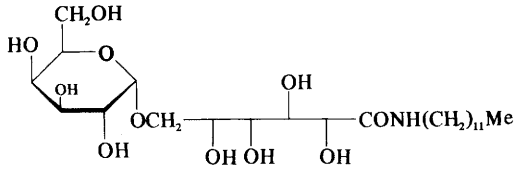
15

Sugar Acids and Lactones

Studies on the kinetics of oxidation of free sugars by various oxidants are referred to in Chapter 2.

1 Aldonic Acids

A new method of preparing aldono-1,5-lactones as their esters involves photolysis of either α- or β-2-oxopropyl glycosides, which can be prepared by standard methods (*e.g.* Koenigs–Knorr synthesis or, in the case of 2,3-unsaturated analogues, from acylated glycals)[1] (*cf.* Chapter 3 for photochemical reactions of other oxoalkyl glycosides). A study of the products of the Fehling's reaction showed that a multicomponent mixture of acids varying from formic to hexonic acids are produced, and that 4.49 equivalents of copper(II) are reduced per mole of glucose oxidized. A reaction scheme for the degradation was proposed.[2] An ingenious method of linking sugars to long-chain aliphatic compounds involved oxidizing disaccharides to the corresponding aldonolactones which were then treated with amines. Compound (1) was prepared in this way from melibiose, and analogues were obtained using lactose, maltose, and cellobiose. They are detergent in action and can lyse erythrocytes.[3]



(1)

The polarographic reduction of D-ribono-γ-lactone has been carried out at potentials varying from −2.49 to −2.99 V,[4] and the alternative reduction process which removes the hydroxy-groups of aldonic acids has been applied using platinum-group metals in the presence of hydrogen iodide in acetic acid. D-Glucono-1,5-lactone gave γ- and δ-caprolactones in 64 and 4% yield, respectively, together with a little caproic acid.[5]

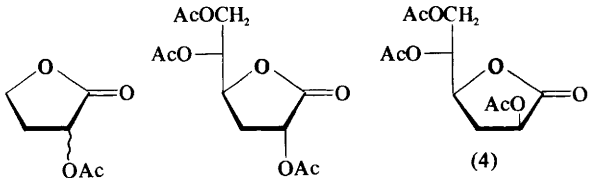
[1] G. Bernasconi, L. Cottier, G. Descotes, and G. Remy, *Bull. Chem. Soc. Fr.*, 1979, Part II, 332.

[2] H. Scherz, *Z. Lebensm.-Unters. Forsch.*, 1979, **168**, 91 (*Chem. Abs.*, 1979, **90**, 204 381*t*).

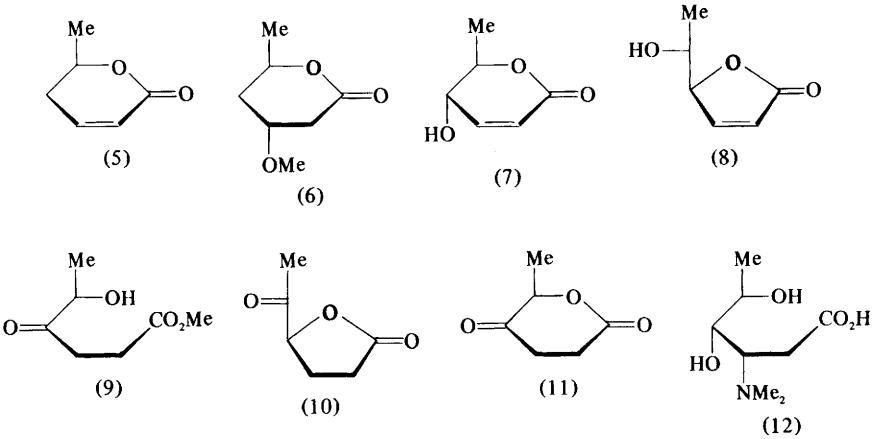
[3] T. J. Williams, N. R. Plessas, I. J. Goldstein, and J. Lönngren, *Arch. Biochem. Biophys.*, 1979, **195**, 145.

[4] V. G. Mairanovskii, J. Swiderski, and A. M. Ponomarev, *Khim.-Farm. Zh.*, 1979, **13**, 62 (*Chem. Abs.*, 1979, **91**, 5425*f*).

[5] Y. Nakamura, *Noguchi Kenkynsho Jiho*, 1978, **21**, 34 (*Chem. Abs.*, 1979, **90**, 103 741*e*).



Alkaline degradation of 2-amino-2-deoxy-D-glucose affords many products, the compounds (2)–(4) being the main ones present after acetylation.[6] The base-catalysed reaction of the lactone (5) with methanol gives the adduct (6). However, the hydroxy-analogues (7) and (8) gave the rearranged acyclic ester (9). Under acidic conditions this product then lactonized to give (10) and some (11) which have potential for the synthesis of racemic 2,3,6-trideoxyhexose derivatives. The furanoid lactone (8) reacted with dimethylamine to give some (12) which is the racemic form of the lactone derivable from megalosamine.[7]

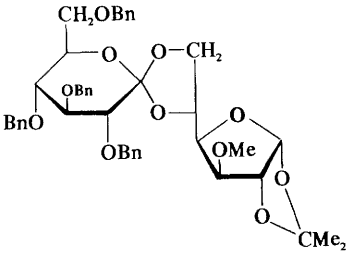


Substituted aldonolactones react with epoxides in the presence of boron trifluoride to give spiro-products which can otherwise be made from diols under dehydrating conditions. By these two approaches both simple products and disaccharide products, *e.g.* (13) and (14), were made.[8] Aldonolactone derivatives also react under Reformatsky conditions to give keto-acid derivatives which are dealt with in the ulosonic acid section below.

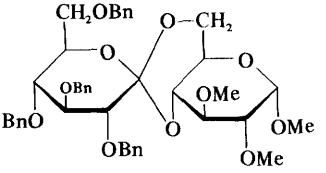
By ^{1}H n.m.r. spectroscopy it has been deduced that tri-*O*-acetyl-D-xylono-1,5-lactone exists in solution in a conformation close to the $^5H_4$ half-chair.[9]

Brominated derivatives are referred to in the ascorbic acid section.

[6] S. J. Eitelman and M. S. Feather, *Carbohydr. Res.*, 1979, **77**, 213.
[7] J. E. Jensen and K. Torssell, *Acta Chem. Scand., Ser. B*, 1978, **32**, 457.
[8] J. Yoshimura and M. Tamaru, *Carbohydr. Res.*, 1979, **72**, C9.
[9] C. R. Nelson, *Carbohydr. Res.*, 1979, **68**, 55.



(13)

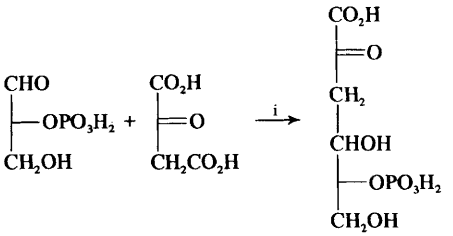


(14)

2 Ulosonic Acids

Oxidation of 2,3 : 4,6-di-*O*-isopropylidene-L-sorbose to the 2-ulosonic acid analogue can be effected using chromium trioxide and celite with the alcohol in ether–dichloromethane.[10]

Szabó and his colleagues have prepared 3-deoxy-D-*erythro*- and D-*threo*-hex-2-ulosonic acid 5-phosphate in the ratio 2 : 1 as illustrated in Scheme 1. The products were isolated in 37 and 23% yield, respectively, as their magnesium salts.[11] In parallel fashion, Russian workers have developed a synthesis of 3-deoxyaldulosonic acids as depicted in Scheme 2.[12] Studies by ^{13}C n.m.r. spectroscopy of α- and β-*N*-acetylneuraminic acid derivatives and determination of $J_{C\text{-}1\text{-}H\text{-}3ax}$ values allowed the conclusion that, in cytidine 5′-phospho-*N*-acetylneuraminic acid, a key intermediate in the biosynthesis of glyconoconjugates, the neuraminic acid moiety has the β-configuration.[13]

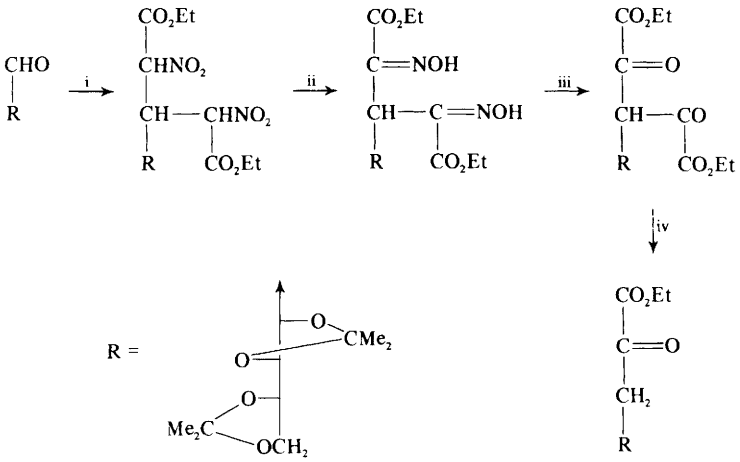


Reagent: i, Ni^{2+}

Scheme 1

Acylation of methyl 3-deoxy-D-*manno*-oct-2-ulosonate leads to furanose derivatives, *e.g.* (15), while similar treatment of the free acid or its ammonium salt affords pyranoid products. Methanolysis of the ester gives mixed 5-membered

[10] S. J. Flatt, G. W. J. Fleet, and B. J. Taylor, *Synthesis*, 1979, 815.
[11] F. Trigalo, R. S. Sarfati, and L. Szabó, *J. Chem. Soc., Perkin Trans. 1*, 1979, 649.
[12] Yu. A. Zhdanov, V. I. Kornilov, and B. B. Paydak, *Bioorg. Khim.*, 1979, **5**, 578 (*Chem. Abs.*, 1979, **91**, 91 896*q*).
[13] J. Haverkamp, T. Spoormaker, L. Dorland, J. F. G. Vliegenthart, and R. Schauer, *J. Am. Chem. Soc.*, 1979, **101**, 4851.

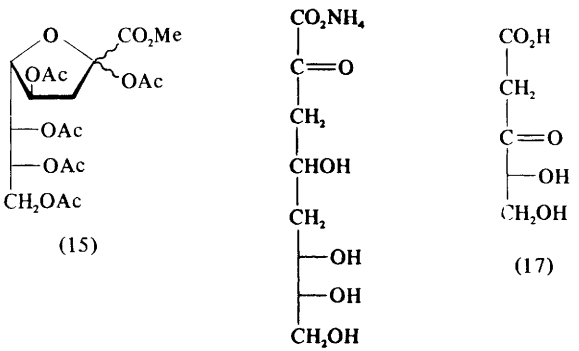


Reagents: i, $O_2NCH_2CO_2Et$; ii, $NaNO_2$; iii, $Pb(OAc)_4$; iv, base

Scheme 2

and 6-membered glycosides.[14] A related ^{13}C n.m.r. study reported that ammonium 3-deoxy-D-*manno*-oct-2-ulosonate exists in aqueous solution mainly as the α-pyranose, but with 11, 20, and 9% of the β-pyranose, α-furanose, and β-furanose, respectively. The 3,5-dideoxyoctulosonate (16) was prepared (to assist with the interpretation of the n.m.r. data) by condensation of 2-deoxy-D-*erythro*-pentose with sodium oxalacetate. The configuration at C-4 of this product was not determined.[15]

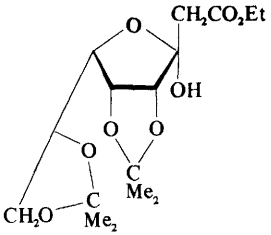
In the area of 3-ulosonic acids, the pentose derivative (17) has been prepared from 2,3-*O*-isopropylidene-D-glyceraldehyde by use of diazoacetic ester,[16] and



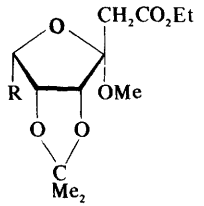
(15) (16) (17)

[14] D. Charon and L. Szabo, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2369.
[15] R. Cherniak, R. G. Jones, and D. S. Gupta, *Carbohydr. Res.*, 1979, 75, 39.

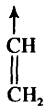
derivatives of more elaborate members of the class are obtainable by application of the Reformatsky reaction to aldonolactones. 2,3 : 5,6-Di-*O*-isopropylidene-D-gulono-1,5-lactone gave the 3-ulosonic acid (18) from which compounds (19)–(21) were derived by standard methods.[17]



(18)

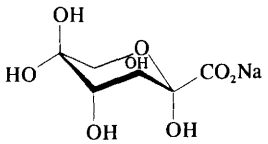


(19) R = 

(20) R = 

(21) R = 

The enolization of the diketo-acid D-*threo*-hex-2,3-diulosonic acid has been studied as a function of pH,[18] and the structure of sodium D-*threo*-hex-2,5-diulosonate has been shown by *X*-ray and ^{13}C n.m.r. methods to be the hydrate α-pyranoid form (22).[19]



(22)

3 Uronic Acids

An authoritative review of the synthesis and chemistry of uronic acids and their derivatives has been published.[20]

[16] F. J. Lopez Aparicio, F. J. Lopez Herrera, and M. Valpuesta Fernandez, *An. Quim.*, 1978, **74**, 1561 (*Chem. Abs.*, 1979, **91**, 108 170*y*).

[17] V. K. Srivastava and L. M. Lerner, *J. Org. Chem.*, 1979, **44**, 3368.

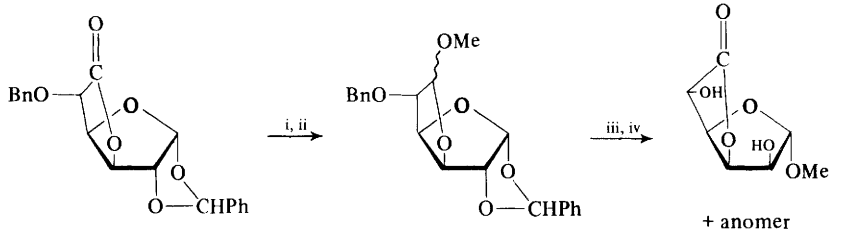
[18] J. Zuluaga and P. Martinez, *Z. Naturforsch., Teil A*, 1978, **33**, 1184 (*Chem. Abs.*, 1979, **90**, 138 122*f*).

[19] G. C. Andrews, B. E. Bacon, J. Bordner, and G. L. A. Hennessee, *Carbohydr. Res.*, 1979, **77**, 25.

[20] D. Keglević, *Adv. Carbohydr. Chem. Biochem.*, 1979, **36**, 57.

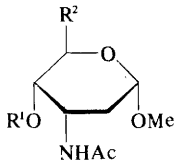
Periodate oxidation of 1-*O*-benzoyl-2,3-*O*-isopropylidene-α-D-mannofuranose has afforded access to several derivatives of D-lyxuronic acid,[21] and glycofuranosyl halides of D-riburonic acid, which are of value in nucleoside synthesis, have been made by way of the corresponding thiofuranosides (see Chapter 7).

An ingenious method of preparing methyl α- and β-L-gulofuranosidurono-6,3-lactone involves reversing the oxidation states of the terminal carbon atoms of a D-glucuronic acid derivative (Scheme 3).[22] The uronoside (23) and the 4,5-unsaturated analogue have been obtained from the bromide (24) by standard methods, the oxidation being achieved by photolysis of the 6-azido compound.[23]



Reagents: i, Bu^i_2AlH; ii, MeI, Ag_2O; iii, H_2–Pd; iv, O_2–Pt

Scheme 3

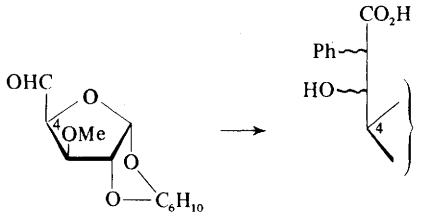


(23) R^1 = H, R^2 = CO_2Me
(24) R^1 = Bz, R^2 = CH_2Br

Isotopic exchange of H-5 occurs in the enzymic oxidation of UDP-D-glucose to the D-glucuronic acid analogue, which is explained by enolization of an intermediate aldehydo-species.[24] Study of the acid-catalysed decarboxylation of penturonic, hexuronic, and hepturonic acids showed that the first two gave one equivalent of carbon dioxide nearly qualitatively, which was not the case with the hepturonic compounds.[25] Derivatives of such hepturonic acids have been prepared by application of the Ivanov reaction as outlined in Scheme 4.[26]

Theoretical studies on the n.m.r. of uronic acids are referred to in Chapter 20.

[21] R. R. Schmidt and P. Hermentin, *Chem. Ber.*, 1979, **112**, 3616.
[22] I. Macher, K. Dax, H. Inselsbacher, and H. Weidmann, *Carbohydr. Res.*, 1979, **77**, 225.
[23] D. Horton, W. Weckerle, and B. Winter, *Carbohydr. Res.*, 1979, **70**, 59.
[24] H. S. Prihar and D. S. Feingold, *FEBS Lett.*, 1979, **99**, 106.
[25] M. A. Madson and M. S. Feather, *Carbohydr. Res.*, 1979, **70**, 307.
[26] Yu. A. Zhdanov, G. V. Bogdanova, and O. V. Doron'kina, *Dokl. Akad. Nauk SSSR*, 1977, **237**, 1367 (*Chem. Abs.*, 1978, **88**, 152 894z).

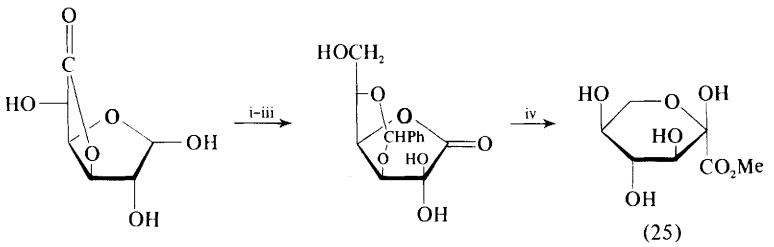


Reagents: i, Mg, Pr^iCl, $PhCH_2CO_2H$

Scheme 4

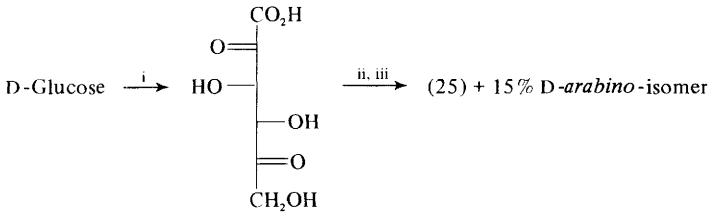
4 Ascorbic Acids

Two new syntheses of L-ascorbic acid have been reported, both giving methyl L-*xylo*-hex-2-ulosonate (25) as immediate precursor. The first used D-glucurono-6,3-lactone (Scheme 5),[27] whereas the second employed D-glucose and an initial bacterial oxidation step (Scheme 6).[28]



Reagents: i, $NaBH_4$; ii, PhCHO, H^+; iii, MnO_2; iv, MeOH, H^+

Scheme 5



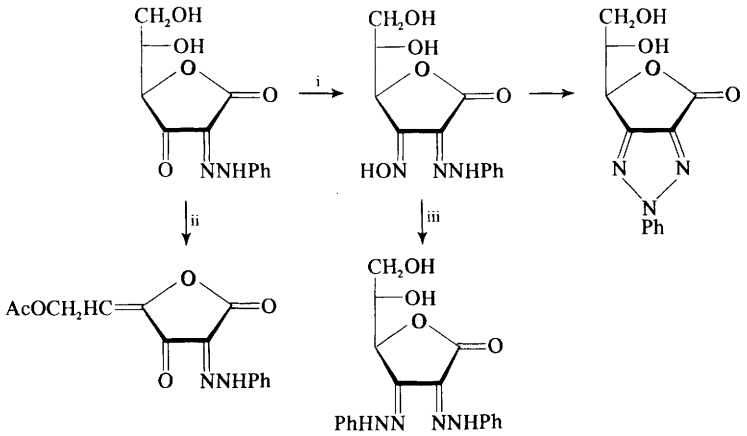
Reagents: i, *Acetobacter*; ii, $NaBH_4$; iii, methylation

Scheme 6

[27] T. C. Crawford and R. Breitenbach, *J. Chem. Soc., Chem. Commun.*, 1979, 388.
[28] G. Andrews, B. Bacon, T. Crawford, and R. Breitenbach, *J. Chem. Soc., Chem. Commun.*, 1979, 740.

The densities, viscosities, and refractive indices of aqueous L-ascorbic acid solutions have been measured in the range 0.01–1.2 M,[29] and a useful study of ^{13}C n.m.r. spectra of L-ascorbic acid and its derivatives has confirmed the previously assigned structures of the 2-sulphate and 3-methyl ether. However, the compound previously described as the 3-phosphate is found to be the 2-isomer. This led to the generalization that substitution, *e.g.* by diazomethane, of the un-ionized acid leads to reaction at O-3, but reaction of the dianion in basic solution occurs at O-2. The structure of the 3-methyl ether was confirmed by hydrogenation, reduction of the lactone, and acetylation to afford penta-*O*-acetyl-3-*O*-methyl-L-mannitol which gave the same m.s. as the similarly substituted D-glucitol derivative.[30] A chemical synthesis of the 2-phosphate (involving rearrangement of a 3-substituted derivative) confirms that this is the product of direct phosphorylation of the acid.[31] A review of the chemical, physiological, and pharmacological properties of the 2-sulphate has appeared in Japanese.[32]

New reactions continue to be reported. A set of nitrogenous derivatives have been produced as outlined in Scheme 7,[33] and bromination with hydrogen

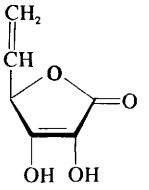


Reagents: i, NH_2OH; ii, Ac_2O, py; iii, $PhNHNH_2$

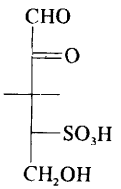
Scheme 7

bromide in acetic acid gave the 6-bromo-6-deoxy derivative and hence the alkene (26) and the 5,6-dideoxy compound. Hexonic acid lactones likewise gave 5-bromo-derivatives but sometimes bromine also entered at C-2.[34] A re-investiga-

[29] M. Shamim and S. B. Khoo, *Aust. J. Chem.*, 1979, **32**, 2293.
[30] T. Radford, J. G. Sweeny, G. A. Iacobucci, and D. J. Goldsmith, *J. Org. Chem.*, 1979, **44**, 658.
[31] J. Jernow, J. Blount, E. Oliveto, A. Perrotta, P. Rosen, and V. Toome, *Tetrahedron*, 1979, 1483.
[32] E. Hayashi and M. Terada, *Shizuoka Yakka Daigaku Kaigaku-25-Shunen Kinen*, 1978, 88 (*Chem. Abs.*, 1979, **91**, 5422c).
[33] M. A. El Sekily and S. Mancy, *Carbohydr. Res.*, 1979, **68**, 87.
[34] K. Bock, I. Lundt, and C. Pedersen, *Carbohydr. Res.*, 1979, **68**, 313.



(26)



(27)

tion of the reaction of L-ascorbic acid with glycine and bisulphite which gives the sulphonate (27) indicates that the yield is much higher (*ca.* 75%) than previously indicated, and suggests the nature of a complex formed between this product and bisulphite which causes strong binding to ion-exchange resin.[35]

The oxidation of ascorbic acid still attracts attention and a mechanism has been proposed for the reaction with photochemically produced $HO_2^{\bullet}$ radicals.[36] An analogous reaction with hydrogen peroxide affords a means of access to L-threonic acid.[37] Heating D-isoascorbic acid in aqueous pyridine in the presence of boric acid gives the decarboxylation products D-arabinose and D-ribose.[38] Two Russian papers have reported on the oxidation of ascorbic acid with copper(II) in the presence and absence of oxygen and light.[39,40] As part of a basic study of the action of oxidase and reductase enzymes, the reduction of Methylene Blue by ascorbic acid has been examined, in the presence of surfactants added to mimic the effects of the protein parts of the enzymes.[41]

A review has appeared on the lactones and reductones derived from cellulose components which are inhibitors or inducers of cellulases with possible use for optimizing the biochemical degradation of cellulose.[42]

[35] B. L. Wedzicha and A. P. Imeson, *J. Sci. Food Agric.*, 1977, **28**, 669 (*Chem. Abs.*, 1978, **88**, 73 270*q*).

[36] A. D. Nadezhdin and H. B. Dunford, *Can. J. Chem.*, 1979, **57**, 3017.

[37] H. S. Isbell and H. L. Frush, *Carbohydr. Res.*, 1979, **72**, 301.

[38] A. Ichikawa and M. Yamate, *Hiroshima Jogakuin Daigaku Ronshu*, 1978, **28**, 139 (*Chem. Abs.*, 1979, **91**, 5424*e*).

[39] E. V. Shtamm, A. P. Purmal, and Yu. I. Skurlatov, *Zh. Fiz. Khim.*, 1977, **51**, 3136 (*Chem. Abs.*, 1978, **88**, 74 499*h*).

[40] E. V. Shtamm, A. P. Purmal, and Yu. I. Skurlatov, *Int. J. Chem. Kinet.*, 1979, **11**, 461 (*Chem. Abs.*, 1979, **81**, 39 762*d*).

[41] M. Seno, K. Kousaka, and H. Kise, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2970.

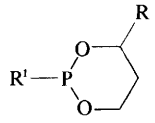
[42] E. E. Bruchmann, *Chem.-Ztg.*, 1978, **102**, 387 (*Chem. Abs.*, 1979, **90**, 98 990*d*).

16

Inorganic Derivatives

1 Carbon-bonded Compounds

Phosphorylation of protected D-glucopyranose and D-galactopyranose with dioxaphosphorinane or 6-methyldioxaphosphorinane gave the corresponding glycosyl phosphorus derivatives (1). Similarly prepared were the phosphonodiamidite and benzyl phosphonoamidite derivatives (2) and (3). Treatment of



(1) R^1 = glycosyl, R = H or Me

$RP(NEt_2)_2$

(2) R = glycosyl

$RP(OBn)NEt_2$

(3) R = glycosyl

(1) with sulphur gave the corresponding thiophosphates. Diastereoisomeric anisochronism was observed in the n.m.r. spectra of (1).[1] 2,3,4-Trideoxy-4-*C*-(phenylphosphinyl)-DL-*glycero*-pentofuranose (4), a phosphorus-ring sugar, has been synthesized by the route shown in Scheme 1.[2] Additions of various phosphorus nucleophiles to the cyano-ene (5) have yielded branched-chain sugar phosphonates such as compounds (6)–(9). Configurations were determined by n.m.r.[3]

Triphenyltin lithium has been used to open the oxiran rings of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allo- and -mannopyranosides to give the tin-bonded altrosides (10) and (11) in 75 and 94% yield respectively. A 43% yield of the C-6 bonded tin derivative (12) was obtained by using the reagent to displace the corresponding tosylate. The n.m.r. of these products is reported in Chapter 20.[4]

2 Oxygen-bonded Compounds

The chemistry of carbohydrate boronates has been reviewed.[5] Borate has been used as a blocking group which could be selectively removed in the presence of isopropylidene acetals. The method was used to synthesize 6-methacrylates as monomers for polymerization.[6] Chelation of pentitols with boric and germanic

[1] D. A. Predvoditelev, M. K. Grachev, M. V. Galakhov, and E. E. Nifant'ev, *Zh. Obshch. Khim.*, 1979, **49**, 285 (*Chem. Abs.*, 1979, **91**, 141 116*h*).

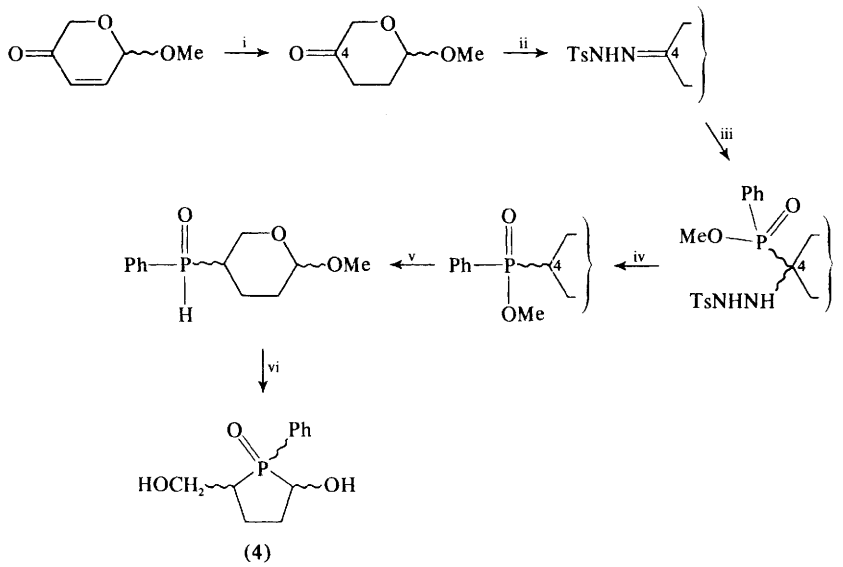
[2] M. Yamashita, M. Yoshikane, T. Ogata, and S. Inokawa, *Tetrahedron*, 1979, **35**, 741.

[3] J. M. J. Tronchet, J.-R. Neeser, L. Gonzalez, and E. J. Charollais, *Helv. Chim. Acta*, 1979, 2022.

[4] L. D. Hall, P. R. Steiner, and D. C. Miller, *Can. J. Chem.*, 1979, **57**, 38.

[5] R. J. Ferrier, *Adv. Carbohydr. Chem. Biochem.*, 1978, **35**, 31.

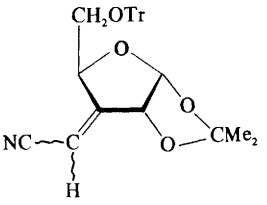
[6] V. L. Lapenko, B. I. Mikhant'ev, and V. N. Prokina, *Zh. Org. Khim.*, 1978, **14**, 1614 (*Chem. Abs.*, 1979, **90**, 23 431*p*).



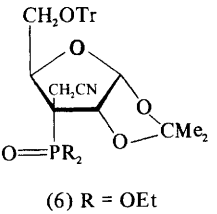
(4)

Reagents: i, H_2–Pd; ii, $TsNHNH_2$; iii, $PhP(O)(OMe)H$; iv, $NaBH_4$; v, $NaAlH_2(OCH_2CH_2OMe)_2$; vi, HCl

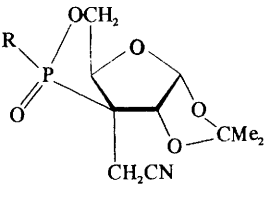
Scheme 1



(5)



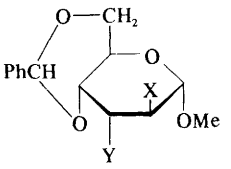
(6) R = OEt
(7) R = Ph



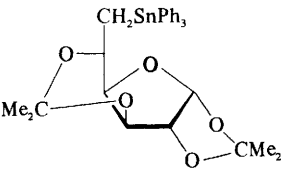
(8) R = OEt
(9) R = Ph

acids has been studied by potentiometry in aqueous acid solution. Both 1:1 and 1:2 chelates were formed and the chelation constants were determined.[7] The association between tellurate and sucrose has been investigated by conductimetry and polarimetry. Besides the expected 1:1 compound, a 4:1 tellurate:sucrose species was formed in large excess of tellurate.[8] The first high-spin tris-chelate monomeric managanese(IV) complex, namely bis(tetramethylammonium)tris(sorbitolato)manganate(IV), has been prepared. Its suggested structure is as shown in (13).[9]

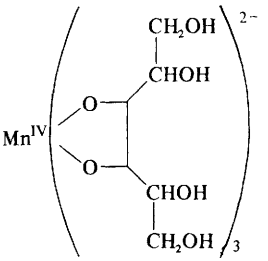
[7] E. Huttunen, *Finn. Chem. Letters*, 1979, **48** (*Chem. Abs.*, 1979, **91**, 57 349*d*).
[8] W. J. Popiel and M. S. Rustom, *J. Inorg. Nuclear Chem.*, 1978, **40**, 921 (*Chem. Abs.*, 1979, **90**, 55 189*g*).
[9] D. T. Richens and D. T. Sawyer, *J. Am. Chem. Soc.*, 1979, **101**, 3681.



(10) X = $SnPh_3$, Y = OH
(11) X = OH, Y = $SnPh_3$



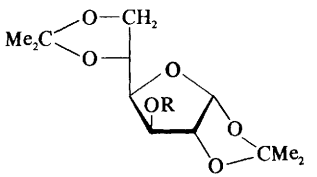
(12)



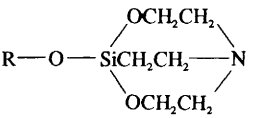
(13)

Reaction of 1,2 : 5,6-di-*O*-isopropylidene-α-D-glucofuranose with triphenylphosphine–diethyl azodicarboxylate and methyl iodide gave the relatively stable alkoxyphosphonium salt (14). The triphenylphosphonium group was displaced by benzoate to give the 3-*O*-benzoyl derivative (15).[10]

Some physicochemical measurements on mono-1-triethyl silatranyl ethers prepared from a range of monosaccharide derivatives have been reported.[11] Treatment of D-glucose, D-galactose, and D-xylose with $(EtO)_3SiH$ followed by tris[$HOCH_2CH_2$)$_3$N] gave the cyclized glycosylsilatranes isolated as their peracetates (16)–(18).[12]



(14) R = $Ph_3\overset{+}{P}I^-$
(15) R = Bz



(16) R = 2,3,4,6-tetra-*O*-acetyl-β-D-glc-*p*
(17) R = 2,3,4,6-tetra-*O*-acetyl-β-D-gal-*p*
(18) R = 2,3,4-tri-*O*-acetyl-β-D-xyl-*p*

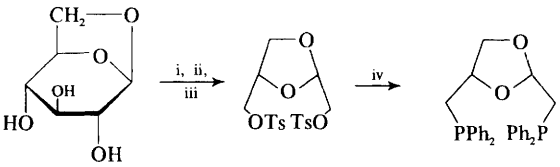
[10] H. Kunz and P. Schmidt, *Z. Naturforsch., Teil B: Anorg. Chem., Org. Chem.*, 1978, **33**, 1009 (*Chem. Abs.*, 1979, **90**, 55 178*c*).

[11] V. I. Kopkov, A. P. Luzin, A. M. Krapivin, V. D. Shcherbukhin, and B. N. Stepanenko, *Zh. Obshch. Khim.*, 1978, **48**, 2611 (*Chem. Abs.*, 1979, **90**, 152 480*c*) (*J. Gen. Chem. USSR*, 1978, **48**, 2370).

[12] V. I. Kopkov, A. P. Luzin, A. M. Krapivin, and B. N. Stepanenko, *Zh. Obshch. Khim.*, 1979, **49**, 220 (*Chem. Abs.*, 1979, **90**, 204 308*s*).

Using the mobilities of various carbohydrates in paper electrophoresis with Ca^{2+} as electrolyte the stereochemical requirement of hydroxy-groups for strong chelation has been demonstrated to be axial–equatorial–axial on a pyranose ring, or three consecutive hydroxy-groups on a furanose ring. The paper contains much discussion on complexation of other species with carbohydrates.[13] Tri-calcium complexes of sucrose have been prepared either from sucrose and calcium chloride with sodium hydroxide or by adding calcium oxide to sucrose solution. Two different types of complexation according to method of preparation were revealed by Raman spectroscopy while i.r. showed the polymeric nature of the complexes.[14] Spectrometry has shown that Al^{III}, In^{III}, and Ga^{III} form 1:1 complexes with D-glucose and D-xylose in acid media. These ions have high catalytic activity in the preparation of furfural from β-D-xylose.[15] The iron(III) chelating ability of carbohydrates has been shown to decrease in the order: fructose, maltitol, sorbitol, xylitol, xylose, maltose, sucrose, glucose. The preparation, isolation and analysis of a series of iron(III) chelates are described.[16]

The syntheses of a series of asymmetric hydrogenation catalysts from sugar diphenylphosphine derivatives and rhodium, capable of giving high enantioselectivity, have been described. A particularly efficient catalyst for stereoselective reduction of α-amino-acid precursors in the presence of bases, rhodium [(1,5-cyclo-octadienato)dioxop]perchlorate, was based on the dioxopan (19) which was synthesized *via* 1,6-anhydro-α-D-glucose as shown in Scheme 2. Other catalysts are reported in this paper.[17] The 2,3-bis-*O*-diphenylphosphino-α- and -β-D-glucosides (20) and (21) have been used to form rhodium complexes.[18,19]

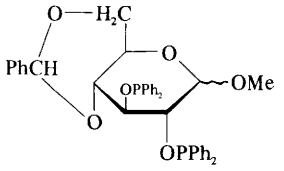


(19)

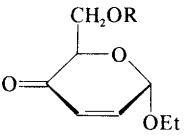
Reagents: i, $NaIO_4$; ii, $NaBH_4$; iii, TsCl; iv, $LiPPh_2$

Scheme 2

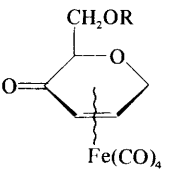
[13] S. J. Angyal and J. A. Mills, *Aust. J. Chem.*, 1979, **32**, 1993.
[14] C. Francotte, J. Vandegans, D. Jacqmain, and G. Michel, *Sucr. Belge Sugar Ind. Abstr.*, 1979, **98**, 137 (*Chem. Abs.*, 1979, **91**, 141 139*t*).
[15] V. I. Krupenskii and T. V. Dolgaya, *Izv. Vyssh. Uchebn. Zaved., Lesn. Zh.*, 1978, **21**, 110 (*Chem. Abs.*, 1979, **90**, 39 140*d*).
[16] M. W. Kearsley, G. G. Birch, and R. A. J. Foyle, *Acta Aliment. Acad. Sci. Hung.*, 1979, **8**, 69 (*Chem. Abs.*, 1979, **91**, 193 532*k*).
[17] D. Lafont, D. Sinov, and G. Descotes, *J. Organomet. Chem.*, 1979, **169**, 87 (*Chem. Abs.*, 1979, **90**, 204 384*w*).
[18] R. Selke, *React. Kinet. Catal. Lett.*, 1979, **10**, 135 (*Chem. Abs.*, 1979, **91**, 91 915*v*).
[19] Y. Sugi and W. R. Cullen, *Chem. Lett.*, 1979, 39.



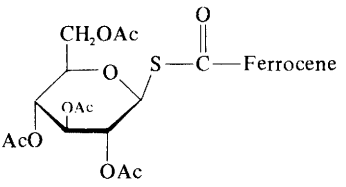
(20) α-glycoside
(21) β-glycoside



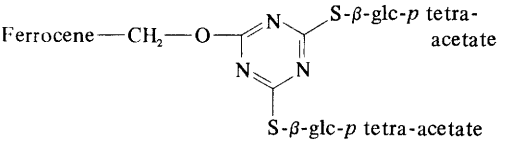
(22) R = H or Tr



(23) R = H or Tr



(24)



(25)

A fourth group has examined the effect of small changes in stereochemistry of the sugar used in the catalyst on the optical yield of the reduced product.[20]

The reaction between the enones (22) and di-iron nonacarbonyl gave the diastereoisomeric complexes (23), isolated by column chromatography using pure solvents and degassed adsorbent under an atmosphere of nitrogen.[21] The sugar–ferrocenyl conjugates (24) and (25) have been prepared.[22]

The polarimetry of complexes of sugars with borate ions and the c.d. of those with cuprammonium ions are referred to in Chapter 21.

The use of stannylene derivatives as synthetic intermediates is mentioned in Chapters 3, 13, and 14, and phosphonium salt derivatives are also referred to in Chapter 11.

[20] R. Jackson and D. J. Thompson, *J. Organomet. Chem.*, 1978, **159**, C29 (*Chem. Abs.*, 1979, **90**, 55 184*b*).
[21] M. B. Yunker and B. Fraser-Reid, *J. Org. Chem.*, 1979, **44**, 2742.
[22] M. J. Adam and L. D. Hall, *J. Chem. Soc., Chem. Commun.*, 1979, 865.

17

Alditols and Cyclitols

1 Alditols

A mixture of 2-*C*-hydroxymethylglycerol, 2,4-bis-*C*-hydroxymethylpentitol, and 3-*C*-hydroxymethylpentitol has been isolated from a calcium hydroxide-catalysed formose reaction from which most of the calcium ions were removed as sparingly soluble salts or chelates at the end of the induction period.[1]

1,2-Anhydro-4-*O*-benzyl-L-threitol has been prepared from diethyl tartrate by standard reactions.[2]

A range of 3,4-di-*O*-alkyl derivatives of 1,2 : 5,6-dianhydro-D-mannitol and 3,4-di-*O*-methyl derivatives of 1,2 : 5.6-dianhydro-D-glucitol, -galactitol, and -L-iditol have been synthesized; 1,6-di-*O*-mesyl-3,4-di-*O*-methyl-D-mannitol was found to be ten times more active as a cytostatic agent than the unmethylated compound.[3] These anhydro hexitol derivatives were also converted to 1,6-dibromo-1,6-dideoxy analogues together with corresponding 1,5-anhydro-6-bromo-6-deoxy compounds by treatment with lithium bromide.[4] 1,6-Dichloro-1,6-dideoxy-D-mannitol and -galactitol have been similarly prepared using hydrochloric acid, and these chloro-derivatives were then converted to the corresponding 1,2-anhydro-6-chloro-6-deoxyhexitols using equimolar amounts of base.[5] Mono- and -di-*O*-benzylidene derivatives of 1,6-dibromo-1,6-dideoxy-D-mannitol have been isolated and their structures determined.[6]

1,3 : 2,4 : 5,6-Tri-*O*-methylene-sorbitol and 1,3 : 2,5 : 4,6-tri-*O*-methylene-mannitol have been prepared in high yields from the hexitol by acid-catalysed reaction with a slight excess of paraformaldehyde.[7] Cyclic bis-phosphite esters of L-arabinitol (1) have been prepared from the pentitol using phosphorus trichloride followed by based-catalysed alkoxylation; ribitol reacted similarly to give 1,4 : 2,3,5-di-*O*-phosphinyl derivatives.[8] Treatment of pentitol cyclic phosphites with hexa-*N*-ethylphosphorous triamide in dry pyridine, yielded the corresponding di-*O*-phophinyl derivatives, *e.g.*, (2).[9] Phosphoramides and thio-

[1] Y. Shigemasa, C. Sakazawa, R. Nakashima, and T. Matsuura, *Origin Life, Proc. I.S.S.O.L. Meet., 2nd*, 1977 (Publ. 1978) 211 (*Chem. Abs.*, 1979, **90**, 82 526*w*).

[2] E. Hungerbühler, D. Seebach, and D. Wasmuth, *Angew. Chem. Int. Ed. Engl.*, 1979, **18**, 958.

[3] J. Kuszmann, *Carbohydr. Res.*, 1979, **71**, 123.

[4] J. Kuszmann, *Carbohydr. Res.*, 1979, **73**, 93.

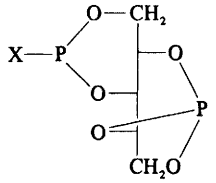
[5] M. Jarman, J. D. Stevens, I. P. Horváth, and L. Hegedüs, *Carbohydr. Res.*, 1979, **72**, 309.

[6] T. Horváth, P. Sohar, and G. Ábrahám, *Carbohydr. Res.*, 1979, **73**, 277.

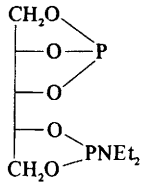
[7] A. C. Poshkus, *J. Org. Chem.*, 1979, **44**, 2794.

[8] Yu. Ya. Efremov, R. Z. Musin, E. T. Mukmenev, and N. A. Makarova, *Khim. Geterotsikl. Soedin.*, 1979, 177 (*Chem. Abs.*, 1979, **91**, 5428*j*).

[9] N. A. Makarova, E. T. Mukmenev, and B. A. Arbuzov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1979, 1167 (*Chem. Abs.*, 1979, **91**, 158 015*x*).



(1) X = Cl, OEt, OMe

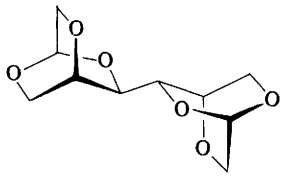


(2)

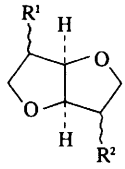
phosphates were also prepared from (1) (X = Cl) and its 1,2,4 : 3,5-substituted stereoisomer obtained from L-arabinitol with phosphorus trichloride.[10]

Lactitol has been prepared by catalytic or borohydride reduction of lactose, and their properties have been compared; lactitol was found to be much less readily consumed by microorganisms, probably reflecting a lower β-galactosidase efficiency with this substrate.[11]

A number of methyl or benzyl ether derivatives of galactitol have been prepared and converted into acetyl derivatives[12] for mass spectral study. ^{1}H N.m.r. investigation of the product obtained from chloroacetaldehyde diethyl acetal and galactitol (see Vol. 1, p. 46) has shown it to be the 1,3 : 4,6-di-*O*-acetal derivative, which on boiling with ethanolic sodium hydroxide yielded the polycyclic acetal-ether (3).[13]



(3)



(4) R^1, R^2 = I, $MeSO_3$

The configurations of a series of 2- and 5-substituted derivatives of 1,4 : 3,6-dianhydro-hexitols (4) have been established from their ^{13}C n.m.r. spectra.[14] Crystal structure measurements have shown that 1,5-anhydro-2,3,4-tri-*O*-benzoyl-xylitol and -ribitol both adopt the expected $^4C_1$(D) conformation, whereas 1,2,3,4-tetra-*O*-benzoyl-β-D-xylopyranoside adopts the alternative $^1C_4$ form, emphasizing the importance of the anomeric effect in the latter case.[15]

Unsaturated alditol derivatives are mentioned in Chapter 12.

[10] N. A. Markarova, E. T. Mukmenev, and B. A. Arbuzov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1979, 1168 (*Chem. Abs.*, 1979, **91**, 175 638*v*).

[11] T. Saijonmaa, M. Heikonen, M. Kreula, and P. Linko, *Milchwissenschaft*, 1978, **33**, 733 (*Chem. Abs.*, 1979, **90**, 72 405*s*).

[12] A. S. Rao and N. Roy, *Carbohydr. Res.*, 1979, **74**, 349.

[13] H. B. Sinclair, *Carbohydr. Res.*, 1979, **72**, 243.

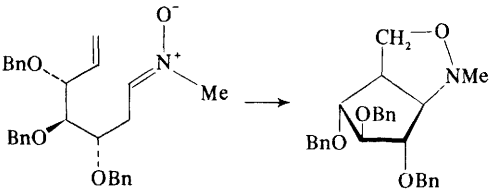
[14] P. Sohar, G. Medgyes, and J. Kuszmann, *Org. Magn. Reson.*, 1978, **11**, 357.

[15] P. Luger, G. Kothe, K. Vangehr, H. Paulsen, and F. R. Heiker, *Carbohydr. Res.*, 1979, **68**, 207.

2 Cyclitols

The synthesis of cyclitols from δ-dicarbonyl sugars has been reviewed.[16]

Treatment of hex-5-enoses with *N*-methylhydroxylamine led to cyclized isoxazolidine-cyclopentane derivatives illustrated in Scheme 1; the reaction provides a good entry to cyclopentane analogues of pentofuranose derivatives, since the products could be converted to a variety of other derivatives.[17] The



Scheme 1

reaction was found to be stereospecific and kinetically controlled.[18,19] *N*-Glycosylhydroxylamines could also be used, leading to the corresponding *N*-glycosyl derivative, *e.g.*, the nucleoside analogue (5).[17] 1,2 : 3,4-Di-*O*-cyclohexylidene-*sn-myo*-inositol has been resolved by the formation of diastereoisomeric *ortho*-acetate derivatives of D-mannose, which could be separated by crystallization or chromatography; the separated ortho-esters were also used to prepare 4-*O*-methyl- and 5-*O*-methyl-*sn-myo*-inositol,[20] and 1-*O*-benzyl-, 3-*O*-benzyl-*sn-myo*-inositol or a mixture of the 4-*O*-benzyl- and 6-*O*-benzyl-*sn-myo*-inositols.[21] The mass spectra of inositol tris(boronate) esters formed from alkyl or benzene boronic acid have been measured, and structures with five-, six-, or seven-membered ester rings could be recognized.[22]

A range of mono- and di-anhydro-inositols have been prepared from the isomeric 3,6-dibromocyclohexadienemono-epoxides (6) by conventional transformations *via* the corresponding anhydroconduritols (7).[23] Another paper reports the synthesis of all the 1,2,3,4- and 1,2,4,5-dianhydro-inositols from inositol disulphonates using sodium methoxide.[24] The hydration of three inososes

[16] D. E. Kiely, J. M. Riordan, G. J. Abruscato, C. E. Cantrell, and W. R. Sherman, Proceedings of the Symposium on Cyclitols Phosphoinositides, ed. W. W. Wells and F. Eisenberg, jun., Academic Press, New York, 1977 (Publ. 1978), 13 (*Chem. Abs.*, 1979, **91**, 157 987*d*).

[17] B. Bernet and A. Vasella, *Helv. Chim. Acta*, 1979, **62**, 1990.

[18] B. Bernet and A. Vasella, *Helv. Chim. Acta*, 1979, **62**, 2400.

[19] B. Bernet and A. Vasella, *Helv. Chim. Acta*, 1979, **62**, 2411.

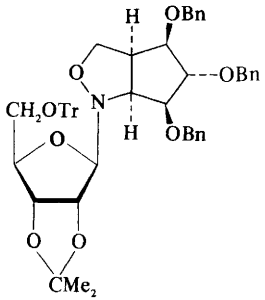
[20] V. N. Krylova, A. I. Lyutik, N. N. Kobel'kova, V. I. Shvets, and R. P. Evstigneeva, *Zh. Org. Khim.*, 1978, **14**, 1858 (*Chem. Abs.*, 1979, **90**, 23 455*z*).

[21] A. P. Kaplun, V. N. Krylova, G. Yu. Soloveichik, B. A. Klyaschchitskii, and V. I. Shvets, *Zh. Org. Khim.*, 1978, **14**, 1863 (*Chem. Abs.*, 1979, **90**, 23 456*a*).

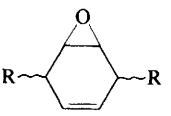
[22] J. Wiecko and W. R. Sherman, *J. Am. Chem. Soc.*, 1979, **101**, 979.

[23] R. Keller, R. Schwesinger, W. Fritsche, H.-W. Schneider, D. Hunkler, and H. Prinzbach, *Chem. Ber.*, 1979, **112**, 3347.

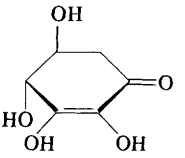
[24] S. Ogawa, S. Oki, and T. Suami, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1095.



(5)



(6) R = Br
(7) R = OH

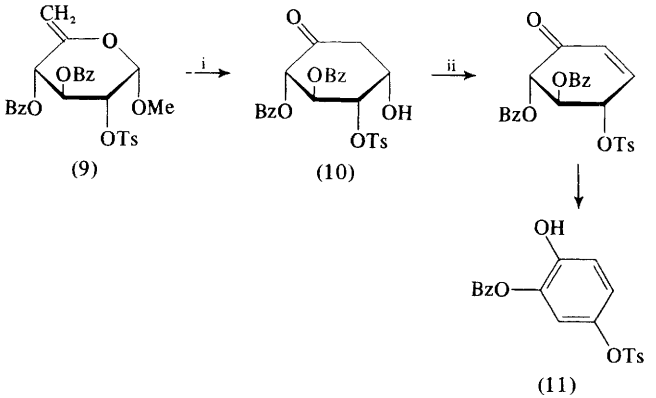


(8)

has been studied by ^{1}H and ^{13}C n.m.r.; whereas the 2,3,4,6/5 isomer exists as the keto form in aqueous solution, the 2,4,6/3,5 isomer is partially hydrated, and the 2,3,5/4,6 isomer is completely hydrated; in alkaline solution all lose water to give the enediol derivative (8), the thermodynamically most stable isomer. On acetylation, compound (8) yielded tetra-acetoxybenzene isomers, and on hydrogenation, cyclohexane pentols.[25]

On treatment with mercuric chloride, the unsaturated glycoside (9) rearranged to give the deoxy-inosose derivative (10), which on acetylation yielded the phenol derivative (11) (Scheme 2).[26]

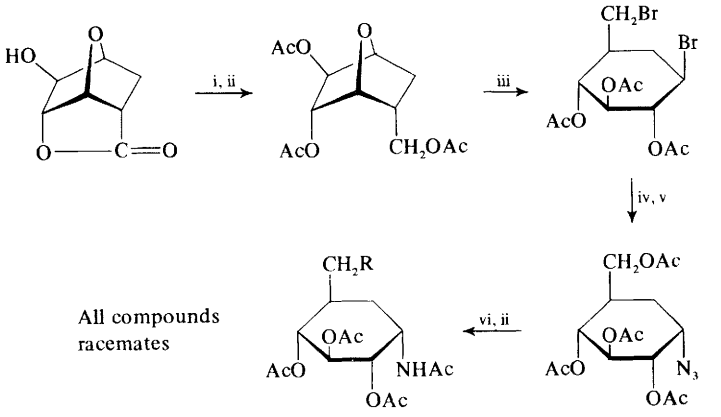
Two closely-related syntheses of penta-acetyl-DL-validamine (12; R = OAc) have been reported from the same laboratory,[27,28] the latter sequence being



Reagents: i, $HgCl_2$–H_2O–Me_2CO; ii, Ac_2O–py

Scheme 2

[25] S. J. Angyal, D. Range, J. Defaye, and A. Gadelle, *Carbohydr. Res.*, 1979, **76**, 121.
[26] R. J. Ferrier, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1455.
[27] S. Ogawa, I. Kasahara, and T. Suami, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 118.
[28] S. Ogawa, K. Nakamoto, M. Takahara, Y. Tanno, N. Chida, and T. Suami, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1174.



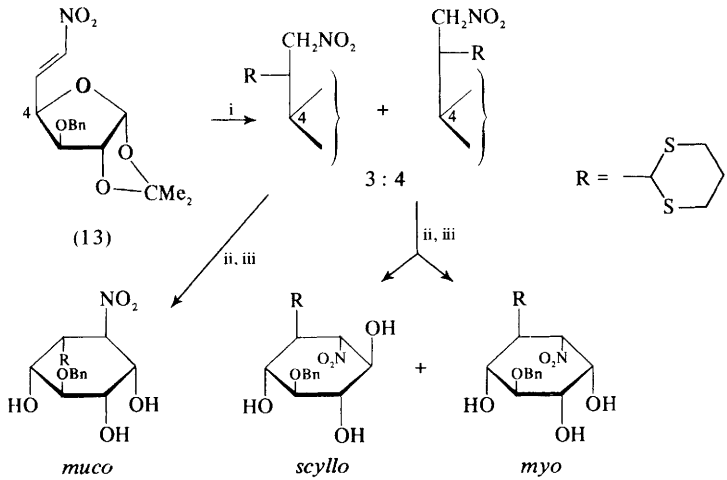
(12) R = OAc

Reagents: i, LAH; ii, Ac_2O–py; iii, HBr–HOAc; iv, NaOAc–$MeOCH_2CH_2OH$; v, N_3^-–DMF; vi, H_2–Ni

Scheme 3

outlined in Scheme 3; the *N*-acetylamino analogue (12; R = NHAc) was also prepared.[27] (See also Vol. 11, p. 150.)

A number of branched chain nitro-cyclitols have been prepared by Michael addition of lithium dithiane to unsaturated nitrosugars followed by cyclization (Scheme 4); conformational effects in the 6-nitro-*aldehydo*-sugar on the stereo-



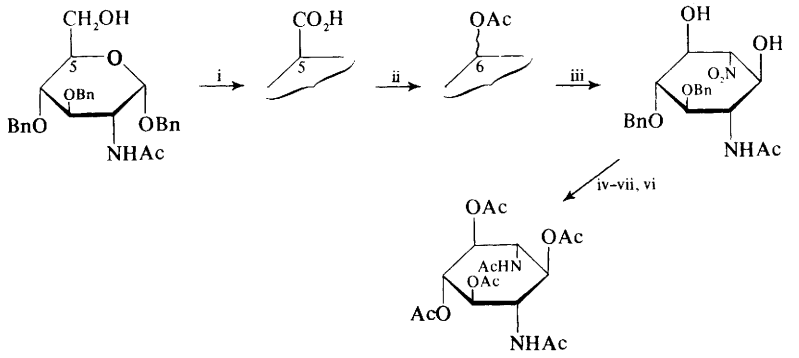
Reagents: i, Li  ; ii, H^+; iii, $NaHCO_3$ or Na_2CO_3

Scheme 4

chemistry of cyclization were discussed.[29] Similarly the *ribo*-isomer of (13) led to *myo*-, *epi*-, and *allo*-inositol analogues.[30]

X-Ray irradiation of partially deuteriated inositol has now been found to yield a primary reduction product, in contrast to an earlier report on non-deuteriated material.[31]

A synthesis of hexa-acetyl streptamine (14) from 2-acetamido-2-deoxy-D-glucose is outlined in Scheme 5.[32] The tritium-labelled 2,6-dideoxystreptamine (15) has been prepared from a trihydroxycyclohexanone derivative by a sequence of standard reactions that included the reduction of the ketone group with sodium borotritide.[33]

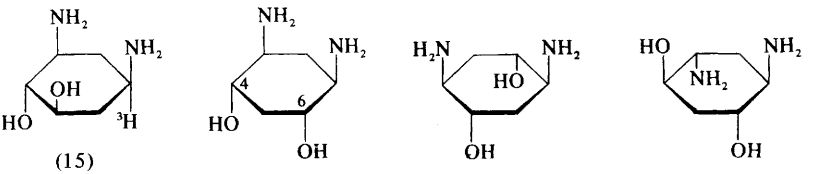


(14)

Reagents: i, CrO_3–H^+; ii, $Pb(OAc)_4$; iii, $MeNO_2$–NaOMe; iv, Ac_2O–BF_3.Et_2O; v, H_2–Ni; vi, Ac_2O–py; vii, H_2–Pd

Scheme 5

Standard transformations have also been used to convert 2,5-dideoxystreptamine (16) to its C-4 and C-6 stereoisomers, and the isomers (17) and (18) were also prepared, starting from cyclohexane diepoxides.[34]



[29] M. Funabashi and J. Yoshimura, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1425.
[30] M. Funabashi, K. Kobayashi, and J. Yoshimura, *J. Org. Chem.*, 1979, **44**, 1618.
[31] E. E. Budinski and H. C. Box, *J. Chem. Phys.*, 1978, **68**, 5296 (*Chem. Abs.*, 1979, **90**, 87 772*h*).
[32] I. Kitagawa, A. Kadota, and M. Yoshikawa, *Chem. Pharm. Bull.*, 1978, **26**, 3825.
[33] D. Mercier, S. D. Gero, and J. E. G. Barnett, *J. Antibiot.*, 1979, **32**, 81.
[34] G. Kavadias and R. Droghini, *Can. J. Chem.*, 1979, **57**, 1870.

D-Ribulose 5-phosphate has been obtained from L-*myo*-inositol-1-phosphate using an enzyme from *Phaseolus aureus*, and a mechanism involving an inos-1,3-diulose leading to a 3-keto-*ribo*-hexonic acid 6-phosphate was proposed.[35]

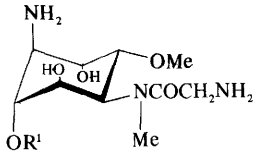
A crystal structural analysis of *muco*-inositol is referred to in Chapter 21. *N*-(2-chloroethyl)-*N*-nitrosoureido derivatives of cyclopentane- and cyclohexane-tetrols are mentioned in Chapter 18. Unsaturated glycosylated cyclitol derivatives are referred to in Chapter 12.

[35] B. P. De and B. B. Biswas, *J. Biol. Chem.*, 1979, **254**, 8717.

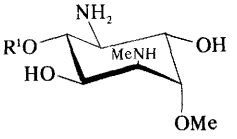
18
Antibiotics

1 Aminoglycoside Antibiotics

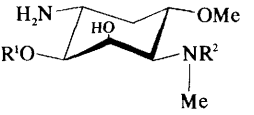
New antibiotics reported this year have included several groups of closely related pseudo disaccharides; fortimicin C, a carbamoyl derivative of fortimicin A (1), fortimicin D, the 6′-demethylated analogue of fortimicin A, and fortimicin KE, the 6′-demethylated derivative of fortimicin B (2), have been isolated from the culture broth of *Micromonospora olivoasterospora*, which also yields fortimicin A and B,[1] and sporaricin A (3, $R^2 = NH_2CH_2CO$–) and sporaricin B (3, $R^2 = H$) have been obtained from the culture broth of *Saccharopolyspora hirsuta*;[2] these are all 1,4-diamino-cyclitol α-glycosides of 6-*epi*-purpurosamine B.



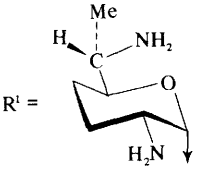
(1)



(2)

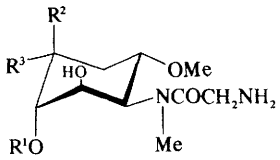


(3)

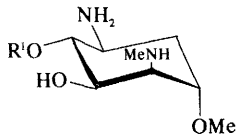


Istamycin A (4) and istamycin B (5) have been isolated from the culture broth of *Streptomyces tenjimariensis*,[3] and sannamycin A (identical with istamycin A) and sannamycin B (6) from the culture broth of *Streptomyces sannanensis*;[4] these are likewise 1,4-diamino-cyclitol α-glycosides of 6-*N*-methylpurpurosamine C. (The synthesis of this amino-sugar is mentioned in Chapter 8.) Derivatives of the amino-sugar and cyclitol constituents of fortimicin B have been prepared by benzyloxycarbonylation and alcoholysis of the antibiotic.[5]

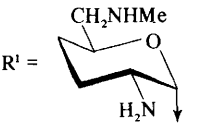
[1] M. Sugimoto, S. Ishii, R. Okachi, and T. Nara, *J. Antibiot.*, 1979, **32**, 868.
[2] T. Deushi, M. Nakayama, I. Watanabe, T. Mori, H. Naganawa, and H. Umezawa, *J. Antibiot.*, 1979, **32**, 187.
[3] Y. Okami, K. Hotta, M. Yoshida, D. Ikeda, S. Kondo, and H. Umezawa, *J. Antibiot.*, 1979, **32**, 964.
[4] T. Deushi, A. Iwasaki, K. Kamiya, T. Mizoguchi, M. Nakayama, H. Itoh, and T. Mori, *J. Antibiot.*, 1979, **32**, 1061.
[5] H. Sano, T. Sakaguchi, and Y. Mori, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2727.



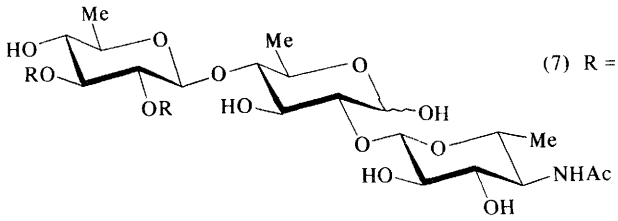
(4) $R^2 = NH_2$, $R^3 = H$
(5) $R^2 = H$, $R^3 = NH_2$



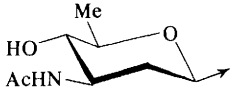
(6)



The structure (7) has been proposed for the heteropentasaccharide, viridopentaose B, isolated from the antibiotic sporoviridin, which also contains two other pentasaccharides.[6] A tetrasaccharide and a trisaccharide (8) composed of quinovose and viosamine were obtained by partial methanolysis of the pentaose (7) by removal of the 2-deoxy sugar units, the latter being characterized as the 3-amino-sugar, D-acosamine.[7]

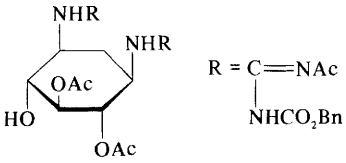


(7) R =



(8) R = H

Recent advances in the synthesis of aminoglycoside antibiotics have been reviewed.[8] Derivatized dihydrostreptomycin has been hydrolysed to give a chiral streptidine derivative (9), which could be condensed with a dihydrostreptobiosaminyl chloride derivative to give α- and β-glycosides, the α-form yielding dihydrostreptomycin again on deprotection.[9]



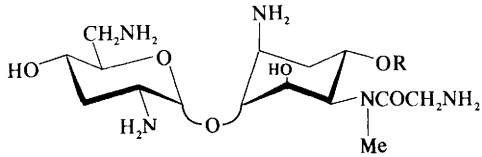
(9)

3′,4′-Dideoxykanamycin B can be prepared on a large scale from kanamycin B by hydrogenation of the corresponding 3′-ene derivative obtained by established procedures *via* the 3′,4′-epoxide.[10] 5-Epikanamycin B has been prepared using

[6] K.-I. Harada, S. Ito, and M. Suzuki, *Tetrahedron Lett.*, 1979, 3965.
[7] K.-I. Harada, S. Ito, and M. Suzuki, *Carbohydr. Res.*, 1979, **75**, C17.
[8] S. Umezawa, *Pure Appl. Chem.*, 1978, **50**, 1453.
[9] T. Yamasaki, T. Tsuchiya, and S. Umezawa, *J. Antibiot.*, 1978, **31**, 1233.
[10] T. Yoneta, S. Shibahara, T. Matsuno, S. Tohma, S. Fukatsu, S. Seki, and H. Umezawa, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1131.

the methanesulphonate displacement procedure to invert the C-5 configuration in the parent antibiotic,[11] and 3′-deoxyseldomycin 5 together with the corresponding 2′-deoxy regioisomer have been obtained by reduction of a 2′,3′-epimino derivative prepared from seldomycin 5.[12] 4′-Deoxy-6′-*N*-methyl-amikacin has been prepared from 1,3-ureido-kanamycin A by Raney nickel treatment of its 4′-thioacetate followed by 6′-*N*-methylation; the activity of this derivative suggests that the 4′-hydroxy- and 6′-amino-groups in amikacins play little part in their defence against inactivating enzymes.[13] Other derivatives of pseudotrisaccharides reported include *NN*-dialkyl derivatives of kanamycins A and B,[14] 1-*N*-(1,3-dihydroxy-2-propyl)kanamycin B, which showed good antibacterial activity,[15] 6′-*N*-[^{14}C]acetyl-tobramycin, which was used to study the binding of the antibiotic to *E. coli* ribosomes,[16] and 2′-*N*-propyl-butirosin A, which proved to be as good an antimicrobial as butirosin A itself.[17]

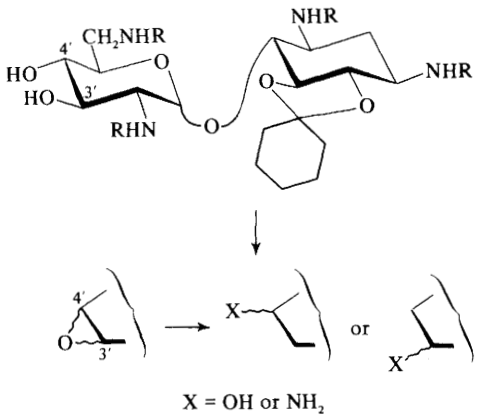
Lividamine has been converted to 2-deoxy-4-*N*-glycyl-6-*O*-α-nebrosaminyl-fortamine (10) and its 3-de-*O*-methyl analogue (11), which are weaker antibacterials than the related sporaricin A mentioned above.[18] 4-*N*-Acyl- and 4-*N*-alkyl derivatives of fortimicin B have also been prepared, and their antibacterial behaviour investigated.[19]



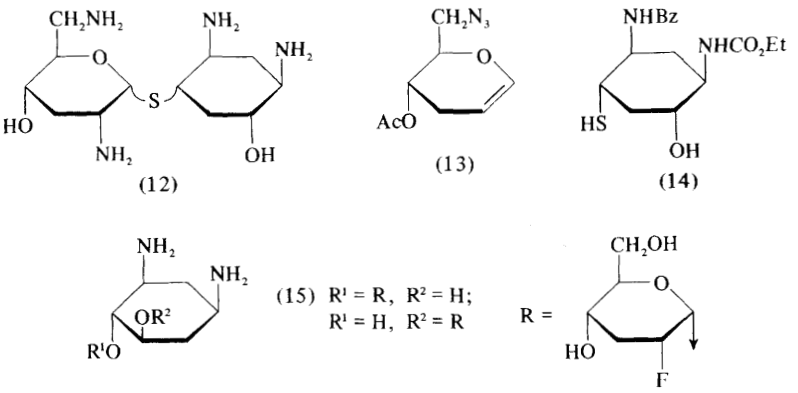
(10) R = Me
(11) R = H

Neamine has been converted to its eight possible 3′- and 4′-amino-deoxy and deoxy-hydroxy analogues by conversion to 3′,4′-anhydro-D-allo and D-galacto intermediates, which were then reduced to 3′- and 4′-deoxy compounds and appropriately transformed (Scheme 1). Some 4′-branched-chain sugar analogues were also prepared.[20] A thio analogue of neamine (12) has been prepared by coupling the nitrosyl chloride adduct of the glycal (13) to the thio-streptamine (14) followed by reduction and deprotection.[21]

[11] T. Suami and K. Nakamura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 955.
[12] H. Matsushima, Y. Mori, and K. Kitaura, *J. Am. Chem. Soc.*, 1978, **51**, 3553.
[13] T. Naito, S. Nakagawa, S. Toda, K.-I. Fujisawa, and H. Kawaguchi, *J. Antibiot.*, 1979, **32**, 659.
[14] V. Kumar and W. A. Remers, *J. Med. Chem.*, 1979, **22**, 432.
[15] K. Richardson, K. W. Brammer, S. Jevons, R. M. Plews, and J. R. Wright, *J. Antibiot.*, 1979, **32**, 973.
[16] F. Le Goffic, M.-L. Capman, F. Tangy, and M. Baillarse, *Eur. J. Biochem.*, 1979, **102**, 73.
[17] K. Nara, K. Yoshioka, and M. Kida, *Chem. Pharm. Bull.*, 1979, **27**, 65.
[18] T. Yamaguchi, Y. Kyotani, I. Watanabe, S. Kato, Y. Takahashi, M. Nagakura, and T. Mori, *J. Antibiot.*, 1979, **32**, 1137.
[19] M. Sato and Y. Mori, *J. Antibiot.*, 1979, **32**, 371.
[20] F. R. Pfeiffer, S. J. Schmidt, C. M. Kinzig, J. R. E. Hoover, and J. A. Weisbach, *Carbohydr. Res.*, 1979, **72**, 119.
[21] G. Kavadias, R. Droghini, Y. Pépin, M. Ménard, and P. Lapointe, *Can. J. Chem.*, 1979, **57**, *1056*.



Scheme 1



2′,3′-Dideoxy-2′-fluoro-pseudodisaccharides (15) have been synthesized using the glycal addition route to glycosides with 3,4,6-tri-*O*-acetyl-2-fluoro-D-glucal.[22]

4′-*N*-propyl sorbistin A_1 has been prepared, and found to be as effective as sorbistin A_1 against Gram positive bacteria.[17]

The mycinose moiety of tylosin can be specifically removed under mild conditions by oxidation of the free hydroxy-group of the sugar in the otherwise protected antibiotic to the corresponding glycos-4-ulose, which with a primary amine hydrochloride is degraded to a pyrrole derivative, liberating the aglycone.[23] Selective *N*-acylation of kanamycin A has been studied, and conditions found for the preferential formation of the mono-6″-*N*-acyl or the 3-*N*-acyl derivative.[24] Base treatment of 3′-chloro-3′-deoxy-pseudotrisaccharides derived

[22] G. Vass, A. Rolland, J. Cleophax, D. Mercier, B. Quiclet, and S. D. Gero, *J. Antibiot.*, 1979, **32**, 670.

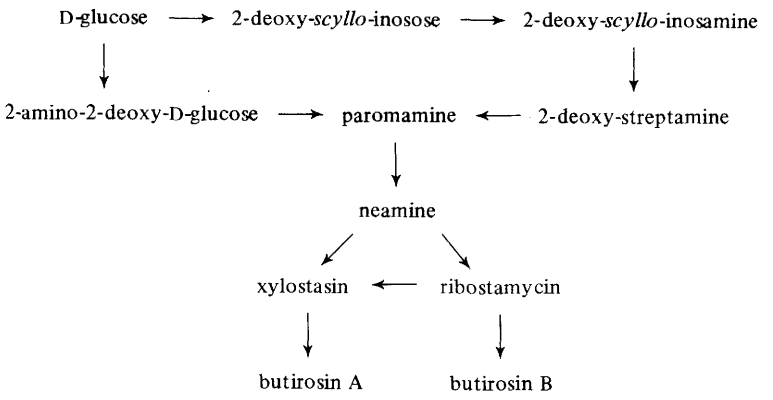
[23] A. A. Nagel and L. A. Vincent, *J. Org. Chem.*, 1979, **44**, 2050.

[24] M. J. Cron, J. G. Keil, J.-S. Lin, M. V. Ruggeri, and D. Walker, *J. Chem. Soc., Chem. Commun.*, 1979, 266.

from butirosin have given new cyclic derivatives containing a 3′,5″-anhydro bridge.[25] An improved procedure for the preparation of triacetyloleandomycin has been reported.[26]

Studies on the biosynthesis of butirosins have suggested the sequence of transformations outlined in Scheme 2.[27,28]

The synthesis of 6-*epi*-purpurosamine B and other amino-sugar components of amino-glycoside antibiotics is referred to in Chapter 8.



Scheme 2

2 Macrolide Antibiotics

The synthesis of stereoisomers of the amino-sugar component of megalomycins, together with full details on the revised structure of these antibiotics reported last year (see Vol. 12, p. 151) are mentioned in Chapter 8.

The antifungal polyene antibiotics levorin A_2 and candicidin D, which contain mycosamine, are identical.[29] The unusual deoxysugar L-digitoxose (2,6-dideoxy-L-ribohexose) has been isolated from the acid hydrolysis products of the macrolide antibiotics nystatin A_3, candidinin, and polyfungin B.[30]

Maridomycin III, in which both ester groups are propionyl derivatives, is the principal component of the maridomycin complex elaborated by a valine resistant mutant of *Streptomyces hydroscopicus* (the six components differ depending on whether acetyl, propionyl, or isovaleryl ester groups are present).[31] The

[25] T. Asako, K. Yoshioka, H. Mabuchi, and K. Hiraga, *Heterocycles*, 1978, **11**, 197 (*Chem. Abs.*, 1979, **90**, 204 395*a*).

[26] M. Khrustov and N. Petkov, *Formatsiya (Sofia)*, 1977, **27**, 1 (*Chem. Abs.*, 1979, **90**, 23 458*c*).

[27] K. Takeda, K. Aihara, T. Furumai, and Y. Ito, *J. Antibiot.*, 1979, **32**, 18.

[28] J. Furumai, K. Takeda, A. Kinumaki, Y. Ito, and T. Okuda, *J. Antibiot.*, 1979, **32**, 891.

[29] J. Zielinski, H. Borowy-Borowski, J. Golik, J. Gumieniak, T. Ziminski, P. Kolodziejczyk, J. Pawlak, E. Borowski, Yu. Shenin, and A. I. Filippova, *Tetrahedron Lett.*, 1979, 1791.

[30] J. Zielinski, E. Jereczek, P. Sowinski, L. Falkowski, A. Rudowski, and E. Borowski, *J. Antibiot.*, 1979, **32**, 565.

[31] K. Miyagawa, M. Suzuki, and M. Uchida, *Agric. Biol. Chem.*, 1979, **43**, 1111.

deltamycins A_1–A_4 are closely related compounds, being 9-oxo analogues of the maridomycins, with the mycarose residue substituted at O-4 by acetyl, propionyl, butyryl, or isovaleryl residues, respectively, and deltamycin A_4 was shown to be identical to carbomycin A.[32] (For earlier studies on the maridomycins and carbomycin A, see Vol. 10, p. 135.)

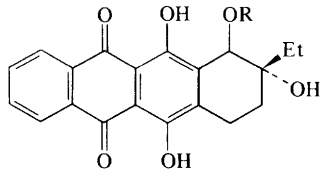
3 Anthracycline Antibiotics

Two new compounds, roseorubicin A and B, have been isolated from the culture broth of *Actinomyces roseoviolaceus*. Roseorubicin A (16) contains a pentasaccharide chain, L-rhodinose → L-rhodinose → 2-deoxy-L-fucose → L-rhososamine → L-rhodosamine, which on mild acid hydrolysis gave roseorubicin B, the corresponding tetracycline disaccharide, which in turn could be selectively hydrolysed to γ-rhodomycin 1, the simple rhodosaminyl glycoside.[33]

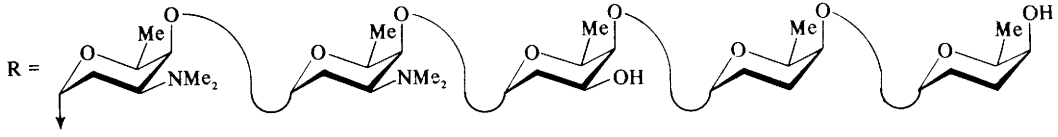
Structures have been reported for the anti-tumour compound aclacinomycin A and its analogues, which are elaborated by *Streptomyces galilaeni*; they contain trisaccharide chains attached to the aglycone (17), in most cases having a deoxysugar (L-amicetose, L-rhodinose) or a ketosugar (D- or L-cinerulose A, L-aculose) attached through 2-deoxy-L-fucose to L-rhodosamine by α-1 → 4 links.[34]

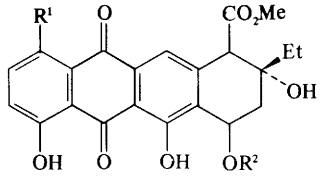
Horton's group has reported syntheses of daunorubicin analogues (18) by appropriate condensation of modified glycosyl halides or glycals with daunomycinone; these include derivatives of 3-amino-2,3,6-trideoxy-α-D-*ribo*-hexopyranoside,[35] 3-amino-2,3,6-trideoxy-α- and -β-D-arabinopyranoside and 3,6-diamino-2,3,6-trideoxy-α-D-*ribo*-hexopyranoside,[36] and 3,6-dideoxy-α- and -β-D-*ribo*- and -L-*lyxo*-hexopyranoside.[37] 4′-*O*-Methyl-daunorubicin (19) and 4′-*O*-methyl-adriamycin (20) together with their 4′-*epi*-isomers have likewise been prepared from appropriately modified sugars by Koenigs–Knorr condensation with the tetracycline.[38] Daunorubicin and adriamycin have been converted to *N*-alkyl and *NN*-dialkyl analogues by cyanoborohydride reduction of derivatives formed from aldehydes or ketones; the *N*-alkyl derivatives showed enhanced anti-tumour activity with reduced cardiotoxicity.[39] 4′-*O*-Tetrahydropyranyl derivatives of daunorubicin, daunomycin, and adriamycin have also been prepared.[40]

[32] Y. Shimauchi, K. Kubo, K. Osumi, K. Okamura, Y. Fukugawa, T. Ishikura, and J. Lein, *J. Antibiot.*, 1979, **32**, 878.
[33] Y. Matsuzawa, A. Yoshimoto, T. Oki, T. Inui, T. Takeuchi, and H. Umezawa, *J. Antibiot.*, 1979, **32**, 420.
[34] T. Oki, I. Kitamura, Y. Matsuzawa, N. Shibamoto, T. Ogasawara, A. Yoshimoto, T. Inui, H. Naganawa, K. Takeuchi, and H. Umezawa, *J. Antibiot.*, 1979, **32**, 801.
[35] D. Horton, R. G. Nickol, W. Weckerle, and E. Winter-Mihaly, *Carbohydr. Res.*, 1979, **76**, 269.
[36] E.-F. Fuchs, D. Horton, W. Weckerle, and B. Winter, *J. Antibiot.*, 1979, **32**, 223.
[37] E.-F. Fuchs, D. Horton, W. Weckerle, and E. Winter-Mihaly, *J. Med. Chem.*, 1979, **22**, 406.
[38] G. Cassinelli, D. Ruggieri, and F. Arcamone, *J. Med. Chem.*, 1979, **22**, 121.
[39] G. L. Tong, H. Y. Wu, T. H. Smith, and D. W. Henry, *J. Med. Chem.*, 1979, **22**, 912.
[40] H. Umezawa, Y. Takahashi, M. Kinoshita, H. Naganawa, T. Matsuda, M. Ishizuka, K. Tatsuta, and T. Takeuchi, *J. Antibiot.*, 1979, **32**, 1082.

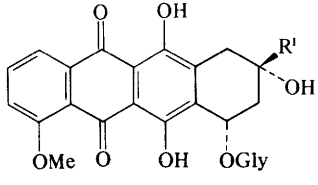


(16)

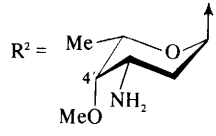




(17) R^1 = H or OH
R^2 = trisaccharide



(18) R^1 = Ac
(19) R^1 = Ac, Gly = R^2

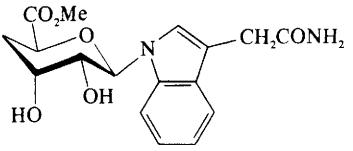


(20) $R^1 = CH_2OH$, Gly = R^2

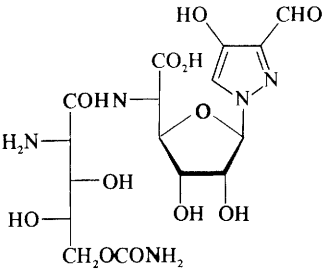
Nogalamycin has been converted to a series of aglycone analogues, and the stereochemistry of the anthracycline ring investigated.[41]

4 Nucleoside Antibiotics

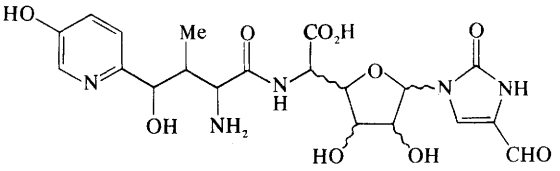
A new antibiotic neosidomycin, isolated from *Streptomyces hygroscopicus*, has been shown to be the indoyl-uronic acid derivative (21) (the sugar may be D or L).[42] A new active polyoxin constituent, polyoxin N, has been isolated from the culture broth of *Streptomyces piomogenus* and assigned structure (22).[43] The antibiotic nikkomycin X has been shown to have the structure (23), an analogue of nikkomycin Z, which is the corresponding uracil-1-yl derivative of the same sugar unit.[44] Sinefungin, an antifungal antibiotic that is a potent inhibitor of tRNA and protein methyl transferases *in vitro*, has the structure (24).[45] Tunicaminyl uracil, a degradation product of tunicamycin, has been shown to have the structure (25).[46]



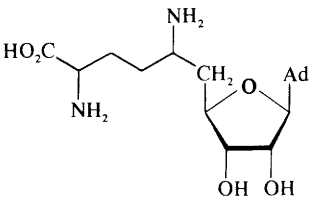
(21)



(22)



(23)



(24)

[41] P. F. Wiley, D. W. Elrod, D. J. Houser, J. L. Johnson, L. M. Pschigoda, W. C. Krueger, and A. Moscowitz, *J. Org. Chem.*, 1979, **44**, 4030.

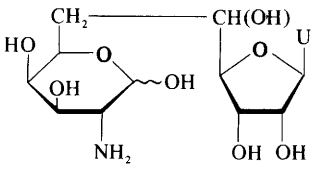
[42] R. Furuta, S. Naruto, A. Tamura, and K. Yokogawa, *Tetrahedron Lett.*, 1979, 1701.

[43] M. Uramoto, J. Uzawa, S. Suzuki, K. Isono, J. G. Liehr, and J. A. McCloskey, *Nucleic Acids Res. Symp. 6th, Spec. Publ.*, 1978, **5**, 327 (*Chem. Abs.*, 1979, **90**, 121 938*d*).

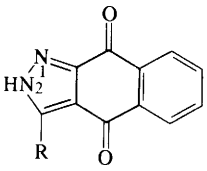
[44] H. Hagenmaier, A. Keckeisen, H. Zähner, and W. A. Konig, *Liebigs Ann. Chem.*, 1979, 1494.

[45] M. Vedel, F. Lawrence, M. Robert-Gero, and E. Lederer, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 371.

[46] T. Ito, Y. Kodama, K. Kawamura, K. Suzuki, A. Takatsuki, and G. Tamura, *Agric. Biol. Chem.*, 1979, **43**, 1187.



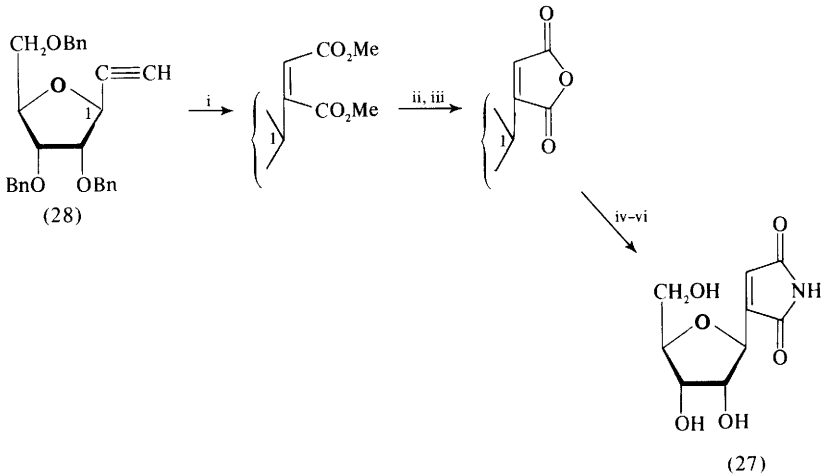
(25)



(26) R = H or CO_2Et

2′-Deoxycoformycin and cordycepin have been isolated from a new strain of *Aspergillus nidulans* Y 176-2 cultured on wheat-bran, the first example of a potent adenosine deaminase inhibitor (2′-deoxycoformycin) occurring in a fungus.[47]

Tubercidin (7-deaza-adenosine) has been synthesized by conventional procedures from 4-chloro-5,7-diazaindoline and tetra-acetyl ribofuranose,[48] and the α-anomer was also prepared.[48,49] A series of glycosyl naphtha [2,3-*c*]pyrazole-4,9-dione derivatives have been prepared from glycals and the parent heterocycle (26), giving either 1-*N*- or 2-*N*-substituted compounds; the former show cytostatic activity against HeLa cells, irrespective of the particular pyranosyl substituent, whereas the 2-*N*-substituted compounds were inactive.[50]



(28) (27)

Reagents: i, MeOH–CO–$PdCl_2$; ii, KOH; iii, $(CF_3CO)_2O$; iv, NH_3; v, AcCl–DMF; vi, H_2–Pd

Scheme 3

[47] K. Kodama, H. Kusakabe, H. Machida, Y. Midorikawa, S. Shibuya, A. Kuninaka, and H. Yoshino, *Agric. Biol. Chem.*, 1979, **43**, 2375.

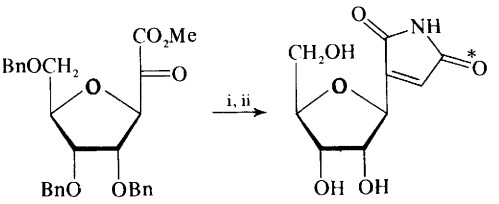
[48] L. V. Ektova, V. N. Tolkachev, M. Z. Kornveits, and M. N. Preobrazhenskaya, *Bioorg. Khim.*, 1978, **4**, 1250 (*Chem. Abs.*, 1979, **90**, 23 501*m*).

[49] L. V. Ektova, V. N. Tolkache, N. L. Radyukina, T. P. Ivanova, Ya. V. Dobrynin, and M. N. Preobrazhenskaya, *Bioorg. Khim.*, 1979, **5**, 1369.

[50] M. T. Garcia-Lopez, F. G. De Las Heras, M. Medrano, E. Sanchez, M. Stud, and G. Alonso, *Eur. J. Med. Chem. – Chim. Ther.*, 1979, **14**, 151 (*Chem. Abs.*, 1979, **91**, 211 734*j*).

Conjugates of 1-β-D-arabinofuranosyl-cytosine have been prepared with cortisol and cortisone,[51] and with prednisolone and prednisone,[52] which show good anti-tumour activity.

A new synthesis of showdomycin (27), outlined in Scheme 3, utilizes the carboxylation of the ribofuranosyl ethyne (28).[53] Showdomycin, labelled with carbon-14 at the β-carbonyl group, has been prepared by Wittig condensation of the 3,6-anhydrohept-2-ulosonic acid derivative (29) with the phosphorane prepared from [1-^{14}C]chloroacetamide (Scheme 4).[54]

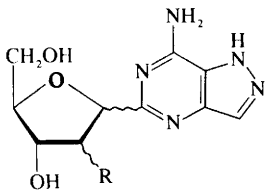


(29)

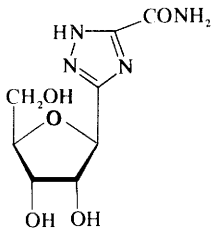
Reagents: i, $Ph_3P{=}CH^{14}CONH_2$; ii, BCl_3

Scheme 4

A number of isoformycins (30) have been prepared from appropriate glycofuranosyl thioformidate derivatives by condensation with 3-cyano-4-aminopyrazole, and their conformations were investigated.[55] A glycosyl thioimidate has similarly been used to prepare the triazole *C*-glycoside analogue (31) of ribavirin by condensation with the hydrazone of ethyl oxamate,[56,57] and the thiazole *C*-glycoside analogue was also synthesized.[57]



(30) R = H or OH



(31)

[51] C. I. Hong, A. Nechaev, and C. R. West, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 1223.

[52] C. I. Hong, A. Nechaev, and C. R. West, *J. Med. Chem.*, 1979, **22**, 1428.

[53] J. G. Buchanan, A. R. Edgar, M. J. Power, and C. T. Shanks, *J. Chem. Soc., Perkin Trans. 1*, 1979, 225.

[54] H. Minato, Y. Katsuyama, T. Nagasaki, J. Irisawa, and K. Igarashi, *J. Labelled Comp. Radiopharm.*, 1978, **14**, 455 (*Chem. Abs.*, 1978, **89**, 215 689*e*).

[55] Huynh Dinh Tam, R. S. Sarfati, J. Igolen, J. M. Neumann, and Tran Dinh Son, *Nouv J. Chim.*, 1978, **2**, 357 (*Chem. Abs.*, 1979, **90**, 55 239*y*).

[56] T. Vaněk, J. Farkaš, and J. Gut, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1334.

[57] T. Vaněk, J. Farkaš, and J. Gut, *Nucleic Acids Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 173 (*Chem. Abs.*, 1979, **90**, 87 812*w*).

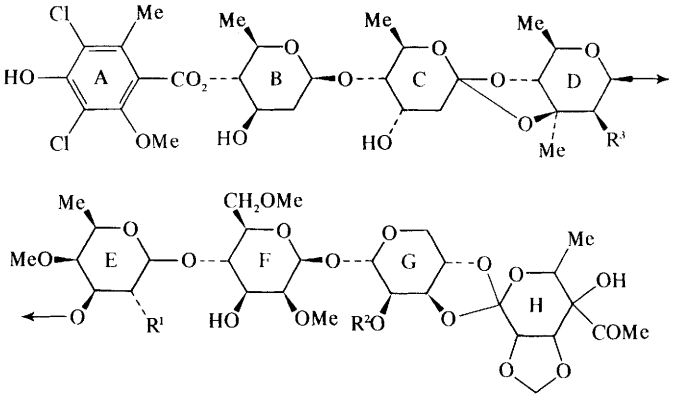
5 Orthosomycin Antibiotics

The above title has been proposed for the oligosaccharide antibiotics, which include the everninomicins, flambamycins, destomysins, avilamycin, curamycin, hygromycin, and related compounds.[58] A review on orthosomycins has appeared.[59]

The structure (32) has been proposed for flambamycin,[58,60] the anomeric configuration of rings, D, F, and G being established by high field ^{13}C n.m.r.;[60] for the latter studies, labilose, (6-deoxy-2,4-di-*O*-methyl-D-galactose), the glycoside component of labilomycin, was also synthesized for comparative purposes. Other papers have recorded ^{13}C n.m.r.[61] and mass spectral[62] data on flambamycin and its derivatives.

Avilamycin A (33) has the same structure as flambamycin except that the sugar evermicose (2,6-dideoxy-3-*C*-methyl-D-*arabino*-hexose) (unit D) in the latter is replaced by evalose (6-deoxy-3-*C*-methyl-D-mannose). Avilamycin C differs from avilamycin A in having a 1-hydroxyethyl side-chain instead of acetyl in unit H.[63,64]

A structure has been proposed for curamycin A (34) based on chemical and spectroscopic studies and comparison with flambamycin and avilamycin.[65]

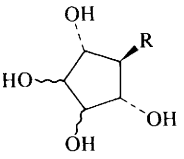


(32) R^1 = OH, R^2 = Me_2CHCO–, R^3 = OH
(33) R^1 = H, R^2 = Me_2CHCO–, R^3 = OH
(34) R^1 = OH, R^2 = Ac, R^3 = H

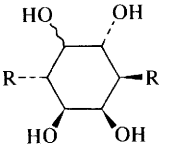
[58] W. D. Ollis, C. Smith, and D. E. Wright, *Tetrahedron*, 1979, 105.
[59] D. E. Wright, *Tetrahedron*, 1979, 1207.
[60] L. Valente, L. E. S. Barata, A. Olesker, R. Rabanal, G. Lukacs, and T. T. Thang, *Tetrahedron Lett.*, 1979, 1149.
[61] W. D. Ollis, I. O. Sutherland, B. F. Taylor, C. Smith, and D. E. Wright, *Tetrahedron*, 1979, **35**, 993.
[62] W. D. Ollis, I. O. Sutherland, B. F. Taylor, C. Smith, and D. E. Wright, *Tetrahedron*, 1979, **35**, 1003.
[63] W. Heilman, E. Kupfer, W. Keller-Schierlein, H. Zähner, H. Wolf, and H. H. Peter, *Helv. Chim. Acta*. 1979, **62**, 1.
[64] W. Keller-Schierlein, W. Heilman, W. D. Ollis, and C. Smith, *Helv. Chim. Acta*, 1979, **72**, 7.
[65] A. K. Ganguly, A. K. Bose, and N. F. Cappuccino, *J. Antibiot.*, 1979, **32**, 1213.

6 Miscellaneous Antibiotics

Methods for synthesizing anisomycin and pentenomycin,[66] and the chemistry and biological transformations of bleomycin and phleomycin have been reviewed.[67] D-Ribo-furanosyl and -pyranosyl derivatives of *N*-methyl-*N*-nitrosourea have been prepared as analogues of streptozotocin [2-deoxy-2-(*N*-methyl-*N*-nitrosoureido)-D-glucose]; they were reported to be more active against L1210 leukaemia in mice and less toxic than the parent antibiotic.[68] Likewise *N*-(2-chloroethyl)-*N*-nitrosoureido derivatives of cyclopentane tetrols (35) and cyclohexane tetrols (36) have been synthesized for comparison with streptozotocin.[69]



(35)



(36)

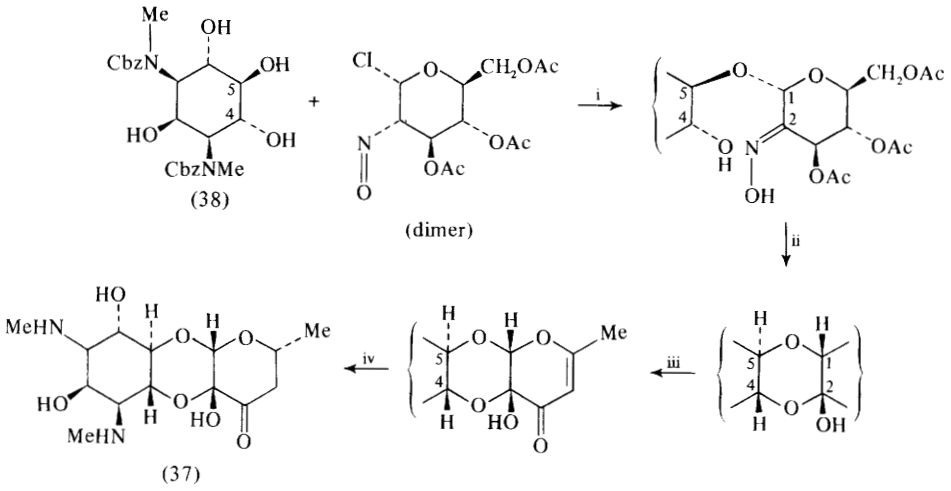
R = $-NHCONCH_2CH_2Cl$
|
NO

Biosynthetic studies have established that D-glycosamine is incorporated into streptozotocin, and L-citrulline and methionine furnish the amide side-chain and *N*-methyl group, respectively, but the source of the nitroso-group was not established.[70]

2-Deoxy-2-ureido-D-glucose, which shows weak antimicrobial activity against some Gram negative bacteria and fungi, has been isolated from *Streptomyces halstedii*; in aqueous solution it slowly cyclized to give the corresponding 1,2-diazolidone derivative. Isomeric ureido derivatives of D-galactose and D-mannose cyclized similarly.[71]

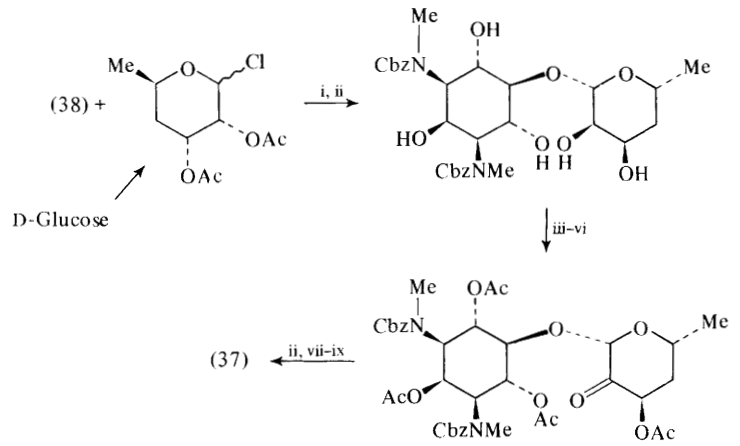
Spectinomycin (37) has been synthesized in two laboratories; one sequence utilized the nitrosyl chloride adduct of glucal with the actinamine derivative (38) illustrated in Scheme 5,[72] whereas the other, outlined in Scheme 6, used a preformed 4,6-dideoxy-D-*ribo*-hexose derivative for the initial glycosidation step; a route that makes elegant use of cyclic blocking groups in the sugar ring to allow selective oxidation of the C-4 and C-3 hydroxy-groups in turn.[73]

[66] J. P. H. Verheyden, A. C. Richardson, R. S. Bhatt, B. D. Grant, W. L. Fitch, and J. G. Moffatt, *Pure Appl. Chem.*, 1978, **50**, 1363.
[67] T. Takita, *Bioact. Pept. Prod. Microorg.*, 1978, 35 (*Chem. Abs.*, 1979, **91**, 141 107*f*).
[68] A. Moruzzi, J. L. Montero, J. Oiry, and J. L. Imbach, *Eur. J. Medicin. Chem. – Chim. Ther.*, 1978, **13**, 421 (*Chem. Abs.*, 1979, **90**, 145 679*w*).
[69] T. Suami, K. Tadano, and W. T. Bradner, *J. Med. Chem.*, 1979, **22**, 314.
[70] S. Singaram, R. S. Lawrence, and U. Hornemann, *J. Antibiot.*, 1979, **32**, 379.
[71] S. Omoto, T. Shomura, H. Suzuki, and S. Inoye, *J. Antibiot.*, 1979, **32**, 436.
[72] D. R. White, R. D. Birkenmeyer, R. C. Thomas, S. A. Mizsak, and V. H. Wiley, *Tetrahedron Lett.*, 1979, 2737.
[73] S. Hanessian and R. Roy, *J. Am. Chem. Soc.*, 1979, **101**, 5839.



Reagents: i, DMF; ii, MeCHO–MeCN–HCl; iii, $KHCO_3$–MeCN; iv, H_2–Pd

Scheme 5

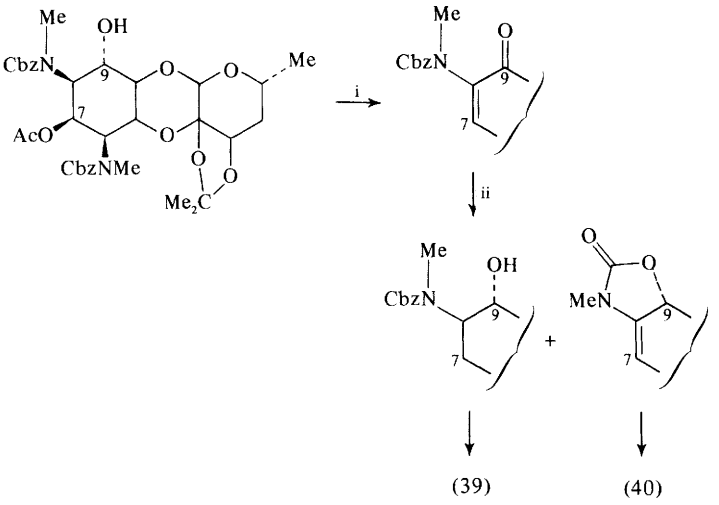


Reagents: i, CF_3SO_3Ag; ii, NaOMe; iii, $MeC(OMe)_3$–H^+; iv, Ac_2O–py–DMAP; v, HOAc–H_2O; vi, CrO_3–py–HCl; vii, Bu_2SnO; viii, Br_2–Bu_3SnOMe; ix, H_2–Pd

Scheme 6

7-Deoxyspectinomycin (39) and 7-deoxy-8-*epi*-4-(*R*)-dihydro-spectinomycin (40) have been prepared by chromic oxide oxidation of a dihydro-spectinomycin derivative at C-9, with concomitant elimination of a C-7 acetoxy-group, to give an unsaturated ketone intermediate, which could in turn be reduced by sodium borohydride to the products as indicated in the part-sequence shown in Scheme 7.[74]

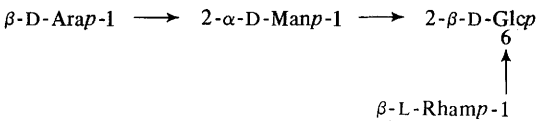
[74] L. Foley, J. T. S. Lin, and M. Weigele, *J. Antibiot.*, 1979, **32**, 418.



Reagents: i, CrO_3-py; ii, $NaBH_4$

Scheme 7

A complete structure has been proposed for the antibiotic ristocetin A, which contains the sugar units β-D-mannosyl, α-L-ristosaminyl, and the tetrasaccharide (41) attached as aryl or benzyl glycosides to a complex macrocyclic core of phenolic amino-acids. Much of the evidence came from a 270 MHz ^{1}H n.m.r. study on the ristosaminyl-core obtained after removal of the other sugar residues by mild acid hydrolysis.[75,76]



(41)

Two further degradation products have been obtained from moenomycin A, suggesting that when the part structures (42) and (43), are linked together they form the complete antibiotic.[77] Another report describes the glucose-glucosamine disaccharide present in fragment (42), which was obtained from moenomycin A by acid hydrolysis.[78] Earlier work on the structure and properties of moenomycin A has been reviewed.[79]

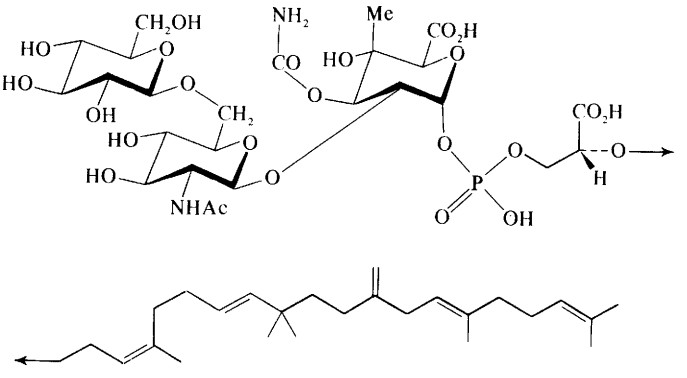
[75] D. H. Williams, V. Rajananda, G. Bojesen, and M. P. Williamson, *J. Chem. Soc., Chem. Commun.*, 1979, 906.

[76] D. H. Williams, V. Rajananda, and J. R. Kalman, *J. Chem. Soc., Perkin Trans. 1*, 1979, 787.

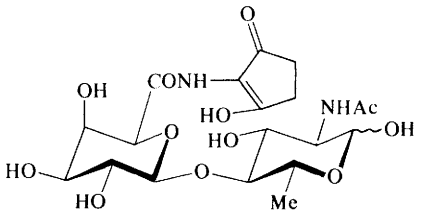
[77] F.-J. Witteler, P. Welzel, H. Duddeck, G. Höfle, W. Riemer, and H. Budzikiewicz, *Tetrahedron Lett.*, 1979, 3493.

[78] R. Tschesche and J. E. Moch, *Tetrahedron Lett.*, 1979, 2119.

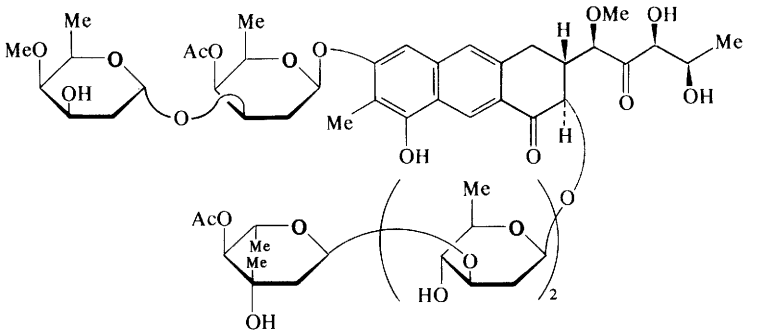
[79] P. Welzel, *Symp. Pap. I.U.P.A.C. Int. Symp. Chem. Nat. Prod. 11th*, 1978, 4 (Part 2), 394 (*Chem. Abs.*, 1979, **91**, 211 687*w*).



(42)



(43)

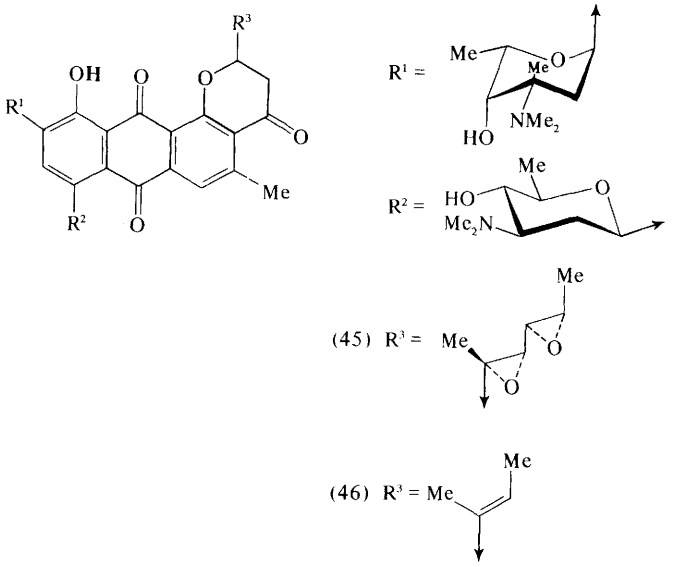


(44)

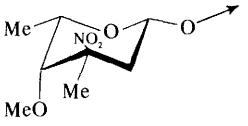
A combination of ^{1}H n.m.r. and ^{13}C n.m.r. techniques has been used to confirm the gross structure of chromomycin A_3, but with the positions of the acetate and glycosidic groups on the disaccharide non-terminal sugar unit interchanged to give the revised structure (44).[80]

[80] J. Thiem and B. Meyer, *J. Chem. Soc., Perkin Trans. 2*, 1979, 1331.

X-Ray crystal analysis has established the structure of the polyether antibiotic carriomycin, which contains 2,3,6-trideoxy-4-*O*-methyl-D-*erythro*-hexose,[81] and has shown that hedamycin is the interesting di-*C*-glycoside (45) involving amino and branched-chain sugars, kidamycin being the closely related analogue (46).[82]



The structure of rubradirin has also been established. This contains the unusual branched-chain nitro-sugar rubranitrose that is linked as a β-glycoside (47) to a complex aglycone, which is structurally related to ansamycin, novobiocin, and everninomicin antibiotics; evernitrose is the L-*arabino* stereoisomer of (47).[83]



(47)

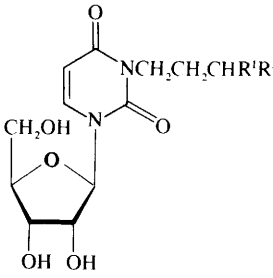
[81] H. Nakayama, N. Otake, H. Mujamae, S. Sato, and Y. Saito, *J. Chem. Soc., Perkin Trans. 2*, 1979, 293.
[82] M. Zehnder, U. Séquin, and H. Nadig, *Helv. Chim. Acta*, 1979, **62**, 2525.
[83] H. Hoeksema, S. A. Mizsak, and L. Baczynskyj, *J. Antibiot.*, 1979, **32**, 773.

19
Nucleosides

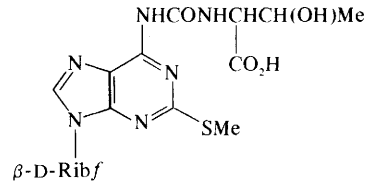
1 Introduction

Reviews have appeared on the general chemistry of nucleosides,[1] and on the conformation[2] and chromatography[3] of nucleosides and nucleotides. In addition, symposium papers on the chemistry and biology of nucleosides and nucleotides, including reviews on the synthesis of nucleosides, the application of ^{13}C n.m.r., and *C*-glycosyl nucleosides, have been published.[4]

Nucleoside X (1) has been isolated from the variable loop of *E. coli* tRNA; the analogues (2) and (3) were prepared from uridine.[5] The modified mammalian tRNA nucleoside (4) has been characterized by mass spectrometry.[6] 2′-*O*-Methyluridine has been isolated from human urine,[7] and a new species of *Streptomyces herbaceus* has been shown to produce Ara-A(9-β-D-arabinosyladenine).[8]



(1) $R^1 = NH_2$, $R^2 = CO_2H$
(2) $R^1 = NH_2$, $R^2 = H$
(3) $R^1 = H$, $R^2 = CO_2H$



(4)

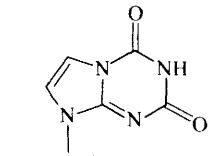
[1] R. T. Walker, in 'Comprehensive Organic Chemistry', Vol. 5, ed. E. Haslam, Pergamon, Oxford, 1979, p. 53.
[2] D. B. Davies, *Prog. Nucl. Magn. Reson. Spectrosc.*, 1978, **12**, 135 (*Chem. Abs.*, 1979, **90**, 55 169*a*).
[3] K. H. Schram and J. A. McCloskey, *Chromatogr. Sci.*, 1979, **9**, 1149 (*Chem. Abs.*, 1979, **91**, 145 986*q*).
[4] 'Chem. Biol. Nucleosides, Nucleotides', [Pap. Symp.], 1976 (publ. 1978), ed. R. E. Harmon, R. K. Robins, and L. B. Townsend, Academic Press, New York.
[5] F. Seela, Q. H. T. Thi, and D. Hasselmann, *Chem. Ber.*, 1979, **112**, 700.
[6] Z. Yamaizumi, S. Nishimura, K. Limburg, M. Raba, H. J. Gross, P. F. Crain, and J. A. McCloskey, *J. Am. Chem. Soc.*, 1979, **101**, 2224.
[7] N. C. De and G. B. Chheda, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, **6**, 371.
[8] J. Awaya, K. Matsuyama, Y. Iwai, S. Omura, H. Oiwa, Y. Hasegawa, and T. Ikai, *J. Antibiot.*, 1979, **32**, 1050.

2 Synthesis

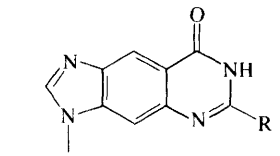
A monograph has appeared that includes new and improved synthetic procedures, methods and techniques, for the synthesis of nucleic acid derivatives including nucleosides.[9]

The mechanism of the Friedel–Crafts-catalysed Hilbert–Johnson reaction has been reviewed, with particular reference to the reaction of silylated uracils and stannic chloride or trimethylsilyl trifluoromethanesulphonate.[10]

Standard methods involving glycosyl halide or ester derivatives in reaction with protected heterocycles have been applied to the synthesis of various glycosyl derivatives of 5-fluoro-uracil and -cytosine,[11] a number of nitroimidazoles and -pyrazoles,[12] thiadiazines and oxadiazolo-thiadiazines,[13] and 5-methylthio derivatives of -uracil, -4-thiouracil, and -cytosine,[14] β-D-ribofuranosyl derivatives of 2-thio-6-azauracil,[15] diethyl 4-hydroxypyrazole-3,5-dicarboxylate,[16] 5-acetyl-uracil,[17] 2,4- and 2,5-thiazolidinediones,[18] 4-thiomethyl-2-azapurine,[19] 2-hydroxypurine,[20] 3-deaza-adenine-8-^{14}C,[21] 1-deaza-8-azaguanine,[22] imidazo-(1,2-*a*)1,3,5-triazenes, *e.g.*, (5),[23] *lin*-benzo-guanosine, -inosine, and -xanthosine (6),[24] and a derivative of *N*-2-(β-D-ribopyranosyl)benzotriazole.[25] Likewise 2′-*O*-



(5)



(6) R = H, OH, or NH_2

[9] 'Nucleic Acid Chem.' Parts 1 and 2, ed. L. B. Townsend and R. S. Tipson, Wiley, New York, 1978.

[10] H. F. Vorbrueggen, U. Niedballa, K. Krolikiewicz, B. Bennua, and G. Hoefle, ref. 4, p. 251.

[11] M. Saneyoshi, M. Inomata, and F. Fukuoka, *Chem. Pharm. Bull.*, 1978, **26**, 2990.

[12] C. Chavis, F. Grodenic, and J. L. Imbach, *Eur. J. Med. Chem. – Chim. Ther.*, 1979, **14**, 123 (*Chem. Abs.*, 1979, **91**, 141 157*x*).

[13] P. Fernandez-Resa, P. Goya, and M. Stud, *Nucleic Acid Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 61 (*Chem. Abs.*, 1979. **90**, 138 129*p*).

[14] E. K. Ryu and T. J. Bardos, *J. Heterocycl. Chem.*, 1979, **16**, 1049.

[15] Y. L. Fu, R. Parthasarathy, and M. Bobek, *J. Carbohydr., Nucleosides, Nucleotides*, 1978, **5**, 79.

[16] A. A. Akhrem, E. I. Kvasyuk, I. A. Mikhailopulo, and N. E. Pupeiko, *Vestsi Akad. Navuk B. SSR, Ser. Khim. Navuk*, 1978, **124** (*Chem. Abs.*, 1979, **90**, 152 528*z*).

[17] A. S. Jones, G. P. Stephenson, and R. T. Walker, *Tetrahedron*, 1979, **35**, 1125.

[18] H. Hřebabecky, Z. Točík, and J. Beránek, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1475.

[19] V. A. Bakuler, V. S. Mokrushin, V. I. Ofitserov, Z. V. Pushkareva, and A. N. Grishakov, *Khim. Geterotsikl. Soedin.*, 1979, 836 (*Chem. Abs.*, 1979, **91**, 175 656*z*).

[20] A. Holý, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2846.

[21] J. A. Montgomery and H. J. Thomas, *J. Labelled Comp. Radiopharm.*, 1978, **15**, (Suppl. Vol.), 539 (*Chem. Abs.*, 1979, **91**, 57 364*e*).

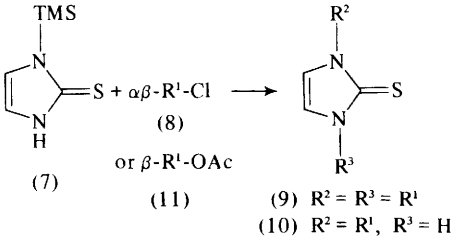
[22] B. L. Cline, R. P. Panzica, and L. B. Townsend, *J. Org. Chem.*, 1978, **43**, 4910.

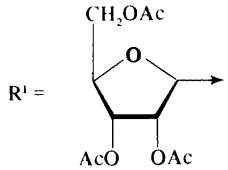
[23] E. J. Prisbe, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, 1978, **43**, 4784.

[24] G. E. Keyser and N. J. Leonard, *J. Org. Chem.*, 1979, **44**, 2989.

benzyl-[26] and 3′-*O*-benzyl-adenosine[27] have been prepared from the corresponding D-ribose monobenzyl ether, and 3-(β-D-ribofuranosyl)uracil from a uracil derivative blocked at N-1 by a cyanoethyl residue, the latter being subsequently removed with sodium methoxide.[28]

High yields of 2′,3′,5′-tri-*O*-acetyl- and -*O*-benzoyl-adenosine have been obtained from the corresponding 1-*O*-acetyl-β-D-ribofuranose derivative by direct reaction with unprotected adenine in acetonitrile in presence of stannic chloride.[29] Ribosylation of the silylated imidazolethione (7) with the glycosyl chloride (8) in presence of stannic chloride or silver perchlorate gave mainly the diribosyl derivative (9) rather than the expected mono-ribosyl nucleoside (10) (2 : 1 ratio), whereas use of the tetra-acetyl sugar (11) gave mainly (10).[30]

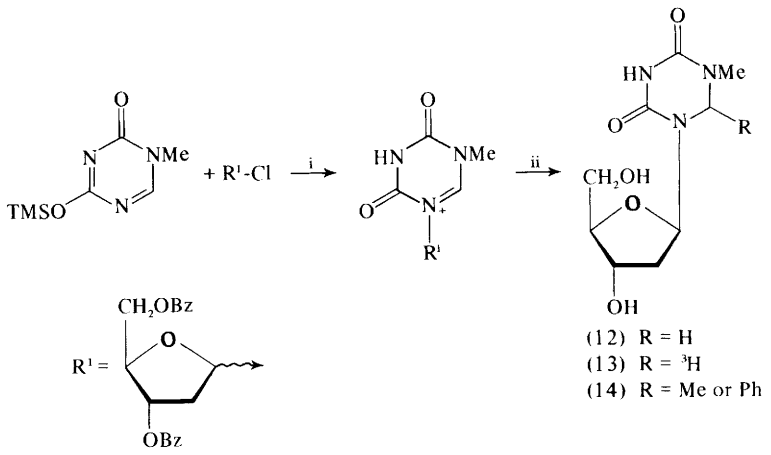




Scheme 1

Nucleosides prepared conventionally from deoxy-sugars have included the antiviral nucleoside dihydro-5-azathymidine (12), the tritiated analogue (13), and the 6-substituted derivatives (14) (Scheme 2),[31] [^{14}C-2] and [^{14}C-4]-2′-deoxyuridine,[32] and 2′-deoxy-ribonucleosides from 5-azapyrimidine derivatives,[33]

[25] M. M. Kul'chitskii and S. V. Bogacheva, *Khim. Geterotsikl. Soedin.*, 1978, 1691 (*Chem. Abs.*, 1979, **90**, 121 963*h*).
[26] R. R. Schmidt and A. Gohl, *Chem. Ber.*, 1979, **112**, 1689.
[27] R. R. Schmidt, A. Gohl, and J. Karg, *Chem. Ber.*, 1979, **112**, 1705.
[28] A. Holý, *J. Carbohydr., Nucleosides, Nucleotides*, 1978, **5**, 487.
[29] M. Saneyoshi and E. Satoh, *Chem. Pharm. Bull.*, 1979, **27**, 2518.
[30] G. Gosselin, J. L. Imbach, L. B. Townsend, and R. P. Panzica, *J. Heterocycl. Chem.*, 1979, **16**, 1185.
[31] H. I. Skulnick and W. Wierenga, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, **6**, 263.
[32] A. Szabolcs, G. Kruppa, J. Sagi, and L. Otvos, *J. Labelled Compd. Radiopharm.*, 1978, **14**, 713 (*Chem. Abs.*, 1979, **90**, 152 519*x*).
[33] A. Piskala, M. Synackova, H. Tomankova, P. Fiedler, and V. Zizkovsky, *Nucleic Acids Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 109 (*Chem. Abs.*, 1979, **90**, 87 808*z*).



Reagents: i, $SnCl_4$–MeCN; ii, $NaBH_4$ or Grignard Reagent

Scheme 2

5-substituted uracils including 5-ethynyl- and (*E*)-5-(2-bromoethenyl)-uracil,[34] and a number of 5-polyfluoroalkyl uracils, including some 4-thio- and 4-amino-analogues of these 2′-deoxynucleosides.[35] The 2′-deoxyribonucleotide of benzimidazole has also been prepared.[36] Nucleosides have also been obtained from standard bases with 6-deoxy-D-allose to give 5′-*C*-methyl analogues of uridine, cytidine, and adenosine,[37] from 2-, 3-, and 5-deoxy-allose derivatives giving the corresponding deoxy-*ribo*-hexofuranosyl cytosine,[38] and from 5-chloro-tetrahydrofuran-2-carboxylic acid esters to give corresponding 2′,3′-dideoxy-α-nucleoside analogues of uridine.[39] The synthesis of 3′- and 4′-hydroxy derivatives of (*RS*)-1-(tetrahydro-2-furanyl)5-fluorouracil (FTORAFUR) has also been reported.[40]

The deoxygenation of intact nucleosides has been used to prepare 2′- and 3′-deoxyguanosine from guanosine *via* chloro-deoxy sugar intermediates (see also below),[41] 5′-deoxy-D-ribo-, -D-arabino-, -D-lyxo-, and -L-lyxo-nucleosides of

[34] R. T. Walker, P. J. Barr, E. De Clercq, J. Deschamps, A. S. Jones, and P. Serafinowski, *Nucleic Acids Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 103 (*Chem. Abs.*, 1979, **90**, 87 807*y*).

[35] S. Ya. Mel'nik, A. A. Bakhmedova, G. I. Vornovitskaya, Ya. V. Dobrynin, T. G. Nikolaeva, T. P. Ivanova, I. V. Yartseva, and M. N. Preobrazhenskaya, *Bioorg. Khim.*, 1979, **5**, 41 (*Chem. Abs.*, 1979, **90**, 187 266*q*).

[36] D. L. Sweeney, J. E. Gearien, L. Bauer, and B. L. Currie, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, **6**, 387.

[37] M. Ya. Karpeisky and S. N. Mikhailov, *Bioorg. Khim.*, 1979, **5**, 895.

[38] S. David and G. De Sennyey, *Carbohydr. Res.*, 1979, **77**, 79.

[39] R. A. Zhuk, A. E. Berzin, V. N. Silin, and E. E. Liepinsh, *Nucleic Acids Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 19 (*Chem. Abs.*, 1979, **90**, 104 265*q*).

[40] A. J. Lin, R. S. Benjamin, P. N. Rao, and T. L. Loo, *J. Med. Chem.*, 1979, **22**, 1096.

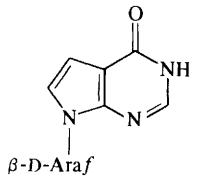
[41] R. Mengel and W. Muhs, *Chem. Ber.*, 1979, **112**, 625.

5-fluoro-uracil from 5-fluoro-uridine, and similarly 5′-deoxy-D-ribo- and -D-arabino-nucleosides of 5-fluorocytidine,[42] and 2′-deoxyribonucleosides of uracil, 5-bromo-uracil, and 5-fluoro-uracil from uridine or 5-fluoro-uridine, which on sequential treatment with acetyl bromide and tributyltin hydride gave the corresponding 2′-deoxynucleoside in high yield[43] (see also below).

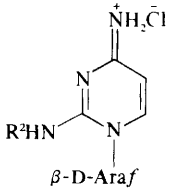
Numerous syntheses have also been reported for arabinofuranosyl nucleoside analogues, prepared either conventionally from arabinofuranosyl derivatives or *via* 2,2′-anhydro-nucleosides obtained from appropriate ribonucleosides. 5-Aza-cytosine-D-arabinoside has been synthesized and found to show similar antiviral activity to Ara-C(arabinosyl-cytosine).[44] 7-α-, 7-β-, 9-α-, and 9-β-arabinofuranosyl derivatives of 3-deazaguanine have also been prepared, but none showed any anti-tumour activity.[45] 9-(α-D-Arabinofuranosyl)-8-aza[2-^{14}C]-adenine,[46] 7-(β-D-arabinofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-4(3*H*)-one (15),[47] 1-(α-D-arabinofuranosyl)- and 1-(β-D-xylofuranosyl)-4-nitropyrazole,[48] and α-arabino-nucleosides of 5-fluoro-cytosine and -uracil derivatives have also been prepared.[49] An improved synthesis of 9-(β-D-arabinofuranosyl)-2-fluoro-adenine has been reported.[50] The ratio of α to β anomers obtained by phase-transfer reaction of 2,3,5-tri-*O*-benzyl-D-arabinofuranosyl bromide with 6-chloro-2-thiomethyl-7-deazapurine varied with the quaternary ammonium salt used as a catalyst, although the β-anomer predominated in every case.[51] 2,2′-Anhydro-nucleosides have been used to prepare 1-β-D-arabinofuranosyl derivatives of 5-alkylthio-uracils,[52] 5-ethyl-cytosine, and 5-ethyl-uracil,[53] 8-alkylamino-purines,[54] and 2-aralkylamino-1,4-dihydro-4-imino-pyrimidine hydrochlorides (16).[55]

L-Arabino-furanosyl or -pyranosyl derivatives of 5-thio-substituted indole[56]

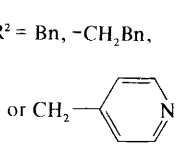
[42] A. F. Cook, M. J. Holman, M. J. Kramer, and P. W. Trown, *J. Med. Chem.*, 1979, **22**, 1330.
[43] J. Brokeš, H. Hřebabecký, and J. Beránek, *Collect. Czech. Chem. Commun.*, 1979, **44**, 439.
[44] J. A. Beisler, M. M. Abbasi, and J. S. Driscoll, *J. Med. Chem.*, 1979, **22**, 1230.
[45] M. S. Poonian, W. M. McComas, and M. J. Kramer, *J. Med. Chem.*, 1979, **22**, 958.
[46] J. A. Montgomery and H. J. Thomas, *J. Labelled Comp. Radiopharm.*, 1978, **15** (Suppl. Vol.), 727 (*Chem. Abs.*, 1979, **90**, 204 409*h*).
[47] U. Lüpke and F. Seela, *Chem. Ber.*, 1979, **112**, 3432.
[48] I. A. Korbukh, L. N. Abramova, B. N. Stepanenko, and M. N. Preobrazhenskaya, *Zh. Org. Khim.*, 1978, **14**, 2169 (*Chem. Abs.*, 1979, **90**, 121 958*k*).
[49] J. A. Montgomery and H. J. Thomas, *J. Heterocycl. Chem.*, 1979, **16**, 353.
[50] J. A. Montgomery, S. D. Clayton, and A. T. Shortnacy, *J. Heterocycl. Chem.*, 1979, **16**, 157.
[51] F. Seela and D. Winkeler, *Angew. Chem. Int. Ed. Engl.*, 1979, **18**, 536.
[52] T. Ueda, S.-I. Watanabe, and A. Matsuda, *J. Carbohydr., Nucleosides, Nucleotides*, 1978, **5**, 523.
[53] T. Kulikowski, Z. Zawadzki, and D. Shugar, *NATO Adv. Study Inst. Ser., Ser. A*, 1978 (Publ. 1979), **A20**, 511 (*Chem. Abs.*, 1979, **91**, 20 950*s*).
[54] J. B. Chattopadhyaya and C. B. Reese, *Nucleic Acids Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 67 (*Chem. Abs.*, 1979, **90**, 104 268*t*).
[55] K. Kondo, A. Kojima, and I. Inoue, *Chem. Pharm. Bull.*, 1978, **26**, 3244.
[56] V. I. Mukhanov, Yu. P. Troyanov, and M. N. Preobrazhenskaya, *Zh. Org. Khim.*, 1978, **14**, 2174 (*Chem. Abs.*, 1979, **90**, 121 926*y*).

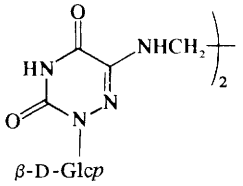


(15)



(16)



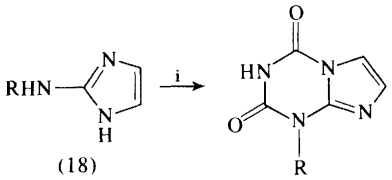


(17)

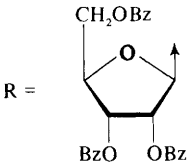
and 5- and 6-nitro-indole[57] have been prepared from L-arabinose by reaction with indoline derivatives followed by dehydrogenation of the indolinyl *N*-glycoside; the nitro-derivatives were also reduced to corresponding amino-compounds.

The dimeric nucleoside analogue (17) has been obtained from *NN'*-bis(6-aza-5-uracilyl)ethylene diamine by reaction with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide followed by deacetylation.[58]

The course and scope of the sugar-exchange reaction of purine nucleosides has been investigated; the exchange, promoted by a glycosylhalide in presence



(18) (19)



Reagents: i, ArOCO(NCO)

Scheme 3

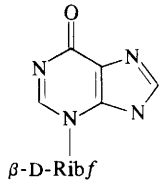
[57] V. I. Mukhanov, I. V. Yartseva, L. V. Ektova, N. D. Chkanikov, T. D. Miniker, and M. N. Preobrazhenskaya, *Khim. Geterotsikl. Soedin.*, 1979, 224 (*Chem. Abs.*, 1979, **91**, 5434*h*).

[58] I. V. Alekseeva, A. S. Shalamai, V. I. Kobylinskaya, and V. P. Chernetskii, *Khim. Geterotsikl. Soedin.*, 1978, 1561 (*Chem. Abs.*, 1979, **90**, 121 940*y*).

of mercuric cyanide, appears to involve an *N*-7, *N*-9-bis-glycosylated purine intermediate, from which the ribose moiety is eliminated as a 1,5-anhydro-sugar, with the resulting *N*-7-glycoside undergoing an N-7 → N-9 transglycosylation, yielding a mixture of *N*-7- and *N*-9-nucleosides.[59]

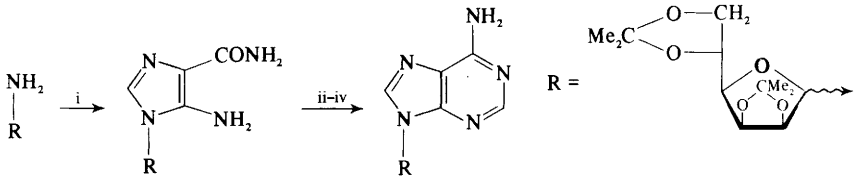
The cyclization of glycosulamine derivatives continues to offer a useful route to nucleoside analogues.

Condensation of the *N*-imidazolyl ribofuranosylamine (18) with aryloxycarbonyl isocyanates gave the corresponding ribofuranosyl-imidazo[1,2-*a*]1,3,5-triazenes (19) (Scheme 3).[60] Likewise 3-hypoxanthosine (20) had been obtained by reaction of 2,3-*O*-isopropylidene-D-ribofuranose with 5-amino-4-carbamoyl-



(20)

imidazole followed by condensation of the glycosylamine product with ethyl formate and deprotection; the 4-thio analogue was similarly prepared.[61] The α- and β-D-mannofuranosyl-adenines have been prepared from αβ-D-mannofuranosylamine as outlined in Scheme 4, and guanine analogues were also synthesized.[62] Scheme 5 summarizes a synthesis of virazole (21) from 2,3,5-tri-*O*-benzyl-D-ribofuranosylhydrazine using a reagent derived from benzyl cyanoformate; alternatively the heterocycle could be formed first, and then condensed with peracetylated ribofuranose conventionally.[63] Treatment of 2,3,4-tri-*O*-acetyl-D-



Reagents: i, $EtOCH{=}NCH(CN)CONH_2$; ii, $POCl_3$; iii, $HC(OEt)_3$; iv, NH_3

Scheme 4

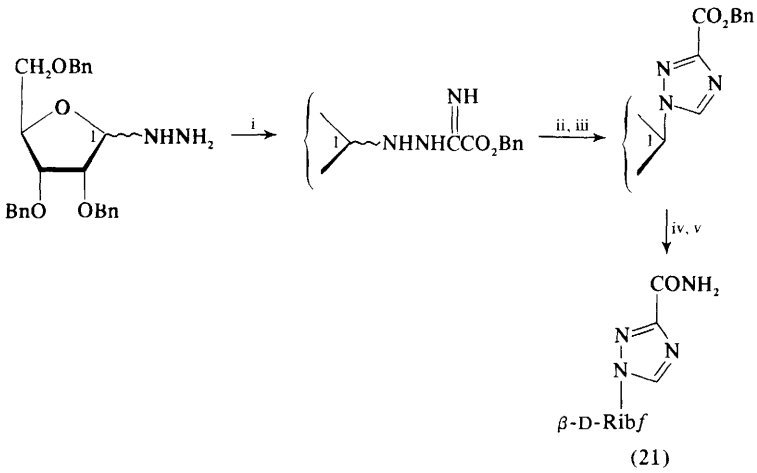
[59] W. Riess and F. W. Lichtenthaler, *Nucleic Acids Res. Symp. 4th., Spec. Publ.*, 1978, **4**, 67 (*Chem. Abs.*, 1979, **90**, 104 269*u*).

[60] E. J. Prisbe, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, 1978, **43**, 4774.

[61] C. De Gourcy, H. M. A. Abdel-Bary, C. Chavis, and J.-L. Imbach, *Carbohydr. Res.*, 1979, **77**, 234.

[62] G. Mackenzie and G. Shaw, *Nucleic Acids Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 55 (*Chem. Abs.*, 1979, **90**, 104 267*s*).

[63] Y. Ito, Y. Nii, S. Kobayashi, and M. Ohno, *Tetrahedron Lett.*, 1979, 2521.



(21)

Reagents: i, $BnOCOC(SEt)=\overset{+}{N}H_2BF_4^-$; ii, $HC(OEt)_3$; iii, TsOH; iv, NH_3; v, H_2–Pd

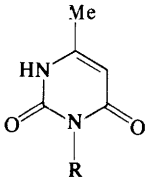
Scheme 5

ribopyranosyl chloride or its furanosyl analogue with anthranilonitrile using a phase-transfer procedure gave the corresponding glycosylamine derivative, which could be cyclized to give quinazoline nucleoside analogues using phenyl-isothiocyanate.[64] 2-Amino-oxazoline derivatives of pentofuranoses have been condensed with diketene to give nucleosides of 6-methyl-uracil (22),[65] and with methyl [2-^{13}C]propynoate leading to 5-fluoro-2′-deoxy-[4-^{13}C]uridine.[66] Glycosyl isothiocyanates have been converted to the *N*-glycosides of a series of heterocycles by similar condensation–cyclization reactions.[67] 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl azide undergoes cycloaddition with halo-propynes or dihalobutynes to give the corresponding triazole derivatives (23).[68]

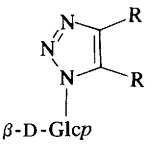
Nucleoside Q has been synthesized by a procedure that attaches the cyclopentene ring to a 7-deaza-9-β-D-ribofuranosyl-purine derivative, and the natural product was thus shown to have the *3S,4R,5S* configuration in the cyclopentenyl substituent.[69]

The formation of nucleosides from cyanamide and potassium nitrite under possible primitive earth conditions has been investigated.[70]

[64] J. P. Ferris, S. Singh, and T. A. Newton, *J. Org. Chem.*, 1979, **44**, 173.
[65] I. Drizina, D. Snikeris, E. Liepins, and E. I. Stankevich, *Khim. Geterotsikl. Soedin.*, 1978, 1115 (*Chem. Abs.*, 1979, **90**, 23 499*s*).
[66] W. H. Dawson and R. B. Dunlap, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 335 (*Chem. Abs.*, 1979, **91**, 158 025*a*).
[67] H. Ogura and H. Takahashi, *Tennen Yuki Kagobutsu Toronkai Koen Yoshishi, 21st*, 1978, 221 (*Chem. Abs.*, 1979, **81**, 39 764*f*).
[68] F. G. De Las Heras, R. Alonso, and G. Alonso, *J. Med. Chem.*, 1979, **22**, 496.
[69] T. Ohgi, T. Kondo, and T. Goto, *J. Am. Chem. Soc.*, 1979, **101**, 3629.
[70] G. Wollin and W. B. F. Ryan, *Biochim. Biophys. Acta*, 1979, **584**, 493.



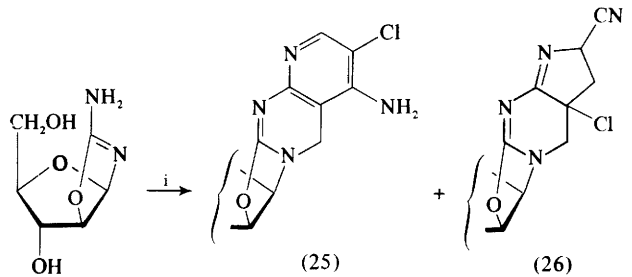
(22) R = β-D-Ara*f*
α-D-Rib*f*



(23) R = CH_2Cl, CH_2Br, H

3 Cyclo- and Anhydro-nucleosides

Treatment of the D-arabinofuranosyloxazoline (24) with 1-chloro-1-cyano-ethene gave the 2,2′-*O*-cyclonucleosides (25) and (26) (Scheme 6).[71] 3,5-Di-*O*-acyl derivatives of 2,2′-*O*-cyclo-β-D-arabinofuranosyl-cytidine have been prepared by reaction of cytidine with the acid chloride or anhydride derivatives of 3-heptyloxypropanoic, 3-dodecyloxypropanoic, and 11-butyrylamino-undecanoic acid; the dimeric derivative (27) was also prepared.[72]



(24) (25) (26)

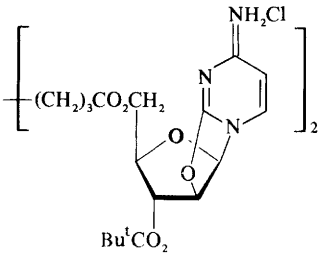
Reagent : i, CH_2=CClCN

Scheme 6

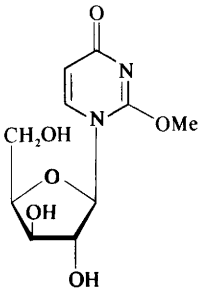
Base-catalysed equilibration of 2,2′-*O*-cyclo-β-D-arabinofuranosyl-uracil with the 2,3′-*O*-cyclo-β-D-xylofuranosyl isomer strongly favours the former compound; 2′,3′-anhydrouridine is an intermediate in the reaction, and with sodium methoxide in boiling methanol the *xylo* and *arabino* isomers of uridine could also be isolated, together with the 2-*O*-methyl-nucleoside (28).[73]

8,2′- and 8,3′-(*S*)-Cyclonucleosides have been prepared from 8-bromo-5′-deoxy-adenosine by sequential tosylation and treatment with sodium hydrosulphide; desulphurization of these anhydro compounds gave the corresponding 2′,5′- and 3′,5′-dideoxyadenosines.[74]

[71] D. Snikeris, I. A. Drizina, E. Liepins, and E. I. Stankevich, *Khim. Geterotsikl. Soedin.*, 1979, 265 (*Chem. Abs.*, 1979, **90**, 204 415*g*).
[72] K. Kondo, T. Nagura, Y. Arai, and I. Inoue, *J. Med. Chem.*, 1979, **22**, 639.
[73] J. G. Buchanan and D. R. Clark, *Carbohydr. Res.*, 1979, **68**, 331.
[74] M. Ikehara, H. Miki, and A. Hasegawa, *Chem. Pharm. Bull.*, 1979, **27**, 2647.



(27)



(28)

A new class of 2,4′-*O*-cyclonucleoside (29) has been obtained as outlined in Scheme 7; 4′-substituted nucleosides prepared from this compound included the methoxy derivative (30), obtained using methanolic silver nitrate, and hence the uridine analogues (31) by reduction.[75]

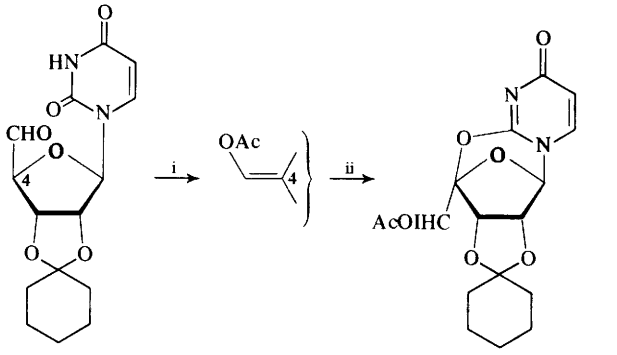
The 2,5′-*O*-cyclonucleoside (32) has been prepared conventionally from the corresponding ribosyl-imidazole derivative.[76]

2′,3′-Anhydro-adenosine has been isomerized to 2′,3′-anhydro-β-D-lyxofuranosyl-adenine in 42% overall yield by the sequence outlined in Scheme 8.[77] Deamination of 1-(3-amino-3-deoxy-β-D-xylofuranosyl)-uracil with nitrous acid did not yield any 2′,3′-anhydro-uridine or products derived from this anhydrosugar derivative, uracil being the major product.[72]

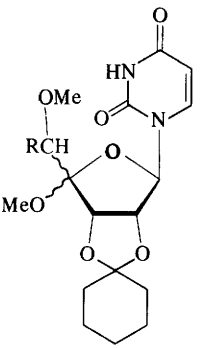
4 *C*-Nucleosides

The 3-β-D-arabinofuranosyl pyrazole derivative (33) has been synthesized from D-mannose by the procedure outlined in Scheme 9; on deprotection with ammonia, (33) epimerized to the β-D-ribofuranosyl isomer.[78] 2,5-Anhydro-D-allonic acid derivatives have been used to synthesize the *C-sym*-triazoloribonucleosides (34) and (35) by condensation with heterocyclic hydrazine derivatives.[79] The diazoketone (36), prepared conventionally from the acid, has been used to prepare the thiazole analogue (37) of ribavirin *via* the intermediate α-bromomethyl ketone (38) by condensation with an imido-thio-oxalic acid derivative,[80] and the indazole nucleoside analogue (39) by cyclization with benzyne (Scheme 10).[81] An acyclic diazoketone analogously prepared from D-ribonic acid similarly gave a *C*-ribonyl-indazole with benzyne, which could be

[75] S. L. Cook and J. A. Secrist, *J. Am. Chem. Soc.*, 1979, **101**, 1554.
[76] H. Tanaka, T. Takahashi, H. Togashi, and T. Ueda, *Chem. Pharm. Bull.*, 1978, **26**, 3322.
[77] R. Mengel and U. Krahmer, *Carbohydr. Res.*, 1979, **75**, 314.
[78] J. G. Buchanan, M. E. Chacon-Fuertes, and R. H. Wightman, *J. Chem. Soc., Perkin Trans. 1*, 1979, 244.
[79] A. Rosenthal and H. H. Lee, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, **5**, 559.
[80] T. Vaněk, J. Farkaš, and J. Gut, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1339.
[81] G. Olonso, E. Garcia-Abbad, M. T. Garcia-López, and M. Stud, *J. Heterocycl. Chem.*, 1979, **16**, 81.



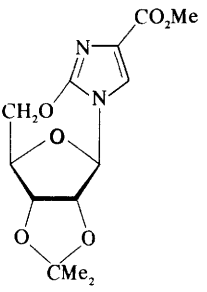
(29)



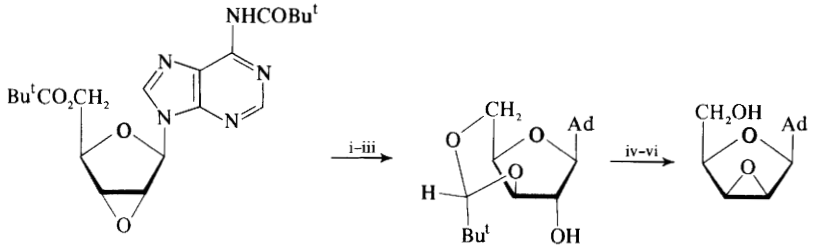
(30) R = OH
(31) R = H

Reagents: i, Ac_2O–Et_3N–*p*-$Et_2NC_5H_4N$; ii, NIS–NaOAc; iii, MeOH–$AgNO_3$

Scheme 7



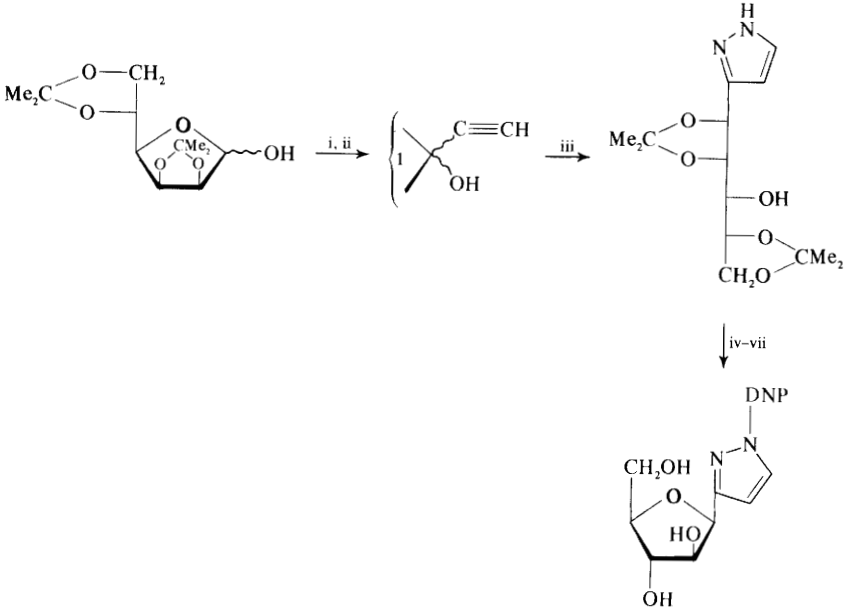
(32)



Ad = Adenin-9-yl

Reagents: i, $BF_3.Et_2O$; ii, $NaBH_4$, iii, NaOMe; iv, MsCl–py; v, HCl; vi, Resin (OH^-)

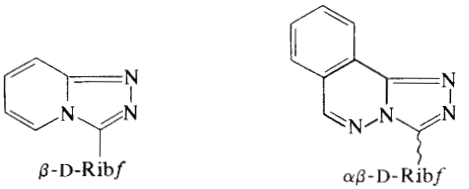
Scheme 8



(33)

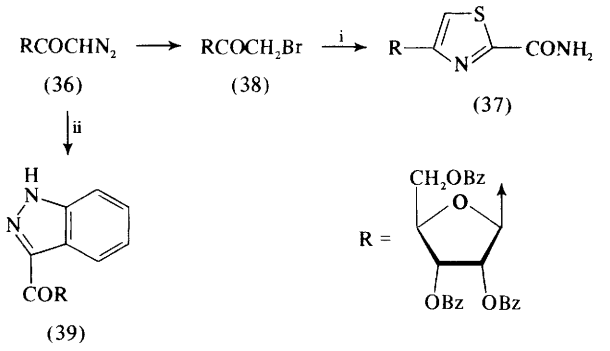
Reagents: i, HC≡CMgBr; ii, MnO_2; iii, $N_2H_4.H_2O$; iv, Me_2CO–H^+; v, DNPF–Et_3N; vi, MsCl–py; vii, $BF_3.Et_2O$

Scheme 9



β-D-Ribf (34)

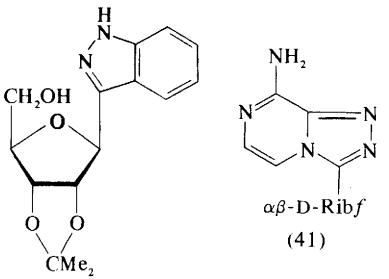
αβ-D-Ribf (35)



Reagents: i, $EtO_2CC(SH)=NH$; ii, C_6H_4

Scheme 10

cyclized to give the corresponding *C*-nucleoside (40) (with some α-anomer).[81] Condensation of an imido-thiolester derivative of 2,5-anhydro-D-allonic or D-altronic acid with 2-chloro-3-hydrazinopyrazine led to the synthesis of the triazolo-pyrazine-*C*-nucleoside (41) and its 2′-deoxy analogue, which are closely related to adenosine and formycin, and an extensive series of derivatives were also prepared.[82] Homopseudo-uridine (42) has been prepared from the chiral lactone (43) by standard reactions, and homopseudo-isocytidine (44) (tautomer shown) was similarly synthesized.[83] Acyclic D-*arabino*-tetrahydroxybutyl-imidazolinethione derivatives have been cyclized to give D-erythrofuranosyl *C*-nucleosides (45); β-anomers predominate on heating, whereas in presence of trifluoroacetic acid, α-anomers could be obtained.[84,85]

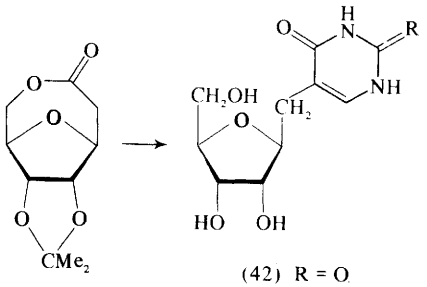


[82] T. Huynh-Dinh, R. S. Sarfati, C. Gouyette, J. Igolen, E. Bisagni, J.-M. Lhoste, and A. Civier, *J. Org. Chem.*, 1979, **44**, 1028.

[83] T. Sato, K. Marunouchi, and R. Noyori, *Tetrahedron Lett.*, 1979, 3669.

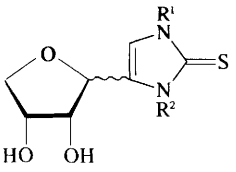
[84] J. Fuentes Mota, M. A. Pradera de Fuentes, and D. Gavilan Ropero, *An. Quim.*, 1977, **73**, 1356.

[85] J. Fuentes Mota and J. M. Pareta Marcano, *An. Quim.*, 1978, **74**, 975 (*Chem. Abs.*, 1979, **91**, 108 174*c*).



(43)

(42) R = O
(44) R = NH



(45) R^1 = H, R^2 = Ph;
R^1 = $(CH_2)_7Me$, R^2 = H

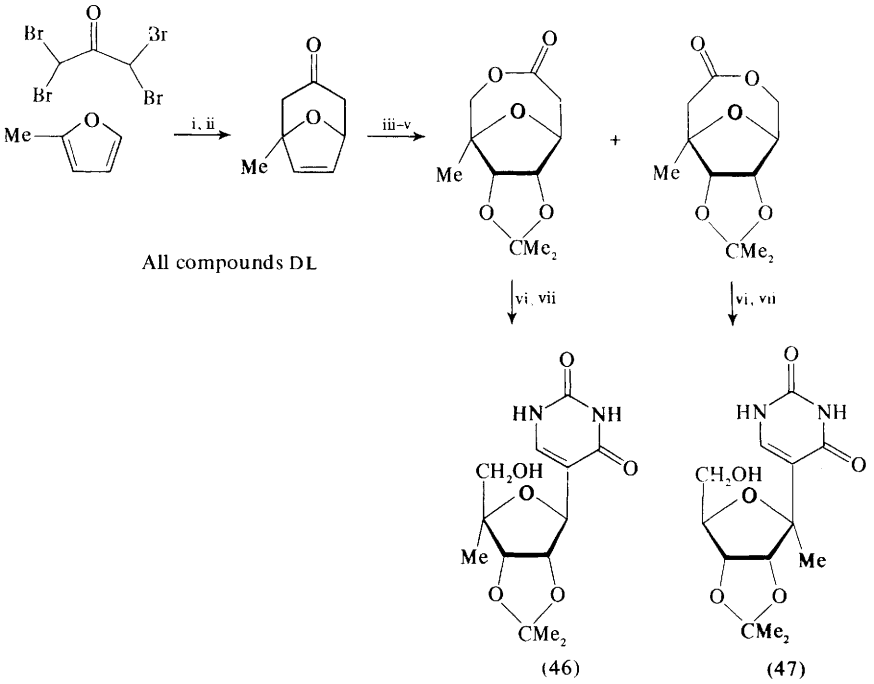
The reaction of 1,1,3,3-tetrabromopropanone with furan derivatives has been used to prepare *C*-methyl analogues (46) and (47) of pseudo-uridine (Scheme 11),[86] and the related *C*-hydroxymethyl analogues (48) were similarly prepared.[87]

5 Amino-sugar Nucleosides and Related Compounds

Syntheses of nucleosides containing amino-sugars either utilize preformed amino- or azido-sugars in reaction with heterocyclic bases, or modify preformed nucleosides.

Syntheses involving standard condensation procedures for heterocyclic bases with amino-sugar derivatives have included the preparation of 2′-azido-, 3′-azido-2′,5′-diazido-, and 3′,5′-diazido-derivatives of *arabino*-uridine,[88] 3′-amino-3′-deoxy-adenosine, -uridine, and -cytidine 5′-phosphates,[89] 2′,3′-bis(2-chloroethyl)-aminophosphoryl-3′-amino-3′-deoxyadenosine (which has anti-tumour activity),[90] 3′-*N*-methyl-*N*-nitrosoureido-3′-deoxy-adenosine and the corresponding 5′-substituted isomer,[91] and 2′-azido-2′-deoxy- and 2′-amino-2′-deoxy-D-arabinofuranosyl-

[86] T. Sato, M. Watanabe, and R. Noyori, *Tetrahedron Lett.*, 1979, 2897.
[87] T. Sato and R. Noyori, *Nucleic Acid Res. Symp. 6th, Spec. Publ.*, 1978, **5**, 257 (*Chem. Abs.*, 1979, **90**, 121 932*x*).
[88] F. M. Unger, R. Christian, and P. Waldstätten, *Tetrahedron Lett.*, 1979, 605.
[89] A. V. Azhayev, A. M. Ozols, A. S. Bushnev, N. B. Dyatkina, S. V. Kochetkova, L. S. Victorova, M. K. Kukhanova, A. A. Krayevsky, and B. P. Gottikh, *Nucleic Acid Res.*, 1979, **6**, 625.
[90] A. Okruszek and J. G. Verkade, *J. Med. Chem.*, 1979, **22**, 882.
[91] J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, 1979, **22**, 1109.



(46) (47)

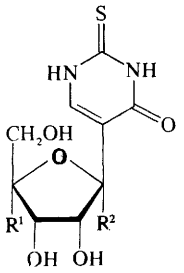
Reagents: i, $Fe_2(CO)_9$; ii, Zn–Cu; iii, H_2O_2–OsO_4; iv, Me_2CO–$CuSO_4$–TsOH; v, CF_3CO_3H; vi, $Bu^tOCH(NMe_2)_2$; vii, $CO(NH_2)_2$–NaOEt

Scheme 11

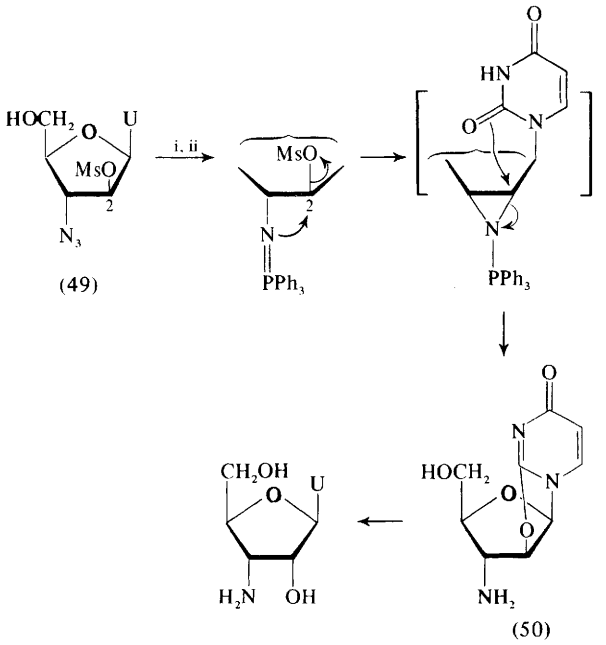
adenine and -guanine.[92] Alternatively nucleosides have been modified by azide-displacement procedures to give 2-amino-2-deoxy-β-D-arabinofuranosyl nucleosides,[93] and 1-(5-amino-5-deoxy-β-D-arabinofuranosyl-)-5-substituted-uracils.[94] A convenient conversion of 2′-azido-2′-deoxy-uridine to 2′-amino-2′-deoxy-ribofuranosyl purines has been described.[95] The conversion of the 3-azido-3-deoxy-arabinofuranosyl uracil derivative (49) to 3′-amino-3′-deoxy-uridine involves the cyclonucleoside (50), which is considered to arise by oxygen attack on an aziridine intermediate formed during the triphenylphosphine reduction step (Scheme 12).[96]

Catalytic reduction of the 3′-azido-3′-deoxy-2′-*O*-mesyl derivative prepared from 2′,3′-anhydro-adenosine yielded an 2′,3′-aziridine of inverted (*lyxo*) con-

[92] M. Bobek, *Carbohydr. Res.*, 1979, **70**, 263.
[93] A. Sato, R. Imai, N. Nakamizo, and T. Hirata, *Chem. Pharm. Bull.*, 1979, **27**, 821.
[94] R. F. Schinazi, M. S. Chen, and W. H. Prusoff, *J. Med. Chem.*, 1979, **22**, 1273.
[95] M. Imazawa and F. Eckstein, *J. Org. Chem.*, 1979, **44**, 2039.
[96] S. V. Popovkina, A. V. Azhayev, M. A. Kharshan, A. A. Krayevsky, I. D. Bobruskin, and B. P. Gottikh, *Bioorg. Khim.*, 1979, **5**, 546.



(48) $R^1 = CH_2OH$, $R^2 = H$;
$R^1 = H$, $R^2 = CH_2OH$



U = Uracil-1-yl

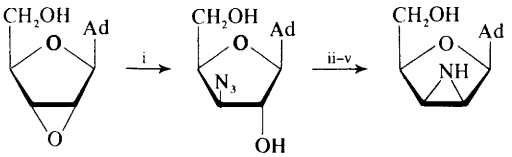
Reagents: i, PPh_3–py; ii, NH_4OH

Scheme 12

figuration (Scheme 13), and the anhydro-lyxose isomer likewise gave the inverted *ribo*-aziridine; these aziridines showed unusual c.d. and ^1H n.m.r. spectra.[97]

Treatment of 2′-deoxy-3′,5′-di-*O*-mesyl-uridine or -thymidine with aqueous alkali yielded the corresponding 3′,5′-oxetan derivative, which with alkali in

[97] M. J. Robins, S. D. Hawrelak, T. Kanai, J.-M. Siefert, and R. Mengel, *J. Org. Chem.*, 1979, 44, 1317.



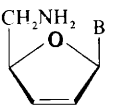
Ad = Adenin-9-yl

Reagents: i, LiN_3; ii, Bu^tCOCl–py; iii, MsCl–py; iv, H_2–Pd; v, NaOMe

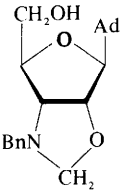
Scheme 13

hexamethylphosphortriamide gave the 2′,3′-unsaturated nucleoside, and this was then converted to the corresponding 5′-amino derivative (51) by a standard sequence.[98]

Treatment of 5′-deoxy-5′-halogeno nucleosides of adenine and uracil with trimethylamine yielded the corresponding 5′-deoxy-5′-trimethylammonium salts, whose interaction with anionic polynucleotides was then examined.[99] Various *N*-alkyl, *N*-cycloalkyl, and *N*-alkaryl derivatives of 3′-amino-3′-deoxy- and 3′,5′-diamino-3′,5′-dideoxy-adenosine have been prepared from the amino-sugar nucleosides; the 3′-benzylamino derivative gave the unusual oxazolidine derivative (52).[100]



(51) B = Uracil-1-yl or thymin-1-yl

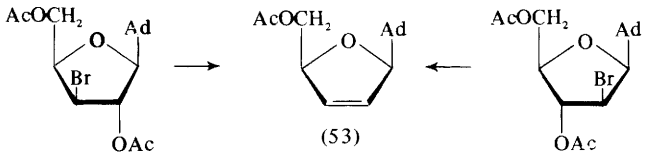


(52)

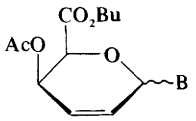
6 Unsaturated and Keto-sugar Nucleosides

The unsaturated nucleoside (53) has been prepared in good yield by electrochemical elimination from either the 2′-bromo-arabino- or the 3′-bromo-xylo-nucleoside indicated in Scheme 14; however, the electrolyte can control the product obtained, for whereas 2′-bromo-2′-deoxy-3′,5′-di-*O*-propanoyl-uracil gave the 2′,3′-unsaturated nucleoside in presence of tetraethylammonium toluene-*p*-sulphonate in methanol, with sodium acetate a mixture of products was obtained; 3′-deoxy-3′-iodo-adenosine yielded 3′-deoxy-adenosine (cordycepin) besides the 2′,3′-unsaturated nucleoside.[101] 1-Acetyl-glycenose derivatives have been condensed with purine and pyrimidine derivatives in presence of antimony

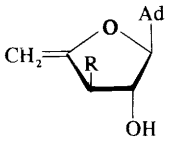
[98] T. Adachi, Y. Yamada, I. Inoue, and M. Saneyoshi, *Carbohydr. Res.*, 1979, **73**, 113.
[99] T. Shimidzu, A. Murakami, and K. Yamana, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 3362.
[100] M. Morr and L. Ernst, *Chem. Ber.*, 1979, **112**, 2815.
[101] T. Adachi, T. Iwasaki, I. Inoue, and M. Miyoshi, *J. Org. Chem.*, 1979, **44**, 1404.



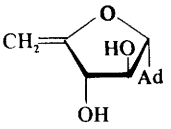
Scheme 14



(54) B = Uracil-1-yl or 6-chloro-purin-9-yl



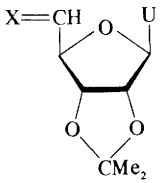
(55) R = H, OH



(56)

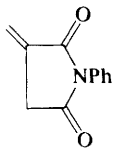
Ad = Adenin-9-yl

pentachloride to give αβ-mixtures of nucleosides, *e.g.*, (54).[102] The 4′,5′-unsaturated nucleosides (55) and (56) have been prepared by treatment of corresponding 5′-deoxy-5′-iodo-nucleosides with DBU.[103] The Wittig reaction has been used to prepare unsaturated nucleosides, *e.g.*, (57), from the dialdose derivative obtained by Pfitzner–Moffatt oxidation of 2′,3′-*O*-isopropylidene-uridine; the *N*-phenyl succinimido derivative (58) showed significant antiviral activity.[104]



(57) X = CHCOMe or $CHCO_2Me$

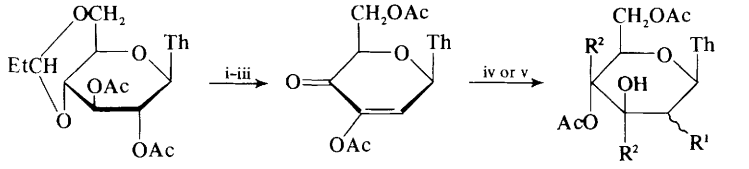
(58) X =



U = Uracil-1-yl

On acetolysis, the 4,6-*O*-ethylidene nucleoside analogue (59) gave the corresponding 2,3,6-tri-*O*-acetyl derivative, which, on Pfitzner–Moffatt oxidation, yielded the unsaturated keto nucleoside (60). The reduction of this intermediate to 2′-deoxy-D-*arabino*-hexo-nucleosides using sodium borohydride in acetic acid was also investigated using deuterium-labelled reagents; sodium borohydride in perdeuteriated acetic acid gave the 2′-deuterio compound (61), whereas sodium borodeuteride in acetic acid gave the 3′,4′-dideuterio-labelled derivative (62) (Scheme 15).[105] Several novel nucleoside analogues prepared from an unsaturated keto-nucleoside derivative are mentioned in Chapter 12.

[102] R. R. Schmidt and R. Angerbauer, *Carbohydr. Res.*, 1979, **72**, 272.
[103] V. K. Srivastava and L. M. Lerner, *J. Med. Chem.*, 1979, **22**, 24.
[104] J. M. J. Tronchet and M. J. Valero, *Helv. Chim. Acta*, 1979, **62**, 2788.
[105] T. Halmo and K. Antonakis, *Carbohydr. Res.*, 1979, **68**, 61.



(59) (60) (61) $R^1 = {}^2H$, $R^2 = H$

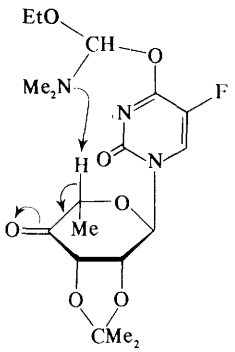
(62) $R^1 = H$, $R^2 = {}^2H$

Th = Thymin-1-yl

Reagents: i, Ac_2O–1% H_2SO_4; ii, 70% HOAc–H_2O; iii, DMSO–DCC; iv, $NaBH_4$–$C^2H_3CO_2{}^2H$; v, NaB^2H_4–CH_3CO_2H

Scheme 15

A useful method for preparing enol-acetates from uracil 4′-keto-nucleosides uses dimethyl formamide diethyl acetal followed by acetic anhydride; 2′-keto isomers, or N-3-substituted analogues, did not react. Further studies supported a mechanism involving O-4 esterification of the nucleoside followed by enolization, which is intramolecularly catalysed by the dimethylamino group, as illustrated in (63).[106]



(63)

7 Halo-nucleosides

4′-Deoxy-4′-fluoro-D-glucopyranosyl thymine[107] and 2′-deoxy-2′-fluoro-D-arabino-furanosyl derivatives of 5-substituted-cytosine and -uracil[108] have been prepared by standard methods from the corresponding fluoro-sugar; the uracil derivatives stopped replication of Herpes simplex virus (HSV-1) *in vitro*. 5′-Deoxy-5′-iodo-adenosine derivatives have also been prepared from iodo sugars.[103]

[106] M. Bessodes, A. Ollapally, and K. Antonakis, *J. Chem. Soc., Chem. Commun.*, 1979, 835.

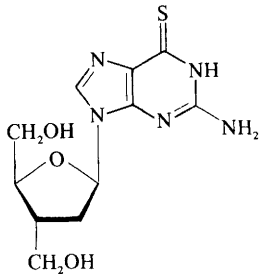
[107] M. Von Janto-Lipinski, G. Etzold, and P. Langen, *Z. Chem.*, 1979, **19**, 106 (*Chem. Abs.*, 1979, **91**, 57 375*j*).

[108] K. A. Watanabe, U. Reichman, K. Hirota, C. Lopez, and J. J. Fox, *J. Med. Chem.*, 1979, **22**, 21.

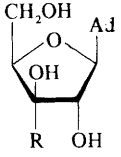
Treatment of nucleosides with thionyl chloride in HMPT gave 5′-chloro-5′-deoxy-2′,3′-*O*-sulphinyl derivatives, which could be converted to 5′-chloro-5′-deoxy-nucleosides on treatment with sodium methoxide in methanol, or to 2,2′-anhydro-nucleosides with imidazole in DMF.[109–112] Likewise 2′-deoxy-adenosine yielded 5′-chloro-2′,5′-dideoxy-adenosine on treatment with a limited amount of the thionyl chloride reagent followed by methanolic ammonia.[113] with thionyl chloride in pyridine, 2′,5′- and 3′,5′-di-*O*-acyl or ether derivatives of adenosine yielded 3′- or 2′-chloro-deoxy analogues with inversion of configuration; the acyl derivatives of these chlorinated sugars gave 2′,3′-anhydro-adenosine with base, and the corresponding deoxy-adenosines with tributyltin hydride; β-D-xylofuranosyladenine with thionyl chloride in HMPT gave the corresponding 5′-chloro-5′-deoxy-nucleoside, not the 2′-isomer previously suggested.[114]

8 Branched-chain Nucleosides

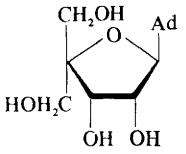
9-(2,3-Dideoxy-3-*C*-(hydroxymethyl)-β-D-*erythro*-pentofuranosyl)-4-thio-guanine (64) has been prepared, and its anti-tumour properties investigated.[115] Likewise, the 3-*C*-hydroxymethyl-β-D-xylofuranosyl analogues (65) of adenosine have been prepared by standard condensation procedures. Both were substrates for adenosine aminohydrolase, showing that the C-3′ branched-chain nucleosides can be deaminated when the branch is *trans* related to the base provided a suitably disposed hydroxy-group is available on the *endo*-side of the furanose ring.[116] The isomeric 4′-*C*-hydroxymethyl-adenosine (66) and analogous derivatives of other



(64)



(65) R = Me or CH_2OH



(66)

[109] J. Beranek and H. Hrebabecky, *Nucleic Acid Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 125 (*Chem. Abs.*, 1979, **90**, 87 810*u*).

[110] H. Hrebabecky and J. Beranek, *Nucleic Acid Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 131 (*Chem. Abs.*, 1979, **90**, 87 811*v*).

[111] H. Hrevabecky and J. Beranek, *Collect. Czech. Chem. Commun.*, 1978, **43**, 3268.

[112] P. Drasar and J. Beranek, *Nucleic Acids Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 179 (*Chem. Abs.*, 1979, **90**, 87 813*x*).

[113] L. M. Beacham, *J. Org. Chem.*, 1979, **44**, 3100.

[114] M. J. Robins, P. Sporns, and W. H. Muhs, *Can. J. Chem.*, 1979, **57**, 274.

[115] E. M. Acton, R. N. Goerner, H. S. Uh, K. J. Ryan, D. W. Henry, C. E. Cass, and G. A. LePage, *J. Med. Chem.*, 1979, **22**, 518.

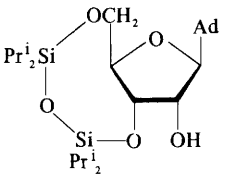
[116] J. M. J. Tronchet and J. F. Tronchet, *Helv. Chim. Acta*, 1979, **62**, 689.

purine and pyrimidine nucleosides have also been synthesized either conventionally from the peracetyl-branched-chain sugar[117] or by base-catalysed reaction of the nucleoside 5′-aldehydes with formaldehyde;[118] in the latter case, epimerization occurred at C-3′ if the vicinal hydroxy-groups at C-2′ and C-3′ were unprotected.

1-(β-D-Apiofuranosyl)-5-fluoro-uracil has been synthesized as part of a study of the ^{13}C n.m.r. spectra of apiosyl pyrimidine nucleosides,[119] and the mass spectra of peracetylated apiosyl-adenine and uracil nucleosides have been compared with ribosyl analogues.[120]

9 Ether and Acetal Derivatives

Bis-chlorodi-isopropylsiloxane has been recommended as a reagent for forming 3′,5′-disilyl derivatives of nucleosides, *e.g.*, (67); 5′-monomethoxytritylated nucleosides gave the corresponding 2′,3′-cyclic ether, from which the trityl ether group could be selectively removed using toluene-*p*-sulphonic acid in dioxan. These reactions provide useful routes to 2′- or 5′-mono-substituted nucleosides.[121,122]



(67)

t-Butyldimethylsilyl ethers continue to provide valuable blocking groups in nucleoside chemistry. Various mono- and di-silyl ethers of cytidine and guanosine have been prepared, and mono-ethers from 5′-*O*-monomethoxytrityl nucleosides provide useful routes to 2′- or 3′-substituted nucleoside derivatives, including 2′,5′-nucleotides, since they are readily separated from each other, and can also be equilibrated in methanol.[123,124] The equilibration of 2′- and 3′-monosilyl ethers of nucleosides is also referred to in Chapter 4. The 2-*O*-t-butyldimethylsilyl derivative of 1,3,5-tri-*O*-benzoyl-α-D-ribofuranose has also been used to prepare nucleosides, which may be debenzoylated to give derivatives suitable for

[117] R. D. Youssefyeh, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, 1979, **44**, 1301.
[118] G. H. Jones, M. Taniguchi, D. Tegg, and J. G. Moffatt, *J. Org. Chem.*, 1979, **44**, 1309.
[119] P. W. Rabidean, D. K. Parikh, and R. R. Watson, *J. Carbohydr., Nucleosides, Nucleotides*, 1978, **5**, 537.
[120] R. M. Thompson, D. K. Parikh, and R. R. Watson, *Biomed. Mass Spectrom.*, 1978, **5**, 367 (*Chem. Abs.*, 1978, **89**, 215 688*d*).
[121] W. T. Markiewicz, *J. Chem. Res. (S)*, 1979, 24.
[122] W. T. Markiewicz and M. Wiewiorowski, *Nucleic Acids Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 185 (*Chem. Abs.*, 1979, **90**, 87 814*y*).
[123] K. K. Ogilvie, A. L. Schifman, and C. L. Penny, *Can. J. Chem.*, 1979, **57**, 2230.
[124] K. K. Ogilvie and N. Y. Theriault, *Tetrahedron Lett.*, 1979, 2111.

the synthesis of nucleotides; the silyl ether group is readily removed using potassium fluoride with 18-crown-6 ether in THF.[125] Treatment of 4-*N*-benzoylcytidine with varying ratios of t-butyldimethylchlorosilane in presence of imidazole allowed the synthesis of all the possible ethers having the 5′-position substituted; the remaining ethers were made from the 5′-monomethoxytrityl nucleoside, and the base-catalysed equilibration of the 2′- and 3′-mono ethers referred to above was again noted.[126]

Tubericidin and nebularin[127] and also 6-azauridine-5′-monophosphate[128] have been bound to polymer supports through cyclic ketal linkages formed between the C-2′ and C-3′ hydroxy-groups and laevulinic acid, which in turn is attached to 6-aminohexyl-agarose by an amide linkage. Conditions for the preparation of 5′-*O*-benzyl and 5′-*O*-glycosyl derivatives of purine nucleosides without concomitant derivatization of the purine unit have been established.[129] The synthesis of nucleoside glycosides is also referred to in Chapter 3.

10 Phosphates and Acyl-esters of Nucleosides

Routine syntheses of nucleotides have not been covered. Syntheses of more unusual derivatives have included adenosine and uridine 5′-(1-amino)ethylphosphonate, which are unaffected by alkaline phosphatase,[130] and 5-fluoro-2′-deoxyuridine 5′-*p*-azidophenylphosphate, used as a photo-affinity label for thymidylate synthetase.[131]

New phosphate ester derivatives of nucleosides useful for oligonucleotide synthesis have included 3′-(2-cyanoethyl, 5-chloro-8-quinolinyl)phosphates[132] and 2,2,2-tribromoethyl, 2,2,2-trichloroethyl phosphates, the latter being selectively dealkylated by electrochemical reduction to give the corresponding 2,2,2-trichloroethyl phosphates.[133] A one-flask method for the synthesis of 5′-phosphordiamidates of nucleosides has been described,[134] and a new direct synthesis of phosphate-blocked nucleotide derivatives of arabinosylcytosine reported.[135] Thymidine has been selectively phosphorylated at the 5′-position using *SS*-diaryl phosphorodithioates in good yield, the protecting thiol ester groups being readily removed by silver acetate in aqueous pyridine.[136]

[125] F. Dumont, R. H. Wightman, J. C. Ziegler, C. Chavis, and J. L. Imbach, *Tetrahedron Lett.*, 1979, 3291.
[126] W. Köhler and W. Pfleiderer, *Liebigs Ann. Chem.*, 1979, 1855.
[127] H. Rosemeyer and F. Seela, *Carbohydr. Res.*, 1979, **74**, 117.
[128] H. Rosemeyer and F. Seela, *J. Med. Chem.*, 1979, **22**, 1545.
[129] F. W. Lichtenthaler, K. Kitahara, and W. Riess, *Nucleic Acids Res. Symp. 4th, Spec. Publ.*, 1978, **4**, 115 (*Chem. Abs.*, 1979, **90**, 121 930*v*).
[130] K. Lesiak, W. J. Slec, and W. S. Zielínski, *Pol. J. Chem.*, 1979, **53**, 327.
[131] L. G. Stephanson and J. M. Whiteley, *J. Med. Chem.*, 1979, **22**, 953.
[132] H. Takaku, T. Nomoto, Y. Sakamoto, and T. Hata, *Chem. Lett.*, 1979, 1225.
[133] J. Engels, *Angew. Chem. Int. Ed. Engl.*, 1979, **18**, 148.
[134] S. Bottka and J. Tomasz, *Tetrahedron*, 1979, **35**, 2909.
[135] S.-H. Kim and A. Rosowsky, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, **6**, 229.
[136] M. Sekine, K. Hamaoki, and T. Hata, *J. Org. Chem.*, 1979, **44**, 2325.

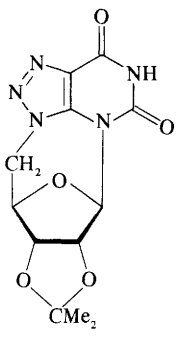
3′- and 5′-Mononucleotides can be readily distinguished by their e.i. mass spectra; in all cases the m/e 501 ion was far more abundant in the 3′-isomer.[137]

2′,3′- and 3′,5′-Di-*O*-acyl derivatives of 9-(β-D-arabinofuranosyl)adenine have been prepared and evaluated as potential antiviral agents; in presence of tetrabutylammonium fluoride in dry THF, the 2′,3′-di-*O*-acyl,5′-*O*-silyl ether derivatives initially prepared, rearranged to give the 3′,5′-di-*O*-acyl nucleoside by 2′→ 5′-acyl migration following desilylation by fluoride ion; protonation of the first formed O-5-anion by a solvent such as acetic acid prevented this rearrangement.[138]

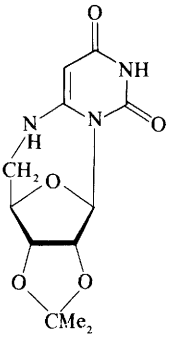
The regioselective deacylation of ribonucleosides is mentioned in Chapter 6; equilibration between 3′,5′- and 2′,5′-di-*O*-acyl derivatives can be achieved on silica gel allowing the 3′,5′-isomer to be obtained as the major product on silica-gel column chromatography.[139]

11 Miscellaneous Nucleosides

A further report on the reactions of 5′-azido-5′-deoxy uridine (see Vol. 12, p. 166) describes its cyclization in presence of DDQ to give the bridged compounds (68) or (69).[140]



(68)



(69)

Treatment of the acetylenic nucleoside (70) with dipolar reagents gave the double-headed nucleoside analogues (71) and (72) (Scheme 16).[141]

The double nucleoside 8,8′-biguanosine (73) has been prepared by irradiation of guanosine with its 8-bromo-derivative, and its crystal structure was also determined.[142]

[137] H. Budzikiewicz and G. Feistner, *Biomed. Mass Spectrom.*, 1978, **5**, 512 (*Chem. Abs.*, 1979, **90**, 152 520*r*).

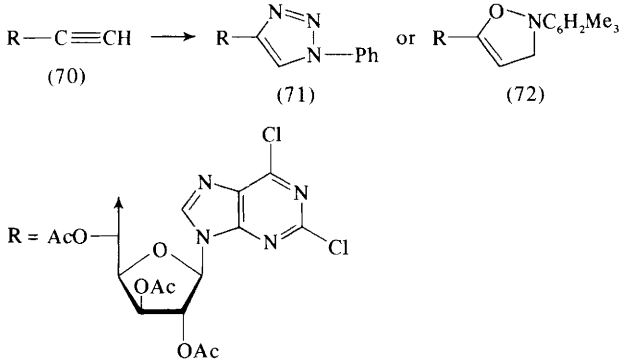
[138] D. C. Baker, T. H. Haskall, S. R. Putt, and B. J. Sloan, *J. Med. Chem.*, 1979, **22**, 273.

[139] Y. Ishido, N. Sakairi, and I. Hirao, *Nucleic Acids Res. Symp. 6th, Spec. Publ.*, 1975, **8**, 263 (*Chem. Abs.*, 1979, **90**, 121 933*y*).

[140] T. Sasaki, K. Minamoto, T. Suzuki, and T. Sugiura, *J. Org. Chem.*, 1979, **44**, 1424.

[141] D. Horton and J.-H. Tsai, *Carbohydr. Res.*, 1979, **75**, 141.

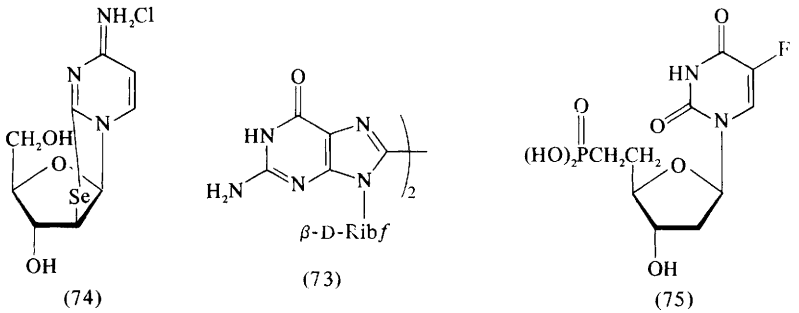
[142] S. N. Bose, R. J. H. Davies, J. C. van Niekerk, D. W. Anderson, and L. R. Nassimbeni, *J. Chem. Soc., Perkin Trans. 2*, 1979, 1194.



Scheme 16

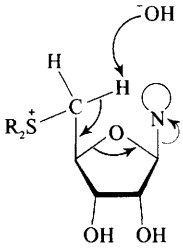
2-Seleno-cytidine gives the novel 2′-cyclo-2-selenocytidine (74) on treatment with 2-acetoxyisobutyryl chloride.[143]

A Wittig condensation has been used to prepare the phosphonate analogue (75) of 2′-deoxy-5-fluoro-uridylic acid from the corresponding 5′-aldehydo nucleoside; the compound is a potent inhibitor of thymidylate synthetase.[144]

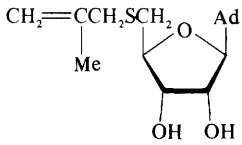


Glycoside hydrolysis of 5′-sulphonium nucleosides such as *S*-adenosyl-methionine occurs very readily in alkaline solution, which is rationalized by a mechanism involving H-5′ proton abstraction and ring opening illustrated in structure (76).[145] A range of analogues of *S*-adenosyl-homocysteine have been prepared, with varying substituents at S-5′, O-2′, O-3′, and N-6; the *S*-isobutenyl analogue (77) was one of the most active inhibitors of the coenzyme *S*-adenosyl-L-methionine prepared.[146] Enzymatic hydrolysis of *S*-adenosyl-L-homocysteine

[143] D. S. Wise and L. B. Townsend, *J. Chem. Soc., Chem. Commun.*, 1979, 271.
[144] J. A. Montgomery, H. J. Thomas, R. L. Kisluik, and Y. Gaumont, *J. Med. Chem.*, 1979, **22**, 109.
[145] R. T. Borchardt, *J. Am. Chem. Soc.*, 1979, **101**, 458.
[146] M. Vuilhorgne, P. Blanchard, C. J. R. Hedgecock, F. Lawrence, M. Robert-Gero, and E. Lederer, *Heterocycles*, 1978, **11**, 495.



(76)

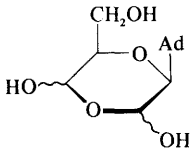


(77)

in deuterium or tritium oxide gave adenosine labelled at C-4′; this, together with other evidence, suggested a mechanistic sequence of 3′-oxidation and β-elimination to give the 4′,5′-dehydro-3′-keto nucleoside, followed by a reverse sequence of addition of water and reduction to give adenosine. 4′,5′-Dehydro-adenosine was also synthesized by standard methods and shown to give adenosine with the same enzyme.[147]

12 Structure and Analysis of Nucleosides

The structures of the dialdehydes obtained by periodate oxidation of adenine nucleosides have been investigated; no free aldehyde was detected, and the major form present in crystalline products was thought to be the di-hemi-acetal hydrate (78).[148]



(78)

The conformation of nucleosides and nucleotides has been reviewed.[149] The conformation of 2-alkylthio-2′,3′-*O*-isopropylidene-adenosine derivatives has been studied by i.r. and c.d. measurements; a strong hydrogen bond exists between *N*-3 and *O*-5′ in carbon tetrachloride, indicating a *syn* conformation for the base; c.d. curves, which are markedly dependent on the sulphur substituent, suggest a *syn* conformation in chloroform and an *anti* arrangement in water.[150] ^{1}H N.m.r. evidence, confirmed by an *X*-ray crystal structure study, indicates that 9-β-D-arabinofuranosyl-8-n-butylaminoadenine adopts the *anti*-conformation in

[147] J. L. Palmer and R. H. Abeles, *J. Biol. Chem.*, 1979, **254**, 1217.
[148] A. J. Grant and L. M. Lerner, *Biochemistry*, 1979, **18**, 2838.
[149] D. B. Davies, 'Progress in Nuclear Magnetic Resonance Spectroscopy', Vol. 12, Pt. 3, Pergamon, Oxford, 1979.
[150] S. Higuchi and K. Kikugawa, *Nucleic Acids Res. Symp. 6th, Spec. Publ.*, 1978, **5**, 367 (*Chem. Abs.*, 1979, **90**, 121 939e).

dimethyl sulphoxide and the crystal, although 8-substituted adenosines are usually expected to be *syn* owing to steric hindrance.[151]

Methods for determining the anomeric configurations of ribonucleosides have been reviewed.[152] The well known anomeric-proton rule for determining anomeric configuration from ^{1}H n.m.r. chemical shifts has been supplemented by the rule, which states that the heterocyclic proton on the carbon next to the glycosidically linked nitrogen is downfield in the isomer in which the base and the C-2′ hydroxy-group are *trans* to each other relative to the same proton in the *cis* isomer. The rule holds for unprotected and acetalated nucleosides, but not for acyl or benzyl derivatives.[153]

The ^{13}C n.m.r. spectra of inosine, 1-methylinosine, and 6-methoxypurine riboside have been completely assigned.[154]

Quantum mechanical calculations on some imidazole and benzimidazole nucleosides do not predict the conformations observed in the crystal, the difference being attributed to stacking forces and intermolecular hydrogen bonds in the latter case.[155] An *ab initio* molecular orbital study of adenosine has been reported.[156]

The mass spectra of benzylated and seleno-nucleosides are mentioned in Chapter 21.

[151] S. Neidle, M. R. Sanderson, A. Subbiah, J. B. Chattopadhyaya, R. Kuroda, and C. B. Reese, *Biochim. Biophys. Acta*, 1979, **565**, 379.

[152] M. J. Robins and M. Maccoss, in 'Chem. Biol. Nucleosides, Nucleotides', [Pap. Symp.] 1976 (Publ. 1978), ed. R. E. Harmon, R. K. Robins, and L. B. Townsend, Academic Press, New York, p. 311 (*Chem. Abs.*, 1979, **91**, 211 683*s*).

[153] V. V. Alenin and V. D. Domkin, *Zh. Obshch. Khim.*, 1978, **48**, 2629 (*Chem. Abs.*, 1979, **90**, 104 276*a*).

[154] K. Maskos, *Acta Pol. Pharm.*, 1979, **36**, 69 (*Chem. Abs.*, 1979, **91**, 91 909*w*).

[155] C. Mitra, A. Saran, and G. Govil, *Indian J. Chem., Sect. B*, 1978, **16**, 132 (*Chem. Abs.*, 1979, **90**, 23 479*k*).

[156] J. S. Kwiatkowski and B. Pullman, *Int. J. Quantum Chem.*, 1979, **15**, 499 (*Chem. Abs.*, 1979, **91**, 20 956*y*).

20

N.m.r. Spectroscopy and Conformational Features

1 Theoretical and General Considerations

Isbell has reviewed the historical development of concepts of ring-conformation and neighbouring-group effects in carbohydrates prior to 1940.[1]

Three analyses of the anomeric effect by means of *ab initio* MO calculations have appeared.[2,3,4] From molecules of general formula XCH_2YH (where X = NH_2, Me, OH, F, or Cl; Y = O or S) and CH_nF_{4-n} the torsional behaviour as a function of X and Y was computed, and, together with bond length, was related to the anomeric effect and the reverse anomeric effect. The methods incorporated the stabilizing effect between the lone pair on Y and the antibonding orbital of XCH_2 and between the highest lying bonding orbital of X and the antibonding orbitals of Y, using a frontier-orbital approach. It was shown that σ^* varies greatly with the nature of X and Y whereas π^* varies very little, therefore supporting the idea that coulombic effects and maximal HOMO–LUMO interactions are the basis for the anomeric effects.[2] Methoxymethyl fluoride and chloride have been subjected to theoretical treatment and the relative anomeric energies and bond lengths were calculated and related to O-5–C-1 bond torsional force constants in sugars.[3] 'Through-bond' and 'through-space' orbital interactions of oxygen lone pairs in dimethoxymethane have been calculated as models for the anomeric effect.[4] The *exo*-anomeric effect has been reviewed.[5] A review on the interaction between ring-oxygen *p*-type lone pairs and adjacent σ-bonds in pyranose derivatives has discussed MO, n.q.r., conformation, and anomeric effects.[6] Classical conformational analysis applied to methylcyclohexane, cyclohexanol, and 3-methylcyclohexene as models has been used for the study of steric dependence of the anomeric and allylic effects. Methods described previously by Allinger, Dodzink, and Engler allowed computation of the interaction energies of groups in pseudoequatorial and pseudoaxial orientations.[7] *Ab initio* calculations of the conformations of D-glucose and β-maltose have allowed quantification of non-bonded interaction energies.[8]

From a model based on two methanol molecules, it has been shown that the maximum probability for the O–H···O hydrogen bond angle is 163°. From eighteen neutron diffraction studies of carbohydrates the mean angle was found

[1] H. S. Isbell, *J. Carbohydr., Nucleosides, Nucleotides*, 1979, **6**, 1.
[2] S. Wolfe, M.-H. Whangbo, and D. J. Mitchell, *Carbohydr. Res.*, 1979, **69**, 1.
[3] G. A. Jeffrey and J. H. Yates, *J. Am. Chem. Soc.*, 1979, **101**, 820.
[4] I. Tvaraska and T. Bleha, *Can. J. Chem.*, 1979, **57**, 424.
[5] R. U. Lemieux, S. Koto, and D. Voisin, *Amer. Chem. Soc. Symp. Ser.*, 1979, **87**, 17.
[6] S. David, *Amer. Chem. Soc. Symp. Ser.*, 1979, **87**, 1.
[7] H. Dodziuk, *Carbohydr. Res.*,1979, **70**, 19.
[8] S. Melberg, K. Rasmussen, R. Scordamaglia, and C. Tosi, *Carbohydr. Res.*, 1979, **76**, 23.

to be 166°. Molecular orbital calculations showed that bifurcation of hydrogen bonds leads to more stable situations than simple linear hydrogen bonding. Computation of the energies of the various possibilities was carried out.[9]

A stereographic spherical representation of the conformational itinerary of carbohydrates using Cremer and Pople puckering parameters has been published.[10]

An improvement to two-dimensional *J* spectroscopy on nulling of the residual solvent resonance has been demonstrated; the technique was applied to the analysis of the spectrum of uridine.[11] Complex ^{1}H n.m.r. spectra have been resolved by three-dimensional plotting of peaks, using computer graphics to tilt the picture through 45°; projections were used to obtain effectively decoupled spectra.[12]

A review of proton spin–lattice relaxation, suggested as a possible new quantitative measure of aglycone–sugar interactions, has appeared.[13] Non-selective proton spin–lattice relaxation rates measured at 400 MHz have allowed a quantitative determination of the geometry of diamagnetic molecules in solution. The comparison of the initial slopes of such relaxation rates for 1,2,3,4-tetra-*O*-trideuterioacetyl-β-D-arabinopyranose, its 5,5-dideuterio derivative, and its two isomeric 5-deuterio derivatives gave specific interproton relaxation contributions arising *via* a dipole–dipole mechanism, from which the magnitude of interproton distances could be calculated. Close agreement with the values obtained by neutron diffraction was found.[14]

Electric field theory has been applied to ^{1}H and ^{13}C n.m.r. of D-galacturonic and D-glucuronic acids. The effects of variation of pH and addition of Ca^{2+} ion on the magnitude of change in chemical shift were measured and considered in the context of the theory. A detailed reconsideration of the field-gradient contribution was carried out.[15] A study of the ionization and mutarotation of hexoses in aqueous alkali, using ^{13}C n.m.r., has been conducted. Some ^{13}C resonances shift considerably with change in pH and n.m.r.-titration curves allowed determination of both pK_a values and the difference in acid strength of the α- and β-anomeric forms. The shifts were not explicable by simple ionization and, either a rapid equilibrium between cyclic and 'pseudo'-cyclic ionized forms, or a non-classical anion were proposed. Base-catalysed mutarotation originates in the weakening of the ring C–O bond in the generated sugar anion.[16]

[9] M. D. Newton, G. A. Jeffrey, and S. Takagi, *J. Am. Chem. Soc.*, 1979, **101**, 1997.
[10] G. A. Jeffrey and J. H. Yates, *Carbohydr. Res.*, 1979, **74**, 319.
[11] L. D. Hall, and S. Sukumar, *Carbohydr. Res.*, 1979, **74**, C1.
[12] L. D. Hall, G. A. Morris, and S. Sukumar, *Carbohydr. Res.*, 1979, **76**, C7.
[13] J. M. Berry, L. D. Hall, D. G. Welder, and K. F. Wong, *Amer. Chem. Soc. Symp. Ser.*, 1979, **87**, 30.
[14] L. D. Hall, K. F. Wong, W. E. Hull, and J. D. Stevens, *J. Chem. Soc., Chem. Commun.*, 1979, 953.
[15] L. W. Jaques, J. B. Macaskill, and W. Weltner, jun., *J. Phys. Chem.*, 1979, **83**, 1412 (*Chem. Abs.*, 1979, **91**, 20 936*s*).
[16] G. Dewit, A. P. G. Kieboom, and H. Van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 1979, **98**, 355 (*Chem. Abs.*, 1979, **91**, 91 860*y*).

Two n.m.r. studies of the hydration of monosaccharides by means of high resolution ^1H n.m.r. in H_2O have been published.[17,18] Under limited conditions, well resolved OH protons can be seen downfield of the H_2O peak. Exchangeable proton spectra for D-glucose, D-galactose, and D-mannose, in which the linewidths were 30–70 Hz, were studied. The exchange was studied in detail for the anomeric protons of D-glucose. The residence times were of the order of 10 ms in the accessible temperature range, with the exchange for the α-anomer being slower. Activation energies were 30–50 kJ mol^{-1} depending on the concentration and type of anomer.[17] The second study showed that the anomeric OH has a lower chemical shift than the other OH resonances, and that an axial anomeric OH had a higher chemical shift than an equatorial. Proton exchange with H_2O was, as expected, strongly acid and base catalysed, but there was also a pH dependent mechanism involved. The effect of temperature and addition of DMSO suggested that each OH bonded on average to two water molecules (a much higher solvation number than suggested by relaxation studies) reflecting residence times for the bound water. Similar results in methanol suggested that there was no special effect owing to water structure.[18] The rate of protonation of the hydroxy-groups of D-glucose has been measured in DMSO.[19] The chemical shifts of all hydroxy-groups and the $J_{OH,CH}$ couplings for α- and β-D-glucose were measured. The rates for the equation $ROH^* + H^+ \xrightarrow[k]{DMSO} \dot{R}\dot{O}H^*H \xrightarrow{fast} ROH + H^{*+}$ were found to be in the order $k_{6\text{-}OH} \sim k_{2\text{-}OH} > k_{3\text{-}OH} > k_{4\text{-}OH} \gg k_{1\text{-}OH}$.

In situ reactions of hydroxylic compounds with trichloroacetylisocyanate have been studied by ^1H n.m.r. Although no carbohydrate examples were given, the reaction, which can be carried out in the n.m.r. tube, has obvious application in this area since it causes shifts in the α-protons.[20]

2 Acyclic Systems

Fourier Transform ^1H n.m.r. at 90 MHz has been used to probe the conformations o per-acetylated L-arabinononitrile, D-mannononitrile, D-gluconononitrile, D-galactononitrile, 6-deoxy-L-galactononitrile, 2-deoxy-D-*arabino*-hexononitrile, and 2-deoxy-D-*lyxo*-hexononitrile. Zig-zag conformations were adopted for all except D-gluconononitrile, which exists in the 'sickle' form.[21] Conformational studies on the four 1-amino-1-deoxy-D-pentitols and their hydrochlorides by 250 MHz ^1H n.m.r. in D_2O have shown that the D-*arabino*- and the D-*lyxo*-isomers adopt the extended zig-zag conformation, whereas the D-*xylo*- and D-*ribo*-isomers, which have unfavourable 1,3-interactions, adopt the non-planar 'sickle' conformation; the results are similar to those obtained by *X*-ray crystallo-

[17] S. Bociek and F. Franks, *J. Chem. Soc., Faraday Trans. 1*, 1979, **75**, 262.
[18] J. M. Harvey and M. C. R. Symons, *J. Solution Chem.*, 1978, **7**, 571 (*Chem. Abs.*, 1979, **90**, 39 141*e*).
[19] B. Gillet, D. Nicole, and J.-J. Delpeuch, *Tetrahedron Lett.*,1979, 1219.
[20] Z. Samek and M. Budesinsky, *Collect. Czech. Chem. Commun.*, 1979, **44**, 558.
[21] L. M. Sweeting, B. Coxon, and R. Varma, *Carbohydr. Res.*, 1979, **72**, 43.

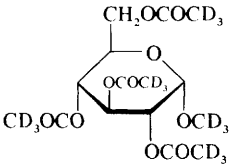
graphy and by study of the related series of acetylated derivatives in organic solvents.[22] ^{13}C N.m.r. spectra of alditols, 1,2-bisphenylhydrazones, dithioacetals, and other acyclic carbohydrates have been reported.[23] The ^{13}C n.m.r. of the oximes of *N*-substituted 1-amino-1-deoxy-hexuloses has been studied as a means of investigating the mechanism of the Amadori rearrangement.[24]

3 Pyranoid Systems

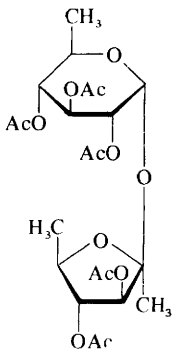
Conformational analysis of pentopyranosyl acetates, benzoates, and halides has been reviewed.[25]

The statistical distribution of the conformational orientations of the hydroxymethyl and esterified hydroxy-groups in glucopyranoses and galactopyranoses in the $^4C_1$ conformation were shown to be *g/g* (60%) and *g/t* (40%) for the former, and *g/t* (58%), *t/g* (34%), and *g/g* (8%) for the latter. The reasons for the unfavourable *t/g* conformation for glucopyranose and *g/g* conformation for galactopyranose were discussed in relation to steric and electronic interactions.[26]

Two-dimensional *J* spectroscopy has been applied to the ^1H 270 MHz spectra of (1) and (2).[27] The influence of *O*-alkyl groups on the chemical shift of the



(1)

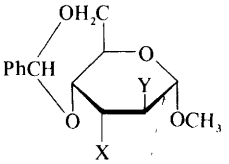


(2)

glycosidic proton in pyranoid systems has been studied using various alkyl β-D-xylopyranosides in D_2O and DMSO at 300 and 360 MHz, respectively. A rationale based on the rotameric state of the aglycone about the C-1–O-1 bond was examined.[28] Solvent shifts in the ^1H n.m.r. 100 MHz spectra of methyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside and its β-anomer have been shown to be very useful for spectral analysis; solvents used were deuterochloroform,

[22] M. Blanc-Muesser, J. Defaye, and D. Horton, *Carbohydr. Res.*, 1979, **68**, 175.
[23] G. W. Schnarr, D. M. Vyas, and W. A. Szarek, *J. Chem. Soc., Perkin Trans. 1*, 1979, 496.
[24] W. Funke, C. von Sonntag, and A. Klemer, *Carbohydr. Res.*, 1979, **71**, 315.
[25] H. Paulsen, P. Luger, and F. R. Heiker, *Amer. Chem. Soc. Symp. Ser.*, 1979, **87**, 63.
[26] R. H. Marchessault and S. Perez, *Biopolymers*, 1979, **18**, 2369.
[27] L. D. Hall, S. Sukumar, and G. R. Sullivan, *J. Chem. Soc., Chem. Commun.*, 1979, 292.
[28] A. De Bruyn, N. Hosten, M. J. O. Anteunis, M. Claeyssens, and C. K. De Bruyne, *Bull. Soc. Chim. Belg.*,1979, **88**, 43 (*Chem. Abs.*, 1979, **91**, 123 962*b*).

[$^2H_6$]benzene, and [$^2H_5$]pyridine.[29] Deshielding of the axial anomeric hydrogen and the shielding of the imino hydrogen has been observed in those *N*-acylglycopyranosylamines with axial hydroxy-groups on C-2.[30] The ^1H and ^{13}C n.m.r. spectra of the tin-bonded carbohydrates (3) and (4) have been measured and the conformations thus deduced. The proton–tin and carbon–tin couplings were reported.[31]

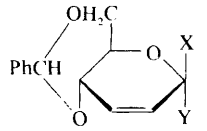


(3) X = $SnPh_3$, Y = OH
(4) X = OH, Y = $SnPh_3$

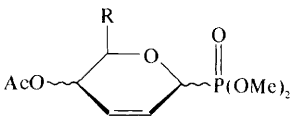
The conformational analysis of the peracetyl 1,5-anhydro-hex-1-enitol system has been carried out using ^1H n.m.r. at 250 MHz: all were shown to adopt the $^4H_5$ (D) conformation except the *lyxo* isomer, which adopted the $^5H_4$ (D) conformation.[32] The conformations of all eight 1,6-anhydro-D-aldohexopyranoses and their triacetates have been shown by ^1H n.m.r. to be the expected flattened $^1C_4$ chair with the flattening being more pronounced when the C-3 substituent is *cis* to the anhydro-bridge.[33] The same group has studied the complete series of 1,6 : 2,3- and 1,6 : 3,4-dianhydro-D-hexopyranoses in a similar manner; all were shown to exist as $^5H_0$ or $^1H_0$ half-chairs.[34] *N*-(2,3,4-Tri-*O*-acetyl-β-D-lyxopyranosyl)-*p*-nitroaniline exists in the $^4C_1$ conformation, whereas the α-anomer adopts the $^1C_4$ conformation.[35] A similar situation has been described for the corresponding D-*ribo* compounds.[36] The non-acetylated *N*-(α-D-ribopyranosyl)-*p*-nitroaniline is in conformational equilibrium between $^1C_4$ and $^4C_1$ chairs.

The ^1H n.m.r. data for 6-deoxy-6-fluoro-α- and -β-D-glucopyranose obtained at 300 MHz in D_2O has been reported with a discussion of chemical shifts and coupling constant values.[37] N.m.r. data on the unsaturated sugar derivatives (5) and (6), as well as on some fructofuranose derivatives useful as intermediates in a new synthesis of sucrose, have been published.[38] The complete elucidation of the ^1H n.m.r. spectra of the 2,3-unsaturated glycosyl phosphonates of type (7)

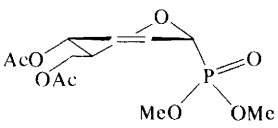
[29] B. Matsuhiro, A. B. Zanlungo, and M. Pieber, *Rev. Latinoam. Quim.*, 1979, **10**, 61 (*Chem. Abs.*, 1979, **91**, 193 574*a*).
[30] A. S. Cerezo, *An. Asoc. Quim. Argent.*,1977, **65**, 173 (*Chem. Abs.*, 1979, **91**, 5430*d*).
[31] L. D. Hall, P. R. Steiner, and D. C. Miller, *Can. J. Chem.*, 1979, **57**, 38.
[32] R. H. Shah and O. P. Bahl, *Carbohydr. Res.*, 1979, **74**, 105.
[33] M. Buděšinský, M. Černý, T. Trnka, and S. Vašičkova, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1965.
[34] M. Buděšinský, T. Trnka, and M. Černý, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1949.
[35] J. Sokolowski and E. Paszkiewicz, *Pol. J. Chem.*, 1979, **53**, 305.
[36] D. Piskorska and J. Sokolowski, *Pol. J. Chem.*, 1979, **53**, 1001.
[37] A. De Bruyn, M. Anteunis, G. Aerts, and C. K. De Bruyne, *Acta Cienc. Indica*, 1978, **4**, 103 (*Chem. Abs.*, 1979, **90**, 104 233*c*).
[38] D. E. Iley and B. Fraser-Reid, *Can. J. Chem.*, 1979, **57**, 653.



(5) X = H, Y = OMe
(6) X = OMe, Y = H

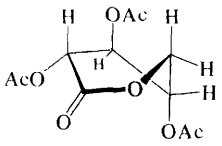


(7) R = H or CH_2OAc



(8)

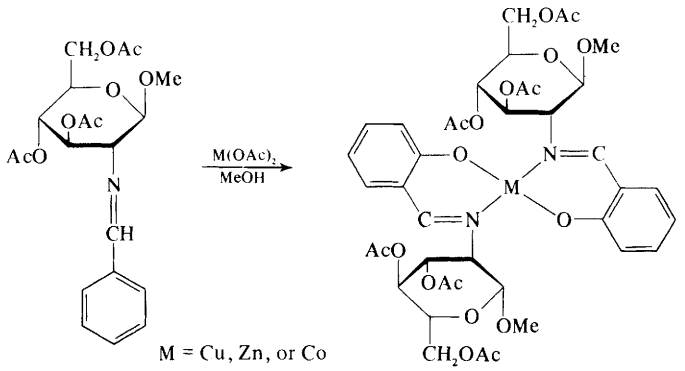
has been described. From the exactly determined proton–proton and carbon–phosphorus coupling constants the conformations were deduced. The crystal structure of the α-D-*erythro*-hexenose compound was determined and shown to have the half-chair conformation (8).[39] Conformational analysis of 2,3,4-tri-*O*-acetyl-D-xylono-1,5-lactone, using ^1H n.m.r., showed that, assuming the validity of a Karplus-type relationship, the C-3 and C-4 acetoxy-groups were antiperiplanar. The results indicated a solution conformation depicted in (9).[40]



(9)

A combination of ^1H, ^{13}C, and ^{31}P n.m.r. has been used to assign the conformations of α- and β-glycopyranosyl phosphates and 1,2-phosphates. For D-glucose, D-galactose, and D-mannose, all derivatives are in the $^4C_1$ conformation, whereas L-fucose is in the $^1C_4$ conformation. UDP-Glucose, UDP-galactose, GDP-mannose, and GDP-fucose were also examined.[41] It has been shown that the signal due to the equatorial proton at C-3 of *N*-acetyl-D-neuraminic acid and its derivatives shows a characteristic dependence on, and is therefore suitable for determination of, the anomeric configuration.[42] ^1H N.m.r. of the anomeric protons of glycosphingolipids in chloroform has been used to determine the anomeric structure of all sugar units by comparison with data obtained from mono-, di-, and tri-glycosyl ceramides.[43] Monomer and dimer units of glycosaminoglycuronan have been studied by ^1H n.m.r. in [$^2H_6$]DMSO solution. Resonances due to N–H and O–H were observed and partially assigned, and their temperature dependence suggested H-bonding to the solvent by all hydroxy- and amino-groups with the exception of HO-4 of sodium D-glucuronate, which was always downfield and non-temperature dependent. This signal was,

[39] G. Adiwidjaja, B. Meyer, H. Paulsen, and J. Thiem, *Tetrahedron*, 1979, 373.
[40] C. R. Nelson, *Carbohydr. Res.*, 1979, **68**, 55.
[41] J. V. O'Connor, H. A. Nunez, and R. Barker, *Biochemistry*, 1979, **18**, 500.
[42] V. Dabrowski, H. Friebolin, R. Brossmer, and M. Supp, *Tetrahedron Lett.*, 1979, 4637.
[43] K.-E. Falk, K.-A. Karlsson, and B. E. Samuelsson, *Arch. Biochem. Biophys.*, 1979, **192**, 164, 177, 191.



(10)

Scheme 1

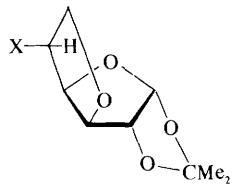
however, concentration dependent showing that HO-4 is involved in dimer formation. Signals for O–H and N–H were also observed in H_2O at sub-zero temperatures.[44] N.m.r. studies of a range of monomers carrying pyruvic acid acetals (see Chapter 5) have shown that the different chemical shifts of the acetalic methyl group may be used in the determination of such groups in naturally occurring polysaccharides.[45] Metal chelates of general formula (10) have been prepared as shown in Scheme 1. Comparison of data from ^{1}H n.m.r., i.r., and e.s.r. indicates that a change from pseudotetrahedral symmetry to flattened tetrahedral symmetry occurs on going from solid to solution phase.[46]

4 Furanoid Systems

Hudson's Rules of isorotation have been applied to furanosides and a correlation with known conformations of methyl aldofuranosides made. The molecular rotations and differences in molecular rotation were tabulated for all aldopentoses, aldohexoses, and hexuloses as their methyl furanosides, and hence their conformations were deduced. The β-D-pentofuranosides were mainly in the $^3T_2$ form with the *O*-methyl group quasi-axial and the side-chain quasi-equatorial, whereas the α-D-pentofuranosides were either in the *E* form or a mixture of *T* forms. The optical rotations for all α-D-furanosides are negative, but for β-D-furanosides they are all positive.[47]

The crystal structure of 6-deoxy-α-L-sorbofuranose has been determined; the furanoid ring had a C-3^1-*exo*-C-4^1-*endo*($^4T_3$) conformation. The paper contains a discussion of the conformations adopted by ketofuranosides.[48] Some $^5J_{H\text{-}2,H\text{-}5}$ couplings of 0.8–0.95 Hz have been observed in 3,6-anhydro-D-idofuranose

[44] F. Heatley, J. E. Scott, and B. Casu, *Carbohydr. Res.*, 1979, **72**, 13.
[45] P. J. Garegg, B. Lindberg, and I. Kvarnstrom, *Carbohydr. Res.*, 1979, **77**, 71.
[46] M. J. Adam and L. D. Hall, *J. Chem. Soc., Chem. Commun.*, 1979, 234.
[47] S. J. Angyal, *Carbohydr. Res.*, 1979, **77**, 37.
[48] P. Swaminathan, L. Anderson, and M. Sundaralingam, *Carbohydr. Res.*, 1979, **75**, 1.

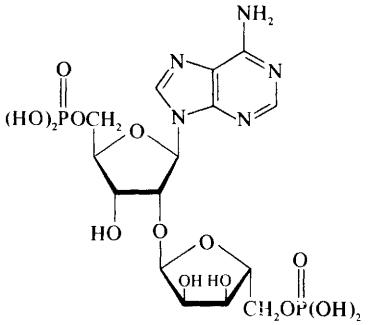


(11) X = H, OH, OBn, or SCH_2CH_2SH

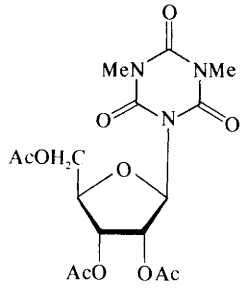
derivatives (11). The couplings, which appeared to be stereospecific since change of configuration at either centre caused loss of coupling, were thought to be through-space rather than through-bond.[49] A study of the anomeric ratio and preponderance of sulphur in the sugar ring in 5-thio- and 6-thio-D-fructose has been carried out using ^1H and ^{13}C n.m.r. in D_2O and O-*H* n.m.r. in DMSO. The results showed that the conformations for both derivatives were the same as for the oxygen in the ring analogues and that the α to β ratio for the thio-ring compounds was about 1:9.[50]

Application of long-range selective proton decoupling using low-power irradiation has enabled the assignment of indirect ^{13}C–^1H coupling in the ^{13}C n.m.r. spectra of the base moieties of purine and pyrimidine nucleosides and their analogues. The values obtained indicated the conformational preference for the *anti* arrangement about the glycosidic bond.[51] The conformations of adenosine 2′,5′- and 3′,5′-diphosphate in D_2O solution have been examined by ^1H, ^{13}C, and ^{31}P Fourier transform n.m.r.[52] Correlations of conformational parameters and equilibrium conformational states in a variety of β-D-arabinonucleosides and analogues have been studied by ^1H n.m.r.[53] The 250 MHz proton spectrum of 2,2′-anhydro-O^2-β-D-arabinofuranosyluracil has provided evidence for long-range coupling over four and five bonds (J = 0.3 Hz) between H-1 and each of H-5 and H-5′.[54] A discussion of conformation of the uracil and cytosine nucleosides of β-D-lyxofuranose based on ^1H n.m.r. spectra has been presented.[55] The ribosyl adenosine 5,5′ bis(phosphate) (12) has been studied by ^1H n.m.r. in aqueous solution.[56] Various 1-(β-D-ribofuranosyl)isocyanuric acid derivatives, *e.g.* (13), have been studied by n.m.r. at a variety of temperatures and the thermodynamic parameters for rotation about the glycosidic bonds calculated. The activation energies were found to be independent of whether the hydroxy-groups at C-2 or C-3 are unsubstituted, acetylated, or involved in isopropylidene rings.[57] The

[49] H. Honig, I. Macher, and H. Weidmann, *Tetrahedron Lett.*, 1979, 2579.
[50] M. Chmielewski and R. L. Whistler, *Carbohydr. Res.*, 1979, **69**, 259.
[51] J. Uzawa and M. Uramoto, *Org. Magn. Reson.*, 1979, **12**, 612.
[52] H. L. Chung and J. P. Horwitz, *J. Carbohydr., Nucleosides, Nucleotides*, 1978, **5**, 469.
[53] I. Ekiel, M. Remin, E. Darzynkiewicz, and D. Shugar, *Biochim. Biophys. Acta*, 1979, **562**, 177.
[54] F. E. Hruska, J. G. Dalton, and M. Remin, *Can. J. Chem.*, 1979, **57**, 2191.
[55] I. Ekiel, E. Darzynkiewicz, G. I. Birnbaum, and D. Shugar, *J. Am. Chem. Soc.*, 1979, **101**, 4724.
[56] F. Inagaki, T. Miyazawa, M. Iwa, H. Saits, and T. Sugimura, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 415.
[57] G. Wegner and J. C. Jochims, *Chem. Ber.*, 1979, **112**, 1941.



(12)



(13)

temperature dependence of the c.d. and n.m.r. spectra of 2-thioribothymidine has been compared with that of ribothymidine. The 3′-*endo-gauche-gauche-anti* form of the 2-thio derivative was found to be more stable than the 2′-conformer over a temperature range of 23–93 °C, a possible contributory factor in the thermostability of thermophile tRNA of which it is a natural constituent.[58]

5 Oligosaccharides

Convergent energy minimization in force-field calculations, carried out initially on α- and β-D-glucopyranose, has been used to predict the conformations of β-maltose[59] and cellobiose.[60] The four conformations predicted for the former were close to those found in *X*-ray structures and n.m.r. experiments, and there was a good correlation with the irregularities known to be present in amylose conformation. There were six local minima identified for cellobiose, the two lowest of which corresponded to the conformations found in *X*-ray structures.

The ^{13}C n.m.r. spectral data for rhamnobioses[61] and for some partially methylated benzyl β-maltosides[62] have been published. A complete unambiguous assignment of all ^{13}C signals of cellobiose and maltose has been achieved using various methods, such as selective proton decoupling, ^{13}C selective spin-labelling, and isotopic chemical shifts induced by deuterium. The results on α- and β-(1 → 4)-oligomers showed that the chemical shift variation of ^{13}C signals is dependent on the degree of polymerization. Solvation and conformational change were responsible for the temperature- and solvent-dependent shift changes.[63] The equilibrium compositions of solutions of maltulose, isomaltulose, sucrose, leucrose, 1-kestose, nystose, inulin, and grass levan have been studied by ^{13}C n.m.r. Observed spin-lattice (T_1) times and n.O.e. factors for the carbon

[58] K. Watanabe, S. Yokoyama, F. Hansske, H. Kasai, and T. Miyazawa, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 671.
[59] S. Melberg and K. Rasmussen, *Carbohydr. Res.*, 1979, **69**, 27.
[60] S. Melberg and K. Rasmussen, *Carbohydr. Res.*, 1979, **71**, 25.
[61] A. Liptak, A. Neszmélyi, and H. Wagner, *Tetrahedron Lett.*, 1979, 741.
[62] E. E. Lee and J. O. Wood, *Carbohydr. Res.*, 1979, **75**, 317.
[63] A. Heyraud, M. Rinaudo, M. Vignon, and M. Vincendon, *Biopolymers*, 1979, **18**, 167.

atoms of the D-fructofuranosyl residues of inulin were used in conjunction with deuteriation experiments as an aid to resonance and linkage assignments.[64]

The ^{1}H n.m.r. spectra of a large number of sugars, including di-, tri-, and tetra-saccharides, in D_2O have been published. The chemical shift value of the anomeric proton was mainly studied. A technique for analysis of oligo- and poly-saccharides was proposed: (a) identify residues by n.m.r.; (b) measure spin-lattice relaxation time, T_1, before and after addition of *e.g.*, Gd^{3+}, as a paramagnetic broadening probe (generally a terminal carboxy-group is first introduced at the reducing terminus *via* the cyanohydrin reaction as a binding site for the metal); (c) identify the sequence of residues from the modified n.m.r. spectrum.[65]

6 ^{13}C N.M.R. Spectroscopy

Two reviews on ^{13}C n.m.r. of carbohydrates have appeared, one in English[66] and the other in Japanese.[67]

A study has been carried out on the effect of solvents on one-bond ^{13}C–^{1}H coupling in carbohydrates; considerable variations with solvent were observed, indicating that structural correlations should be made using the same solvent.[68] The ^{13}C chemical shifts induced by deuteriation of the hydroxy-groups of a range of free sugars, glycosides, disaccharides, and mono-saccharide derivatives have been measured using a dual coaxial cell with the samples in H_2O solution in one compartment and in D_2O in the other. Carbons bearing the hydroxy-group suffered shifts of 0.14 p.p.m. (0.11 p.p.m. at the anomeric centre); carbons vicinal to carbons carrying hydroxy-groups underwent shifts of 0.03–0.06 p.p.m. This novel technique has obvious applicability in assignment work, and the paper, which contains much valuable ^{13}C n.m.r. data, also has a very good bibliography.[69] A new technique, given the acronym CASS (*C*arbon *A*ssignment by *S*elective *S*aturation), involving selective radiofrequency saturation with gated-proton decoupling and Fourier Transform difference spectroscopy, gives fully coupled ^{13}C n.m.r. spectra from individual carbon sites. The method was applied to methyl 2,3-di-*O*-allyl-α-D-glucopyranoside.[70]

In a study of the ^{13}C n.m.r. of glycosides, a significant influence of the stereochemistry of the adjacent hydroxy-group on the chemical shift of the glycosyl-carbon was observed.[71] Fifteen methyl aldofuranosides have been examined in aqueous solution by ^{13}C n.m.r. Measurement of the coupling of ^{13}C-1 and ^{13}C-2 with the protons of the furanose ring, together with a consideration of ^{1}H–^{1}H

[64] H. C. Jarrell, T. F. Conway, P. Moyna, and I. C. P. Smith, *Carbohydr. Res.*, 1979, **76**, 45.
[65] J. H. Bradbury and J. G. Collins, *Carbohydr. Res.*, 1979, **71**, 15.
[66] H. Sugiyama, *Heterocycles*, 1978, **11**, 615 (*Chem. Abs.*, 1979, **90**, 204 375*n*).
[67] Y. Inoue, *Kagaku No Ryoiki*, 1979, **33**, 552 (*Chem. Abs.*, 1979, **91**, 211 685*u*).
[68] K. Bock and C. Pedersen, *Carbohydr. Res.*, 1979, **71**, 319.
[69] P. E. Pfeffer, K. M. Valentine, and F. W. Parrish, *J. Am. Chem. Soc.*, 1979, **101**, 1265.
[70] G. T. Andrews, I. J. Colquhoun, B. R. Doggett, W. McFarlane, B. E. Stacey, and M. R. Taylor, *J. Chem. Soc., Chem. Commun.*, 1979, 89.
[71] K. Yamasaki, C. Kihara, K. Itano, R. Kasai, O. Tanaka, and J. H. Kim, *Tennen Yuki Kagobutsu Toronkai Kaen Yoshishu, 21st*, 1978, 504 (*Chem. Abs.*, 1979, **90**, 121 957*j*).

coupling, gave important information on the shapes adopted by five-membered ring derivatives of sugars. The presence of stabilizing factors in *quasi*-equatorial side-chains and magnitudes of anomeric effects were revealed by the study.[72]

The ^{13}C n.m.r. spectra of acetylated methyl α- and β-glycopyranosides of D-glucose, D-xylose, D-galactose, L-arabinose, L-fucose, and L-rhamnose in $CDCl_3$ have been reported.[73] Mono- and di-methyl ethers of methyl α-D-galactopyranoside have been examined by ^{13}C n.m.r. and the β-effect due to the neighbouring methoxy-group determined. When the latter was axial a positive β-effect was produced, whereas a negative effect was apparent when this group was equatorial.[74] Natural abundance ^{13}C n.m.r. spectra of seven di- and tri-*O*-(3-nitropropanoyl)-α- and -β-D-glucopyranoses have been treated by linear regression analysis to obtain best-fit substituent parameters for prediction of chemical shifts at ring carbons.[75] The ^{13}C chemical shifts and $^{1}J_{C,H}$ values for methyl 2,3-anhydro-α- and -β-D-lyxo- and -ribo-pyranosides, together with those of methyl 4,6-*O*-benzylidene-α- and -β-D-manno- and -allo-pyranosides have been determined. The carbon atoms in the positions β and γ to the oxiran ring show distinctive chemical shifts, while large $^{1}J_{C,H}$ values for *cis*-C–H bonds adjacent to the oxiran relative to those for their *trans*-counterparts were noted. All anomeric carbons of the furanosides resonate at lower field than those of the pyranosides, thus providing a useful distinction. In addition, the $^{1}J_{C\text{-}2,H\text{-}2}$ and $^{1}J_{C\text{-}3,H\text{-}3}$ values were larger for the furanoside oxirans.[76] The relationship between the ^{13}C chemical shifts and the structure of all possible 1,6 : 2,3- and 1,6 : 3,4-dianhydro-β-D-hexopyranoses has been outlined. Inversion of the oxiran ring from *endo* to *exo* causes an upfield shift at C-2 or C-4, respectively. The report contains a discussion of chemical shifts related to steric and polar interactions.[77]

^{13}C N.m.r. data for methyl 4,6-*O*-benzylidene-D-hexopyranosides and their 2,3-disulphonate esters together with some related nucleosides has been published.[78] A study of ^{13}C chemical shift and $^{1}J_{C,H}$ for acetalic carbons and hydrogens in benzylidene acetals has shown a correlation between these values and the ring-size of the acetal ring. Axial substituents at positions 4 and 6 in 2-phenyl-1,3-dioxan rings or 4 and 7 in substituted 2-phenyl-1,3-dioxepan rings were readily detected. In the nineteen carbohydrate derivatives studied, the ring-size of the benzylidene acetal could be assigned unambiguously.[79] The ^{13}C n.m.r. spectra of diastereoisomeric pairs of nine 2,3-*O*-benzylidene-α-L-rhamno- and

[72] N. Cyr and A. S. Perlin, *Can. J. Chem.*, 1979, **57**, 2504.
[73] A. I. Kalinovskii and E. V. Evtushenko, *Khim. Prir. Soedin.*, 1979, 6 (*Chem. Abs.*, 1979, **91**, 74 841*a*).
[74] A. S. Shashkov, A. I. Usov, S. V. Yarotskii, and A. B. Rabovskii, *Bioorg. Khim.*, 1978, **4**, 1489 (*Chem. Abs.*, 1979, **90**, 87 828*f*).
[75] P. E. Pfeffer, K. M. Valentine, B. G. Moyer, and D. L. Gustine, *Carbohydr. Res.*, 1979, **73**, 1.
[76] K. S. Kim, D. M. Vyas, and W. A. Szarek, *Carbohydr. Res.*, 1979, **72**, 25.
[77] T. Trnka, M. Černý, A. Ya. Shmyrina, A. S. Shashkov, A. F. Sviridov, and O. S. Chizhov, *Carbohydr. Res.*, 1979, **76**, 39.
[78] H. Sugiyama, T. Yamazaki, Y. Senda, J. Ishiyama, K. Matsuda, and S. Seto, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 3659.
[79] T. B. Grindley and V. Gulasekharam, *Carbohydr. Res.*, 1979, **74**, 7.

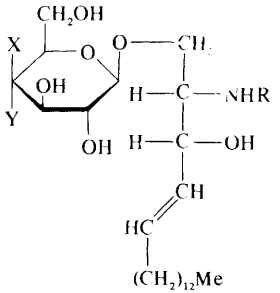
-α-D-manno-pyranosides have been assigned. The chemical shift of the acetal carbon was found to be diagnostic; the *endo*-isomer gave 103.9–104.7 p.p.m., whereas the *exo*-isomer gave 102.8–103.4 p.p.m. Deshielding of C-3 was more pronounced in the *exo*-isomer than in the *endo*, while the reverse was the case for the deshielding of C-2. In the *exo*-isomer C-4 was shielded, but in the *endo*-isomer it was deshielded.[80]

Studies on the ^{13}C n.m.r. of α-cellotriose, α-cellotetraose, and α-cellopentaose,[81] and on methyl β-cellobioside[82] deuteriated in various positions have appeared.[82] All carbon resonances of the β-D-fructofuranosyl residue in sucrose, melezitose, levan, inulin, palatinose, and D-fructose have been assigned. *O*-Substitution of the β-D-fructofuranosyl moiety caused a downfield displacement of the corresponding linked-carbon atom.[83] In an investigation into the structure of teichoic acid from the cell wall of *Streptomyces antibioticus* 39 the monomeric units were characterized by ^{13}C n.m.r. and the spectrum of α-Gal-(1→3)-β-*N*-acetylgal-(1→1)-glycerol was interpreted.[84] Phenolic glycosides from chestnut galls including dimethyl crenatin, cretanin, neocretanin, chesnatin, and chestanin have been structurally investigated by ^{13}C n.m.r.[85] By studying the ^{13}C n.m.r. of α- and β-anomeric pairs of D-mannopyanosides and L-rhamnopyranosides some useful correlations have been found for determination of anomeric configurations of such carbohydrates in otherwise difficultly determined plant glycosides.[86] ^{13}C N.m.r. data for fourteen naturally occurring xanthone *O*- and *C*-glycosides and glycones, useful for determining substitution patterns, have been published.[87]

Chemical shifts and spin–lattice relaxation times have been reported for $CDCl_3$–CD_3OD solution of D-galactosylsphingosine derivatives (14) and (15),[88] while the β-pyranosyl structure of the related glucocerebroside (16) has been proved by ^{13}C n.m.r.[89] A paper dealing with the ^{13}C n.m.r. of flambamycin and its derivatives has provided useful data for the study of other members of the orthosomycin family of antibiotics.[90]

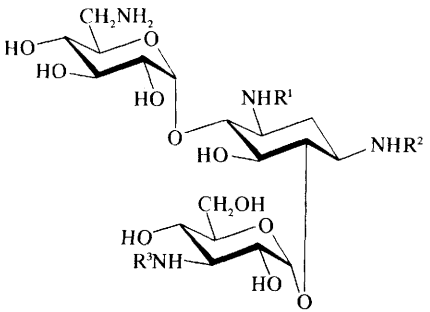
Papers on the structure of bleomycin and its zinc complex,[91] the ^{13}C spin–lattice relaxation times for bleomycin-A_2 at 67.89 MHz,[92] and the Fe^{2+}–

[80] A. Lipták, P. Fügedi, P. Nanasi, and A. Neszmélyi, *Tetrahedron*, 1979, **35**, 1111.
[81] B. Capon, D. S. Rycroft, and J. W. Thomson, *Carbohydr. Res.*, 1979, **70**, 145.
[82] G. K. Hamer, F. Balza, N. Cyr, and A. S. Perlin, *Can. J. Chem.*, 1978, **56**, 3109.
[83] F. R. Seymour, R. D. Knapp, J. E. Zweig, and S. H. Bishop, *Carbohydr. Res.*, 1979, **72**, 57.
[84] A. S. Shashkov, M. Sh. Zaretskaya, S. V. Yarotsky, I. B. Naumova, O. S. Chizhov, and Z. A. Shabarova, *Eur. J. Biochem.*, 1979, **102**, 477.
[85] T. Ozawa and Y. Takino, *Agric. Biol. Chem.*, 1979, **43**, 1173.
[86] R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, and O. Tanaka, *Tetrahedron*, 1979, 1427.
[87] I. Miura, K. Hostettmann, and K. Nakanishi, *Nouv. J. Chim.*, 1978, **2**, 653 (*Chem. Abs.*, 1979, **90**, 168 910*d*).
[88] P. Tkaczuk and E. R. Thornton, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 1415.
[89] T. A. W. Koerner, L. W. Cary, S.-C. Li, and Y.-T. Li, *J. Biol. Chem.*, 1979, **254**, 2326.
[90] W. D. Ollis, I. O. Sutherland, B. F. Taylor, C. Smith, and D. E. Wright, *Tetrahedron*, 1979, **35**, 993.
[91] N. J. Oppenheimer, L. O. Rodriguez, and S. M. Hecht, *Biochemistry*, 1979, **18**, 3439.
[92] N. R. Krishna, J. L. Dassas, E. S. Mooberry, T. T. Sakai, R. C. Allen, G. C. Levy, and J. D. Glickson, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 363.



(14) X = OH, Y = H, R = Ac
(15) X = OH, Y = H, R = $CO(CH_2)_7CH{=}CH(CH_2)_7Me$
(16) X = H, Y = OH, R = $CO(CH_2)_{13}CH{=}CH(CH_2)_7Me$

bleomycin complex[93] have appeared. The iron was found to be chelated to the α-amino-group of the terminal diaminopropionic acid and to the pyrimidine ring. A revision of one of the glycosidic linkages in chromomycin A_3, determined by ^{13}C n.m.r., has been published.[94] The ^{13}C n.m.r. of the analogues of the antibiotic butikacin shown in formulae (17) to (21) have been recorded, assigned, and discussed.[95]



X = (*S*)-4-amino-2-hydroxybutyl
Y = (*R*)-4-amino-2-hydroxybutyl

(17) $R^1 = R^2 = R^3 = H$
(18) $R^1 = R^3 = H$; $R^2 = X$
(19) $R^1 = R^3 = H$; $R^2 = Y$
(20) $R^2 = R^3 = H$; $R^1 = X$
(21) $R^1 = R^2 = H$; $R^3 = X$

Structural correlations and conformations about the glycosidic bond of selected adenine nucleosides have been suggested from a ^{13}C n.m.r. study.[96] The published assignments for C-3′ and C-4′ in the ^{13}C n.m.r. spectra of nucleoside 3′,5′-cyclic phosphates should be reversed;[97] the results have been confirmed by

[93] R. K. Gupta, J. A. Ferretti, and W. J. Caspary, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 534.
[94] J. Thiem and B. Meyer, *J. Chem. Soc., Perkin Trans. 2*, 1979, 1331.
[95] M. W. Coleman, M. Kinns, and M. J. Sewell, *J. Antibiot.*, 1979, **32**, 355.
[96] A. A. Akhrem, I. A. Mikhailopulo, and A. F. Abramov, *Org. Magn. Reson.*, 1979, **12**, 247.
[97] S. Uesugi, S. Tanaka, and M. Ikehara, *Org. Magn. Reson.*, 1979, **12**, 143.

selective decoupling experiments.[98] The interaction of Mn^{2+} with *N*-9-(β-D-ribofuranosyl)purine-6-yl carbamoyl threonine, studied by ^{13}C n.m.r., has suggested that such residues, which are found at the 3′-end of the anticodon sequences of nearly all tRNA molecules that respond to codons beginning with adenosine, may be the Mn^{2+} binding site in tRNA.[99]

As references for structures present in glycoproteins, the natural-abundance ^{13}C n.m.r. of spectra of β-*N*-acetylglucosamine-asparagine and -serine have been determined.[100]

7 Lanthanide Shift Reagents

The ^1H n.m.r. of methyl 2,3,5-tri-*O*-acetyl-α- and -β-L-arabinofuranosides in the presence of $Eu(fod)_3$, using the Abraham modification of the Karplus equation, has shown that the former adopts the $^1T_0$ conformation and the latter the E_1 form.[101] The same worker has reported the ^1H n.m.r. spectra of both anomers of methyl and phenyl D-xylopyranoside and L-arabinopyranoside in the presence of $Eu(fod)_3$. It was shown that the H-2, H-3, and H-4 signals were more shifted than others by the reagent. The bound shifts of H-4 in α-D- and β-L-glycosides and H-3 in β-D- and α-L-glycosides were much greater than in the corresponding hexopyranosides. In equimolar mixtures of two sugars, preferential co-ordination of the β-D-xylo- and β-L-arabino-pyranosides with europium was found to occur in comparison with α-anomers of the corresponding hexopyranosides.[102]

Interpretation of ^{13}C n.m.r. and ^1H n.m.r. shifts in the spectra of the 3-*O*-methyl ether and two disaccharide derivatives of methyl 4,6-*O*-benzylidene-D-glucopyranosides obtained in the presence of $Eu(fod)_3$ has been carried out. The positions of substituents were determined by using a computer search programme on the europium 'complex' with europium in the calculated position predicted from shift values.[103]

8 Other Nuclei

The ^2H Fourier transform n.m.r. of [6-^{2}H]*N*-hexadecanoyl galacto-ceramides has been investigated. The quadrupole echo method at 34.1 MHz gave two sets of quadrupole splittings, which were thought to be due to *R* and *S* configurational isomers.[104] The conformation of cyclic AMP in solution has been studied by the n.m.r.-DESERT (*D*euterium *S*ubstitution *E*ffect on *R*elaxation *T*imes) method.[105]

[98] M. Kainosho, *Org. Magn. Reson.*, 1979, **12**, 548.
[99] M. P. Schweizer and D. W. Hamill, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 1367.
[100] K. Dill and A. Allerhand, *FEBS Lett.*, 1979, **107**, 26.
[101] K. Izumi, *Carbohydr. Res.*, 1979, **77**, 218.
[102] K. Izumi, *Agric. Biol. Chem.*, 1979, **43**, 95.
[103] P. McArdle, J. O. Wood, E. E. Lee, and M. J. Conneely, *Carbohydr. Res.*, 1979, **69**, 39.
[104] R. Skarjune and E. Oldfield, *Biochim. Biophys. Acta*, 1979, **556**, 208.
[105] F. Hayashi, K. Akasaka, and H. Hatano, *Biochim. Biophys. Acta*, 1979, **588**, 181.

^{3}H N.m.r. studies have shown that the regiospecificity of the tritium label in [*n*-^{3}H]D-glucopyranose (*n* = 1, 2, 3, 4, 5, and 6), available commercially in aqueous solution, was 100% in each case. After storage some label appears in the water or as a non-glucose impurity.[106]

A conformational study of nucleic acid phosphate esters has been carried out using ^{31}P n.m.r. and the temperature-dependent ^{31}P chemical shifts used to give information about the geometry of the phosphodiester linkage. Compounds studied were 3′- and 5′-*O*-methyl phosphates of uridine and adenosine, and the 3′-*O*-methyl phosphate of 6-*N*-dimethyladenosine.[107] It has been reported that the ^{31}P n.m.r. of *myo*-inositol hexakisphosphate has four resonances that shift and broaden as a function of pH.[108] Reference to the use of ^{31}P n.m.r. in the configurational determination of cyanomethyl-branched phosphonates is made in Chapter 16.

[106] J. A. Elvidge, J. R. Jones, R. B. Mane, V. M. Chambers, E. A. Evans, and D. C. Warrell, *J. Labelled Comp. Radiopharm.*, 1978, **15** (Suppl.) 141 (*Chem. Abs.*, 1979, **91**, 57 315*q*).

[107] C. A. G. Haasnoot and C. Altona, *Nucleic Acid Res.*, 1979, **6**, 1135.

[108] E. R. P. Zuiderweg, G. G. M. Van Beek, and S. H. De Bruin, *Eur. J. Biochem.*, 1979, **94**, 297.

21

Other Physical Methods

1 I.R. and Raman Spectroscopy

In the Raman and i.r. spectra of crystalline methyl 3,6-dideoxy-β-D-*ribo*-hexopyranoside measured at low temperatures, four O–H bands were observed in the O–H stretching region. These were correlated with four distinct hydrogen bonds determined from *X*-ray data, and deuterium-isotope dilution methods were used to assist the analysis.[1] An interesting study of the far-i.r. spectrum of a pyroelectric sucrose single crystal has been carried out, and the temperature dependence was correlated with polarization of the low-frequency mode (49.5 cm^{-1}) along the binary axis.[2]

Studies of carbohydrates in solution have included an investigation of the intramolecular hydrogen bonding of methyl 4,6-*O*-benzylidene-α-D-hexopyranoside derivatives. Diols, monomethyl ethers, and monodeoxy-compounds with various configurations were studied in the 3600 cm^{-1} region.[3] A similar investigation was carried out on methyl ethers and benzylidene derivatives of D-aldopyranoses.[4] A laser Raman study of D-fructose in aqueous solution indicated that furanose forms could be distinguished from pyranoses and that at equilibrium the ratio of the two forms was 41:59.[5]

2 Mass Spectrometry

Too many papers contain mass spectrometric data for individual mention; the following reports were concerned primarily with the technique applied to various carbohydrate derivatives.

An interesting study of anion mass spectra of thirteen acetylated glucopyranosyl esters showed that molecular ions were observed whereas, in most cases, cationic spectra did not show such ions. It was indicated that comparison of anion and cation spectra can yield significant structural information.[6] A further point of general interest is that the 'active-hydrogen' content of carbohydrates (usually the hydroxy- and amino-groups) can be determined by chemical ionization mass spectrometry using deuteriated ammonia as reagent gas with examination of the ammonia adduct ions and deuteriated analogues.[7]

[1] J. Umemura, G. I. Birnbaum, D. R. Bundle, W. F. Murphy, H. J. Bernstein, and H. H. Mantsch, *Can. J. Chem.*, 1979, **57**, 2640.

[2] B. Wyncke, J. Serrier, F. Brehat, and A. Hadni, *J. Phys. C*, 1978, **11**, 2639 (*Chem. Abs.*, 1979, **90**, 72 395*p*).

[3] H. Hönig and H. Weidman, *Carbohydr. Res.*, 1979, **73**, 260.

[4] V. N. Gritsan, *Zh. Prikl. Spektrosk.*, 1978, **29**, 562 (*Chem. Abs.*, 1979, **90**, 55 179*d*).

[5] M. Mathouthi, C. Luu, and D.-V. Luh, *C.R. Hebd. Seances Acad. Sci., Ser. C*, 1979, **288**, 359.

[6] D. Voigt and M. Lehmann, *J. Prakt. Chem.*,1979, **321**, 243 (*Chem. Abs.*, 1979, **91**, 141 117*j*).

[7] Y. Y. Lin and L. L. Smith, *Biomed. Mass Spectrom.*, 1979, **6**, 15 (*Chem. Abs.*, 1979, **91**, 119 748*u*).

The mass spectra of 1,2- and 2,3-unsaturated compounds together with a 1,5-diene,[8] and of trimethylsilyl ethers of 2- and 3-uloses have been examined.[9] The latter hexose or heptose compounds were mainly acyclic structures. The effects of the halogen atoms on the fragmentation of the tetra-acetates of 3-bromo- and 3-chloro-3-deoxy-β-D-glucopyranose and the penta-acetates of 3-bromo- and 3-chloro-3-deoxy-D-glucitol have been examined.[10] Thioglycosides of 6-deoxy-6-amino-α-DL-mannopyranose have been studied.[11] Several amino-sugars have been analysed by the 'in-beam electron-impact method'.[12] Amino-sugars derived from bacterial polysaccharides have been identified by mass spectrometry of the *N*-acetyl TMS ethers,[13] and related work has been carried out on glycosylamines and their products of Amadori rearrangement.[14] In the nucleoside field, monobenzylated derivatives have been examined, showing that the site of the substituent may be located.[15] Various selenobases and selenonucleosides have been studied.[16] Papers reporting other mass spectral studies of nucleosides are noted in Chapter 19.

Glycoside derivatives to have been examined are methyl arabinopyranoside methyl ethers[17] and C-1–C-5 deuteriated analogues,[18] methyl tetra-*O*-methyl-β-D-galactopyranoside (chemical ionization),[19] methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-glucopyranoside and the phenyl β-analogue (high-resolution work with deuterium labelling revealed methoxy migrations),[20] and various flavonoid *O*-glycosides.[21] Various glycosides of methyl uronate acetates and analogous glycosyl esters and halides have been studied[22] as have glycosides of uronic acid amides[23] and some sugars esterified with lysergic acid and its 9,10-dihydro-derivative.[24]

[8] G. Descotes, P. Boullanger, Tachi-Dung, and J.-C. Martin, *Carbohydr. Res.*, 1979, **71**, 305.

[9] T. Okuda, S. Saito, and M. Hayashi, *Carbohydr. Res.*, 1979, **68**, 1.

[10] J. Kiburis, A. B. Foster, M. Jarman, and J. H. Westwood, *Chem. Chron.*, 1979, **8**, 77 (*Chem. Abs.*, 1979, **91**, 91 885*k*).

[11] E. Baranowska, W. Priebe, A. Zamojski, and K. Walocha, *Pol. J. Chem.*, 1979, **53**, 277.

[12] M. Ohashi, S. Yamada, H. Kudo, and N. Nakayama, *Biomed. Mass Spectrom.*,1978, **5**, 578 (*Chem. Abs.*, 1979, **91**, 20 925*n*).

[13] D. V. Bowser, R. G. Teece, and S. M. Somani, *Biomed. Mass Spectrom.*, 1978, **5**, 627 (*Chem. Abs.*, 1979, **91**, 20 920*g*).

[14] W. Funke, D. Henneberg, C. Von Sonntag, and A. Klemer, *Org. Mass Spectrom.*, 1979, **14**, 220.

[15] H. T. Cory, K. Yamaizumi, D. L. Smith, D. R. Knowles, A. D. Broom, and J. A. McCloskey, *J. Heterocycl. Chem.*, 1979, **16**, 585.

[16] J. G. Liehr, C. L. Weise, P. F. Crain, G. H. Milne, D. S. Wise, L. B. Townsend, and J. A. McCloskey, *J. Heterocycl. Chem.*, 1979, **16**, 1263.

[17] V. Mihálov, V. Kováčik, and P. Kováč, *Carbohydr. Res.*, 1979, **73**, 267.

[18] A. Ott, B. M. Zolotarev, and O. S. Chizhov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1979, 191 (*Chem. Abs.*, 1979, **90**, 168 918*n*).

[19] V. I. Kadentsev, A. Ott, and O. S. Chizhov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1978, 1204 (*Chem. Abs.*, 1978, **89**, 110 258*k*).

[20] N. Kyriakidis, T. C. Smale, and E. S. Waight, *Org. Mass Spectrom.*, 1079. **14**, 532.

[21] K. M. Biswas, M. E. Ali, A. H. Jackson, and D. E. Games, *J. Indian Chem. Soc.*, 1978, **55**, 1240 (*Chem. Abs.*, 1979, **91**, 193 572*y*).

[22] V. Mihálov, V. Kováčik, and P. Kováč, *Chem. Zvesti*, 1979, **33**, 377.

[23] V. Mihálov, V. Kováčik, and P. Kováč, *Carbohydr. Res.*, 1979, **70**, 239.

[24] J. Schmidt, K. Seifert, and S. Johne, *Pharmazie*, 1979, **34**, 77 (*Chem. Abs.*, 1979, **91**, 141 118*k*).

A wide range of aldose derivatives containing amino-, and acetamido-, deoxy-, and thio-groups were examined as their acetylated aldononitriles, and it was shown that the chemical ionization method best gave access to molecular weight data, while electron-impact studies revealed the positions and natures of the functional groups.[25] Analogous work was carried out on benzoylated *O*-benzyl-oximes of mono- and di-saccharides of clinical interest. Molecular ions were observable, whereas the spectra of alditol perbenzoates did not yield such ions.[26] Other acyclic compounds to have been examined are partially methylated alditol acetates,[27] and bisphenylboronates of various tetraols derived from tetroses, pentoses, and hexoses.[28]

Disaccharides to have been examined are two D-ribofuranosyl-D-ribitols produced from two bacterial capsular polysaccharides,[29] and several $\alpha\alpha$-trehalose derivatives prepared as model bacterial 'cord factors' [F.D. cationization with cesium iodide gave intense (M + Cs) ions].[30] The same group of workers have also reported on this method applied to the natural 'cord factor' of *Corynebacterium diphtheriae*, and they found at least 22 species differing in the chain lengths of the two trehalose acyl substituents.[31] A further report describes the determination of the sites of substitution of the acyl groups.[32] Daunosamine-containing disaccharides have been examined by negative ion, positive ion, chemical ionization, and electron-impact methods.[33]

More complex carbohydrates to have been studied are a methylated derivative of a 3,6-dimannopyranosyl-D-mannitol obtained from fetuin, the major glycolipid of fetal calf serum, and a related *N*-acetylglucosamine trisaccharide derived from the same source.[34] Analogous work reports studies of methylated oligosaccharides with up to six monosaccharide units;[35] underivatized oligosaccharides up to pentasaccharide have been studied by negative chemical ionization methods. Methane affords (M^-) ions for mono-, di-, tri-, and tetra-saccharides, but pentasaccharides gave only fragment ions under these conditions. Other gases used included dichlorodifluoromethane (Freon 12).[36] The use of mass spectrometry to determine the location of *N*-acyl chains in amino-glycosides is mentioned in Chapter 6.

[25] F. R. Seymour, E. C. M. Chen, and S. H. Bishop, *Carbohydr. Res.*, 1979, **73**, 19.
[26] R. M. Thompson and D. A. Cory, *Biomed. Mass Spectrom.*, 1979, **6**, 117 (*Chem. Abs.*, 1979, **91**, 211 689*y*).
[27] A. S. Rao and N. Roy, *Carbohydr. Res.*, 1979, **74**, 349.
[28] C. J. Griffiths, I. R. McKinley, and H. Weigel, *Carbohydr. Res.*, 1979, **71**, 1.
[29] B. A. Fraser, F.-P. Tsui, and W. Egan, *Carbohydr. Res.*, 1979, **73**, 59.
[30] J. C. Prome and G. Puzo, *Isr. J. Chem.*, 1978, **17**, 172 (*Chem. Abs.*, 1979, **90**, 121 904*q*).
[31] G. Puzo, G. Tissie, C. Lacave, H. Aurelle, and J. C. Prome, *Biomed. Mass Spectrom.*, 1978, **5**, 699 (*Chem. Abs.*, 1979, **91**, 91 869*h*).
[32] G. Puzo and J. C. Prome, *Biomed. Mass Spectrom.*, 1978, **5**, 146.
[33] H. S. El Khadem and A. Liav, *Carbohydr. Res.*, 1979, **74**, 199.
[34] B. Nilsson, N. E. Nordén, and S. Svensson, *J. Biol. Chem.*, 1979, **254**, 4545.
[35] E. G. de Jong, W. Heerma, J. Havercamp, and J. P. Kamerling, *Biomed. Mass Spectrom.*, 1979, **6**, 72 (*Chem. Abs.*, 1979, **91**, 158 008*x*).
[36] A. K. Ganguly, N. F. Cappuccino, H. Fujiwara, and A. K. Bose, *J. Chem. Soc., Chem. Commun.*, 1979, 148.

3 *X*-Ray and Neutron Diffraction Crystallography

Jeffrey, with two different co-authors, has published two reviews on carbohydrates; the first deals with mono-, di-, and poly-saccharides,[37] the second[38] updates the bibliographies of the structures of carbohydrates and nucleosides, which have recently appeared in *Advances in Carbohydrate Chemistry and Biochemistry*.

Specific crystal structures have been reported as follows, neutron diffraction studies being identified by the letters 'n.d.';

Free Sugars and Simple Derivatives Thereof. – β-D-Arabinopyranose (n.d.),[39] β-D-lyxopyranose (n.d.),[40] β-D-xylopyranose tetrabenzoate and 1,5-anhydro-2,3,4-tri-*O*-benzoylxylitol,[41] α-D-glucopyranose (a refinement based on previously published n.d. data),[42] 1,2-*O*-isopropylidene-α-D-glucofuranose,[43] α-L-sorbopyranose (n.d.),[44] 6-deoxy-α-L-sorbofuranose,[45] and α-D-*manno*-heptulopyranose.[46]

Glycosides and Simple Derivatives Thereof. – Methyl β-D-arabinopyranoside,[47] methyl α- and β-D-galactopyranoside (n.d.),[48] methyl 3,6-dideoxy-β-D-*ribo*-hexopyranoside,[49] methyl 2,3 : 4,5-di-*O*-isopropylidene-α-D-glucoseptanoside,[50] methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-gluco- and β-D-galacto-pyranoside (thermodynamically less stable stereoisomers with phenyl groups axial),[51] methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-(*N*-triphenylphosphinoepimino)-α-D-alloside toluene-*p*-sulphonate,[52] and methyl 4,6-*O*-benzylidene-2,3-*O*-dibutylstannylene-α-D-glucopyranoside.[53]

Halogen- and Nitrogen-containing Compounds (Other than Nucleosides). – 2,3,4-Tri-*O*-acetyl- and -*O*-benzoyl-β-D-xylopyranosyl fluoride ($^4C_1$ and $^1C_4$ conformations, respectively),[54] D-glucose oxime (exists as the cyclic β-gluco-

[37] G. A. Jeffrey and A. D. French, *Mol. Struct. Diffract. Methods*, 1978, **6**, 183 (*Chem. Abs.*, 1979, **91**, 74 790*h*).
[38] G. A. Jeffrey and M. Sundaralingham, *Adv. Carbohydr. Chem. Biochem.*, 1977, **34**, 345.
[39] S. Takagi, S. Nordenson, and G. A. Jeffrey, *Acta Crystallogr.*, 1979, **B35**, 991.
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[41] P. Luger, G. Kothe, K. Vangehr, H. Paulsen, and F. R. Heiker, *Carbohydr. Res.*, 1979, **68**, 207.
[42] G. M. Brown and H. A. Levy, *Acta Crystallogr.*, 1979, **B35**, 656.
[43] S. Takagi and G. A. Jeffrey, *Acta Crystallogr.*, 1979, **B35**, 1522.
[44] S. Nordenson, S. Takagi, and G. A. Jeffrey, *Acta Crystallogr.*, 1979, **B35**, 1005.
[45] P. Swaminathan, L. Anderson, and M. Sundaralingam, *Carbohydr. Res.*, 1979, **75**, 1.
[46] J. A. Kanters and L. M. J. Batenburg, *Acta Crystallogr.*, 1979, **B35**, 1152.
[47] J. F. McConnell, A. Schwartz, and J. O. Stevens, *Cryst. Struct. Commun.*, 1979, **8**, 33 (*Chem. Abs.*, 1979, **90**, 131 127*c*).
[48] S. Takagi and G. A. Jeffrey, *Acta Crystallogr.*, 1979, **B35**, 902.
[49] G. I. Birnbaum and D. R. Bundle, *Biochim. Biophys. Acta*, 1979, **582**, 515.
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[51] J. C. Barnes, J. S. Brimacombe, B. H. Nichols, and T. J. R. Weakley, *Carbohydr. Res.*, 1979, **69**, 47.
[52] K. B. Lindberg, A. Kalman, J. Kovacs, A. Messmer, and I. Pinter, *Cryst. Struct. Commun.*, 1978, **7**, 607 (*Chem. Abs.*, 1979, **90**, 64 820*h*).
[53] S. David, C. Pascard, and M. Cesario, *Nouv. J. Chim.*, 1979, **3**, 63 (*Chem. Abs.*, 1979, **90**, 152 544*b*).
[54] G. Kothe, P. Luger, and H. Paulsen, *Acta Crystallogr.*, 1979, **B35**, 2079.

pyranosylhydroxylamine) and *syn*- and *anti*-D-arabinose oximes (both acyclic),[55] *N*-methyl-*N*-β-D-xylopyranosylurea,[56] and *N*-benzyl-*N*-methyl-1-amino-1-deoxy-β-D-*arabino*-hexo-2-ulopyranose.[57]

Unsaturated Compounds. – Tri-*O*-acetyl-D-glucal[58,59] and the 2-acetoxy-derivative,[59] tri-*O*-benzyl-D-glucal,[60] ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside,[60] 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl dimethyl phosphonate,[61] and methyl 3,4-dideoxy-α-DL-*threo*-hex-3-enopyranoside.[62]

Anhydro-compounds. – 2-*O*-Acetyl-1,6 : 3,5-dianhydro-α-L-idofuranose,[63] 1,6-anhydro-2,4-dideoxy-2,4-difluoro-β-D-glucopyranose,[64] and an isopropylidene derivative of a dimer of 1,6-anhydro-D-*arabino*-hex-3-ulopyranose (see Chapter 4).[65]

Acid Derivatives. – Calcium di-DL-glycerate,[66] manganese(II) D-gluconate,[67] sodium α-D-*threo*-hex-2,5-ulosonate hydrate,[68] and ethyl 3,7-anhydro-6,8-*O*-benzylidene-4-deoxy-2-ethylenedithio-D-*talo*-2-octulosonate.[69]

Acyclic and Alicyclic Compounds. – Pentaerythritol,[70,71] D-galactitol and D-mannitol diarsenite (both 1,2,4 : 3,5,6-structures),[72] and muco-inositol.[73]

Disaccharides and Higher Saccharides. – 4-*O*-β-D-Glucopyranosyl-D-glucitol,[74] 2-*O*-β-D-glucopyranosyl-α-D-glucopyranose (sophorose),[75] 1′,2 : 4,6-di-*O*-iso-

[55] A. Mostad, *Acta Chem. Scand., Ser. B*, 1978, **32**, 733.
[56] B. K. Sadybakasov, M. Yu. Afanas'ev, and Zh. A. Dzhamanbaev, *Kristallografiya*, 1978, **23**, 1267 (*Chem. Abs.*, 1979, **90**, 121 893*k*).
[57] E. Moreno, S. Perez-Garrido, P. Villares, and R. Jimenez-Garay, *Cryst. Struct. Commun.*, 1978, **7**, 547 (*Chem. Abs.*, 1979, **90**, 32 311*b*).
[58] J. W. Krajewski, Z. Urbanczyk-Lipkowsk, P. Głuzinski, J. Bleidelis, and A. Kemme, *Acta Crystallogr.*, 1979, **B35**, 1248.
[59] K. Vangehr, P. Luger, and H. Paulsen, *Carbohydr. Res.*, 1979, **70**, 1.
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[61] G. Adiwidjaja, B. Meyer, H. Paulsen, and J. Thiem, *Tetrahedron*, 1979, 373.
[62] J. W. Krajewski, Z. Urbanczyk-Lipkowska, and P. Gluzinski, *Acta Crystallogr.*, 1979, **B35**, 519.
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[64] W. Choong, *Cryst. Struct. Commun.*, 1979, **8**, 27 (*Chem. Abs.*, 1979, **90**, 178 456*z*).
[65] P. Köll and J. Kopf, *Carbohydr. Res.*, 1979, **68**, 189.
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[67] T. Lis, *Acta Crystallogr.*,1979, **B35**, 1699.
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[70] D. Eilerman and R. Rudman, *Acta Crystallogr.*, 1979, **B35**, 2458.
[71] M. F. C. Ladd, *Acta Crystallogr.*, 1979, **B35**, 2375.
[72] R. E. Ballard, A. H. Haines, A. A. Khandar Shahabad, and M. Elder, *Carbohydr. Res.*, 1979, **68**, C1.
[73] D. C. Craig and V. J. James, *Cryst. Struct. Commun.*, 1979, **8**, 629 (*Chem. Abs.*, 1979, **91**, 123 325*w*).
[74] W. P. J. Gaykema and J. A. Kanters, *Acta Crystallogr.*, 1979, **B35**, 1156.
[75] R. Ohanessian, F. Longchambon, and F. Arene, *Acta Crystallogr.*, 1978, **B34**, 3666.

propylidenesucrose tetra-acetate,[76] methyl 2,3-anhydro-6-bromo-6-deoxy-4-*O*-(4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranosyl)-α-D-allopyranoside,[77] and *O*-α-D-mannopyranosyl-(1→3)-*O*-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-α-D-glucopyranose.[78]

Nucleosides, Nucleotides, Derivatives, and Related Compounds. – 5-Dimethylaminouridine,[79] 5-methylaminomethyl-2-thiouridine,[80] 2′,3′-*O*-isopropylidene-5-methylaminomethyl-2-thiouridine,[81] 2′-deoxy-5-isopropyluridine,[82] 5′-amino-2′,5′-dideoxy-5-iodouridine,[83] 5-nitro-1-(β-D-ribofuranosyluronic acid)uracil,[84] uridine 3′-phosphate,[85] and uridine 5′-diphosphate dipotassium salt.[86]

2′-*O*-Methylcytidine (correction to previous paper),[87] platinum complex of cytidine 3′-phosphate,[88] 1-β-D-arabinofuranosylcytosine 5′-phosphate (2 independent analyses),[89] 2,2′-anhydro-1-β-D-arabinofuranosyl-5-dimethylsulphonio-6-oxocytosine chloride,[90] 2,2′-anhydro-1-(3′,5′-di-*O*-acetyl-β-D-arabinofuranosyl)-5-chloro-6-oxocytosine,[90] 2,2′-anhydro-1-β-D-arabinofuranosylcytosine 2′,5′-diphosphate,[91] and 6,2′-anhydro-1-β-D-arabinofuranosyl-6-hydroxycytosine.[92]

Adenosine 5′-triphosphate disodium salt,[93] adenosine 5′-methylphosphate,[94] adenosine 5′-methylphosphonate,[95] 8-methyladenosine 3′-phosphate,[96] 2-azaadenosine,[97] 9-α-D-arabinofuranosyladenine,[98] 8,2′-anhydro-9-β-D-arabino-

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[91] Y. Yamagata, Y. Suzuki, S. Fujii, T. Fujiwara, and K. Tomita, *Acta Crystallogr.*, 1979, **B35**, 1136.
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furanosyladenine,[99] 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine 5′-phosphate,[100] 8,3′-anhydro-8-mercapto-9-β-D-xylofuranosyladenine,[101] and adenosine 5′-phosphorothioate *p*-nitrophenyl ester.[102]

Platinum(diethylenetriamine)guanosine perchlorate,[103] Ni^{II} and Co^{II} complexes of 2′-deoxyguanosine 5′-phosphate,[104] and an 8,8-linked dimer of guanosine.[105]

Platinum (diethylenetriamine)inosine[106] and inosine 5′-phosphate copper complex.[107]

β-D-Ribofuranosylpyrimidine-2-one,[108] 7-(methyl 2-acetamido-6-*O*-acetyl-2,3,4-trideoxy-β-D-*erythro*-hex-2-enopyranosid-4-yl)theophylline,[109] 7-(methyl 2-acetamido-6-*O*-acetyl-2,3,4-trideoxy-α-D-*threo*-hex-2-enopyranosid-4-yl)theophylline,[110] 5,6-dimethyl-1-(α-D-ribofuranosyl)benzimidazole[111] and 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (and its 5′-phosphate).[112]

Antibiotics. – Erythromycin cyclic carbonate,[113] daunomycin butanol complex,[114] hedamycin (see Chapter 18),[115] and olgose (a major degradation product of the oligosaccharide antibiotic everninomicin D).[116]

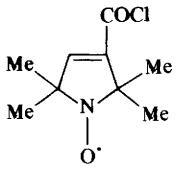
Other Compounds. – Affinoside B (a cardiac glycoside with a 4,6-dideoxy-3-*O*-methyl-2-hexulose unit linked through a C-1 and C-2).[117]

4 E.S.R. Spectroscopy

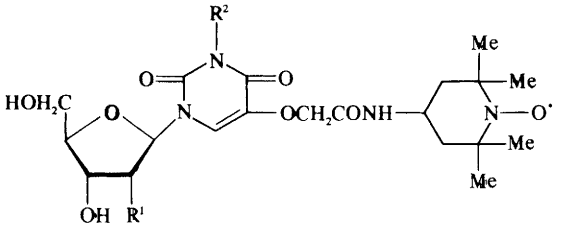
E.s.r. and ENDOR techniques have enabled the detection of five specific radicals produced by *X*-ray irradiation of methyl α-D-glucopyranoside,[118] and e.s.r. tech-

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[100] K. Tanaka, S. Fujii, T. Fujiwara, and K. Tomita, *Acta Crystallogr.*, 1979, **B35**, 929.
[101] M. Yoneda, K. Tanaka, T. Fujiwara, and K. Tomita, *Acta Crystallogr.*, 1979, **B35**, 2355.
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[110] B. Kojić-Prodić, *Acta Crystallogr.*, 1979, **B35**, 1162.
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[112] D. A. Adamiak and W. Saenger, *Acta Crystallogr.*, 1979, **B35**, 924.
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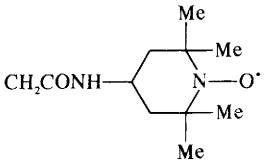
niques have been applied to a study of the metal binding properties of bleomycin A_2[119–124] and tallysomycin.[123,124] The stable radical species (1) has been advocated as a spin label for bonding to primary amino-groups,[125] and, in analogous work, the spin-labelled uridine derivatives (2)–(5) have been prepared.[126]



(1)



	R^1	R^2
(2)	OH	
(3)	H	
(4)	OH	H
(5)	H	H



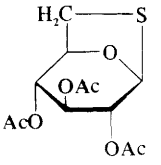
5 Polarimetry and Circular Dichroism Studies

The complexings of D-glucose, D-fructose, and maltose with borate anions have been studied by polarimetric, conductivity, and pH measurements,[127] and circular dichroism methods have been used to investigate the complexing of cuprammonium ions with carbohydrate diols and amino-alcohols.[128] An examination of the c.d. spectra of fifteen methyl 3-acetamido-3,6-dideoxyhexopyranoside derivatives measured in methanol, 2,2,2-trifluoroethanol, and acetonitrile suggests that the amide group is not completely planar and may show chirality.[129] The $n \rightarrow \pi^*$ transition of the carboxylic acid carbonyl groups at

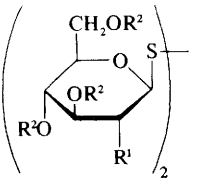
[119] Y. Sugiura, K. Ishizu, and K. Miyoshi, *J. Antibiot.*, 1979, **32**, 453.
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225 nm of glycosides and oligosaccharides containing neuraminic acid residues gives rise to a c.d. band, the sign of which can be correlated with the configuration of the glycosidic linkages.[130]

A helicity rule has been proposed for 1-thio-D-glucopyranosides which allows the c.d. band at 220 nm ($n \rightarrow \sigma^*$ of sulphur) to be interpreted in terms of the stereochemical relationship at C-1 and C-2.[131] The o.r.d. and c.d. characteristics of the thioanhydro-compound (6) and the disulphides (7) and (8) have been measured at different temperatures and discussed.[132]



(6)



	R¹	R²
(7)	NHAc	H
(8)	OAc	Ac

Chapter 6 contains references to the o.r.d., c.d., and u.v. studies of glucopyranosyl xanthates.

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22

Separatory and Analytical Methods

1 Chromatographic Methods

Gas–Liquid Chromatography. – A review of g.c. and h.p.l.c. of sugars and alditols has been published.[1] A standardized method for analysing trimethylsilyl ethers of mixtures of sugars has been proposed using a 3 ft SE 30 column coupled to a mass spectrometer.[2] Similarly, a modified g.c. method has been reported for the analysis of methyl glycosides as TMS ethers, and was employed for the analysis of cellular carbohydrates of *S. mutans* NCTC 10832.[3] The use of glass support-coated open tubular capillary columns for the analysis of monosaccharides, alditols, cyclitols, sucrose, and quinic and shikimic acids as their TMS ethers has been reported.[4] The g.c. assay of ribose is best conducted by conversion to the TMS ether of its oxime, which affords a single peak well separated from those due to D-glucose, D-gluconic acid, and ribitol.[5] The g.c. of *O*-acetylated-*O*-methylated oximes of hexosamines has been presented in a preliminary communication.[6]

A convenient g.c. analysis of hexosamines involves sequential nitrous acid deamination, mercaptalation, and conversion to the TMS ethers, followed by g.c.[7] G.c. in combination with mass spectrometry has been used to distinguish between the acetylated alditols of seventeen methyl ethers of L-*glycero*-D-*manno*-heptose and six methyl ethers of D-*glycero*-D-*manno*-heptose, both of which occur as components of lipopolysaccharides.[8] A technique for the g.c. analysis of permethylated glycoproteins and glycopeptides involves methanolysis and conversion to the TMS ethers of the *O*-methylalditols, including 2-deoxy-2-*N*-methylacetamido-alditols. This permitted the analysis of a twenty-two component mixture of methylated mannose, galactose, and *N*-acetylglucosamine.[9] The retention times of the methyl glycopyranosides derived from methylated D-glucose, D-galactose, D-mannose, 6-deoxy-D-glucose, L-fucose, D-rhamnose, D-xylose, L-arabinose, and D-lyxose have been tabulated in order to facilitate the use of g.c. in the analysis of polysaccharides.[10] A comparative

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[10] Yu. N. Elkin, *J. Chromatogr.*, 1979, **180**, 163.

study of the g.c. separation of methyl ethers of L-rhamnose by prior conversion to either their methyl glycosides, their trifluoroacetylated L-rhamnitols, or their acetylated rhamnononitriles has been conducted. The authors conclude that the former derivatives give the best separations.[11] Low-bleeding columns containing 0.3–0.4% OV-225 on a surface-modified chromosorb have been recommended for the g.c. analysis of methylated alditols. Short retention times permit rapid analysis at 200–210 °C.[12]

The method by which the absolute configuration of aldoses may be determined by conversion to glycosides of (−)-butan-2-ol followed by g.c. (see Vol 12, p. 207) has been successfully applied to the hydrolysates of several polysaccharides and glycoproteins.[13]

High Pressure Liquid Chromatography. – In addition to the review mentioned previously,[1] the principles of h.p.l.c. have been discussed as well as its application to natural products, including carbohydrates.[14] The separation of multicomponent mixtures of mono- and di-saccharides has been accomplished by h.l.p.c. using a colorimetric method of detection based on orcinol–sulphuric acid.[15] The semi-preparative separation of water-soluble carbohydrates has been achieved using refractive index detection, and the method was applied to mixtures of chlorinated and methylated sucroses.[16] Additionally, the h.p.l.c. separation of mixtures of oligo- and mono-saccharides has been achieved on the ion exchanger, DEAE-spheron, using stepwise elution with various concentrations of borate.[17] H.p.l.c. using ion-exchange resins has been employed for the separation of ionic compounds, *e.g.*, chondroitin 4- and 6-sulphate,[18] and for the separation of neutral and acidic oligo- and mono-saccharides.[19,20] Modification of the surface of silica gel with amines, particularly tetraethylene pentamine, improves the resolution of sugars in h.p.l.c.[21]

Applications of h.p.l.c. to benzoyl derivatives of alditols, glycosides, aminosugars, aldoses, and alditols has been examined; the presence of benzoyl groups has the advantage that u.v. detection methods can be used,[22] and indeed a method for the simultaneous determination of picomole levels of gluco- and galacto-cerebrosides involves benzoylation followed by h.p.l.c.[23] Similarly, tritiated mannitol and fucitol were easily separated after *O*-benzoylation.[24]

[11] F. Janeček, R. Toman, S. Karacsonyi, and D. Anderle, *J. Chromatogr.*, 1979, **173**, 408.
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[19] M. H. Simatupang, *J. Chromatogr.*, 1979, **178**, 588.
[20] J. Kumanotani, R. Oshima, Y. Yamauchi, N. Takai, and Y. Kurosu, *J. Chromatogr.*, 1979, **176**, 462.
[21] B. B. Wheals and P. C. White, *J. Chromatogr.*, 1979, **176**, 421.
[22] C. A. White, J. F. Kennedy, and B. T. Golding, *Carbohydr. Res.*, 1979, **76**, 1.
[23] G. Nonaka and Y. Kishimoto, *Biochim. Biophys. Acta*, 1979, **572**, 423, 432.
[24] P. F. Daniel, *J. Chromatogr.*, 1979, **176**, 260.

Ion Exchange Chromatography. – Separations of many aldoses and alditols on the calcium form of cation exchange resins are improved by the addition of methanol to the eluting solvent and by the use of lower temperatures.[25] Good separations of mixtures of glucose, gluconic acid, glucuronic acid, guluronic acid, and glucaric acid were achieved on the strongly basic Dowex 1 × 8 resin.[26] Acidic sugars, such as the 2-keto-3-deoxyoctonic acids, are readily separable from neutral sugars by chromatography on Amberlite IR-45 (H^+ form) resin. Subsequently the acidic sugars may be eluted from the resin using sodium formate.[27] Ketoses, being less readily retained, can be separated from aldoses by chromatography on polyethyleneimine ion exchanger using water for elution.[28] The analysis of mixtures of neutral sugars has been achieved after their conversion to glycamines. The sugars were first reductively aminated using ammonia and sodium cyanoborohydride, and the resulting glycamines then separated on an amino-acid analyser.[29] The separation and determination of uronic acids and α-linked oligogalacturonides has been described using an automated system.[30]

Thin-layer Chromatography. – A t.l.c. method for the analysis of eleven carbohydrates commonly found in foodstuffs has been described using silica gel plates, which are eluted three times with acetonitrile–water (85 : 15). The spots were visualized using a diphenylamine dip and estimated spectrophotometrically.[31]

A method for the separation of several mono- and di-saccharides of biochemical interest involves two dimensional t.l.c. using solvents containing either boric acid or phenylboronic acid. The latter enhances the rate of migration of some sugars, whereas the former can slow it down.[32] A t.l.c. method for the determination of sucrose, fructose, and glucose in molasses employs silica gel plates treated with potassium phosphate, the components again being visualized with diphenylamine and estimated spectrophotometrically.[33] The t.l.c. separation of sugar phosphates and sulphates has been described,[34] and a method for the determination of lactulose in the presence of other oligosaccharides based on t.l.c. and polarography has been reported.[35]

Malonamide has been recommended as a spray reagent for the fluorimetric detection of reducing sugars on t.l.c. plates,[36] and *o*-aminobenzene sulphonic

[25] S. J. Angyal, G. S. Bethel, and R. J. Beveridge, *Carbohydr. Res.*, 1979, **73**, 9.
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[30] H. Konno, Y. Yamasaki, K. Yoshida, and J. Ozawa, *Agric. Biol. Chem.*, 1979, **43**, 2387.
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[35] E. Virag, T. Eros Takacs, A. Tioth, and K. Bittera, *Gyogyszereszet.*, 1979, **23**, 170 (*Chem. Abs.*, 1979, **91**, 44 556*u*).
[36] S. Honda, Y. Matsuda, and K. Kakehi, *J. Chromatogr.*, 1979, **176**, 433.

acid permits the simultaneous fluorimetric and colorimetric detection of reducing sugars.[37]

2 Other Analytical Methods

An improved procedure for the Morgan–Elson assay for 2-amino-2-deoxy aldoses has been described.[38] A study of the titrimetric determination of reducing sugars by $Cu^{II}SO_4$ has resulted in modifications to the original (1923) method of Lane and Eynon.[39] A simple sensitive assay of 2-amino-2-deoxyaldoses involves sequential treatment with nitrous acid and 3,5-diaminobenzoic acid to give a stable fluorescent product. The method is reported to be superior to the Elson–Morgan reaction, and glucose and other aldoses do not interfere.[40] The reductive amination of oligosaccharides with 2-aminopyridine affords a series of fluorescent derivatives that are separable by paper electrophoresis, enabling the detection of *ca.* 1 nmol of derivative.[41]

[37] F. IInuma, Y. Hiraga, T. Kinoshita, and M. Watanabe, *Chem. Pharm. Bull.*, 1979, **27**, 1268.
[38] E. Enghofer and H. Kress, *Carbohydr. Res.*, 1979, **76**, 233.
[39] T. H. Khan, *Analyst (London)*, 1979, **104**, 261 (*Chem. Abs.*, 1979, **91**, 101 579*e*).
[40] J. E. Scott, *Biochem. J.*, 1979, **183**, 91.
[41] S. Hase, T. Ikenaka, and Y. Matsushima, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 257.

23

Stereospecific Syntheses from Carbohydrates

Hanessian has provided a useful review of this rapidly developing subject covering examples in which carbohydrate rings are retained in tetrahydrofuran and tetrahydropyran moieties of the products, and some in which sugars are used to provide the bases of acyclic synthons.[1]

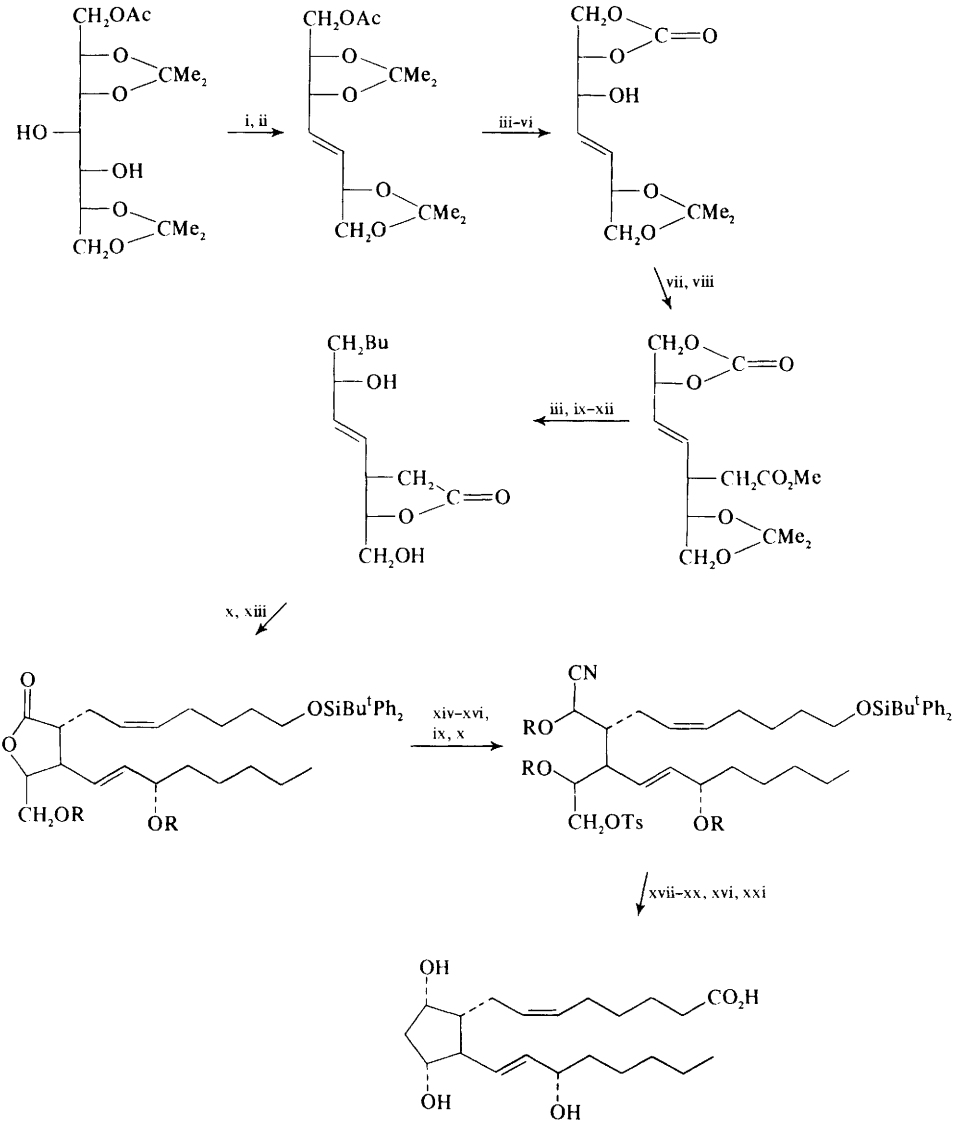
Pride of place must be given to Stork's synthesis of prostaglandin $F_{2\alpha}$ from D-glucose that is outlined in Scheme 1, and which represents a landmark in synthetic organic chemistry illustrating most powerfully the value of carbohydrates as chiral synthons.[2]

Three publications from Bernet and Vasella have made an important contribution to the topic of synthesis of functionalized cyclopentanes from carbohydrate compounds (see Chapter 17).

L-*erythro*-β-Hydroxyhistidine, required for the total synthesis of bleomycin B_2, has been prepared from *N*-acetyl-D-glucosamine by standard methods *via* the aldehydrolactone (1), which existed as a tricyclic dimer. Treatment of (1) with ammonium acetate followed by copper(II) acetate and formaldehyde gave *N*-acetyl-L-*erythro*-β-hydroxyhistidine (2), which was deacetylated to give the required product.[3] A further acyclic compound to have been synthesized is (+)-cirulenin (3), an antibiotic substance, which has been prepared in two laboratories. Both groups adopted the same approach of synthesizing a 2,3-anhydro-γ-lactone with a suitable 8-carbon side-chain (extended from a formyl group[4] and a vinyl group,[5] respectively), and then opening the lactone ring to give an amide and oxidizing the released hydroxy-group to the corresponding ketone. A somewhat analogous synthesis (Scheme 2) gave (+)chrysanthemum dicarboxylic acid (4), the enantiomer of this product being obtained by application of an epimerization at C-2 after hydrolysis of the pyranosyl ring.[6]

Considerable work has been carried out on the preparation of component parts of chiral macrocycles. The acid (5) has been prepared for the synthesis of pyridomycin by the procedure outlined in Scheme 3.[7] The complete antibiotic antimycin A_3 (6) has been synthesized from methyl 2-butyl-2,5-dideoxy-β-L-arabinofuranoside (7) (Scheme 4),[8] and the C-1 → C-9 segment (8) of the 16-membered ring of carbomycins and leucomycins has been made from D-glucose by the sequence shown in Scheme 5.[9]

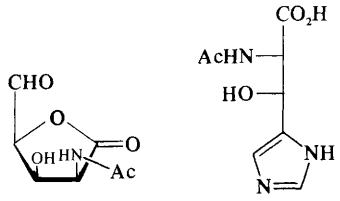
[1] S. Hanessian, *Acc. Chem. Res.*, 1979, **12**, 159.
[2] G. Stork, T. Takahashi, I. Kawamoto, and T. Suzuki, *J. Am. Chem. Soc.*, 1978, **100**, 8272.
[3] S. M. Hecht, K. M. Rupprecht, and P. M. Jacobs, *J. Am. Chem. Soc.*, 1979, **101**, 3982.
[4] N. Sueda, H. Ohrui, and K. Kuzuhara, *Tetrahedron Lett.*, 1979, 2039.
[5] M. Pietraszkiewicz and P. Sinäy, *Tetrahedron Lett.*, 1979, 4741.
[6] B. J. Fitzsimmons and B. Fraser-Reid, *J. Am. Chem. Soc.*, 1979, **101**, 6123.
[7] M. Kinoshita, H. Hamazaki, and M. Awamura, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 3595.
[8] S. Aburaki and M. Kinoshita, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 198.
[9] K. C. Nicolaou, M. R. Pavia, and S. P. Seitz, *Tetrahedron Lett.*, 1979, 2327.



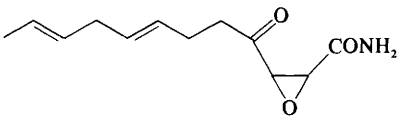
R = –CHMe(OEt)

Reagents: i, $HC(OMe)_2NMe_2$; ii, Ac_2O; iii, MeO^-; iv, $ClCO_2Me$–Py; v, $CuSO_4$–H_2O–MeOH; vi, Me_2CO–H^+; vii, $MeC(OMe)_3$; viii, Δ; ix, TsCl–py; x, $EtOCH{=}CH_2$; xi, $LiCuBu_2$; xii, H_2SO_4; xiii, *cis*-$BrCH_2CH{=}CH(CH_2)_3OSiBu^tPh_2$–$LiN(TMS)_2$; xiv, Bu^i_2AlH; xv, HCN; xvi, HOAc; xvii, $KN(TMS)_2$; xviii, F^-; xix, CrO_3–py–CH_2Cl_2; xx, $AgNO_3$–KOH; xxi, Lithium Selectride

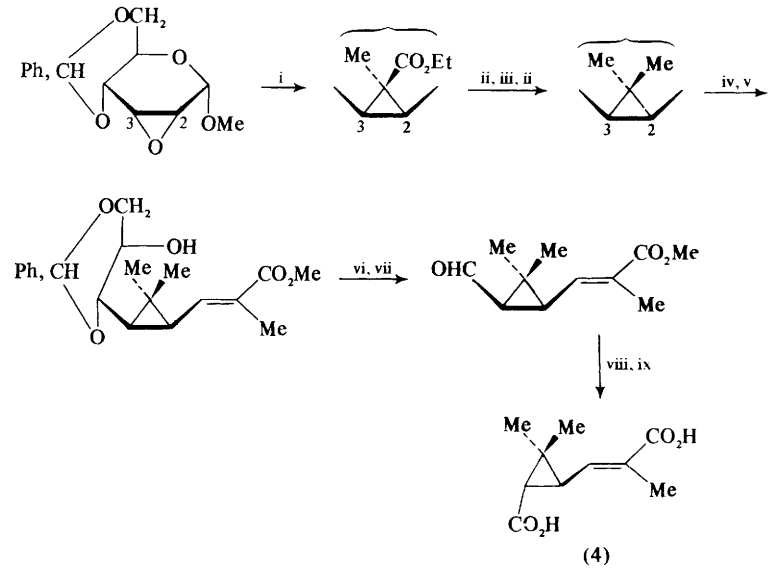
Scheme 1



(1) (2)

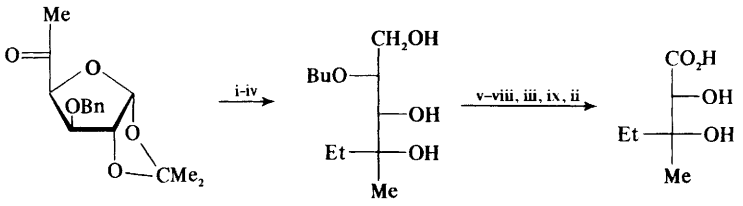


(3)



Reagents: i, $(EtO)_2POCH(Me)CO_2Et$; ii, LAH; iii, MsCl–DMF; iv, H_2O; v, $Ph_3P{=}C(Me)CO_2Et$; vi, H^+; vii, IO_4^-; viii, MeO^-; ix, Ag_2O–NaOH

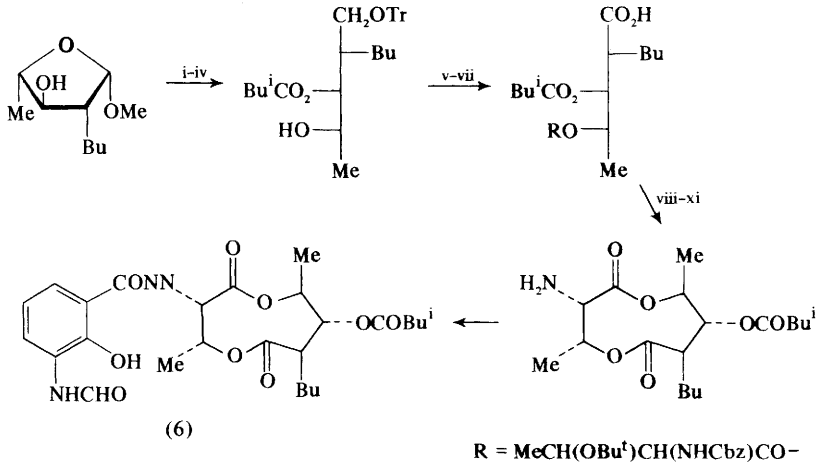
Scheme 2



(5)

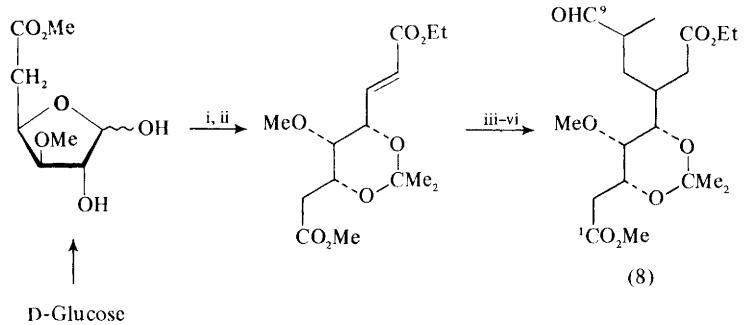
Reagents: i, EtMgBr; ii, H^+; iii, IO_4^-; iv, LAH; v, BzCl–py; vi, $Me_2C(OMe)_2$–H^+; vii, MeO^-; viii, H_2–Pd; ix, NaOI

Scheme 3



Reagents: i, $(Bu^iCO)_2O$–py; ii, HCl; iii, $NaBH_4$; iv, TrCl–py; v, MeCH(OBu^t)
v, MeCH(OBu^t)CH(NHCbz)CO_2H–DCC; vi, HOAc; vii, CrO_3; viii, CF_3CO_2H;
ix, (2-py-S)$_2$–Ph_3P; x, $AgClO_4$; xi, H_2–Pd

Scheme 4



Reagents: i, $Ph_3P{=}CHCO_2Et$; ii, $Me_2C(OMe)_2$–H^+; iii, $LiCu[CH_2C(Me){=}CH_2]_2$; iv, BH_3;
v, H_2O_2–OH^-; vi, $C_5H_5N.CrO_3.HCl$

Scheme 5

Some Hungarian work, similar to that reported in Vol. 12 (Chapter 23, ref. 9), has appeared on the synthesis of crown ethers by linking the hydroxy-groups of separate molecules of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside with diethyleneglycol bridges.[10]

The use of asymmetric hydrogenation catalysts formed from sugar diphenylphosphine derivatives is mentioned in Chapter 16.

[10] L. Toke, L. Fenichel, P. Bako and J. Szejtli, *Acta Chim. Acad. Sci. Hung.*, 1978, **98**, 357 (*Chem. Abs.*, 1979, **90**, 138 111*b*).

Part II

MACROMOLECULES

By

J. F. Kennedy
I. M. Morrison
C. M. Sturgeon
R. J. Sturgeon

1

Introduction

BY J. F. KENNEDY

The introduction to the Part II Report was revised extensively in the previous volume[1] and that chapter, which covered in detail: 'Scope and Coverage of the Report'; 'Organization, Nomenclature, and Use of the Report'; 'Significant Advances in Macromolecular Carbohydrate Chemistry'; and 'Conclusions and Readership', is classified as 'essential reading' for any new reader of this Series of Part II Reports.

This present Chapter essentially builds upon the corresponding Chapter of the previous Volume[1] which may be regarded as the ground state.

1 Scope and Coverage of the Report[1]

This is largely unchanged, but in the last few years a number of additonal biochemical journals have been published and these have been added to the journal coverage list. The inclusion of reports of principal carbohydrate (-containing) macromolecules in the new Specialist Periodical Reports series entitled 'Macromolecular Chemistry'[2,3] is not a duplication of, or a competitor to, 'Carbohydrate Chemistry, Part II'. For the specialists, and the readers who want information on the broader field of both synthetic and natural macromolecules, the similarities and differences between them, and an overview of the uses of all types of macromolecules including carbohydrate, proteins, and nucleic acids, 'Macromolecular Chemistry' is appropriate.

A text,[4] which is a complete treatment of all phases of Macromolecular Chemistry and Physics at the undergraduate level, includes a chapter on biological macromolecules, which are treated with exceptional thoroughness.

2 Organization, Nomenclature, and Use of the Report

Whereas organization and use of Part II Report are unchanged, clearly the subject of nomenclature is one of a state of flux. By the end of 1980, many of the draft documents of the IUB–IUPAC Joint Commission on Biochemical Nomenclature relating to carbohydrates should have been finalized. At that

[1] J. F. Kennedy in, 'Carbohydrate Chemistry', Part II, Macromolecules, ed. J. F. Kennedy and N. R. Williams (A Specialist Periodical Report), The Royal Society of Chemistry, London, 1981, Vol. 12, p. 215.

[2] A. D. Jenkins and J. F. Kennedy, 'Macromolecular Chemistry', ed. J. F. Kennedy and A. D. Jenkins (A Specialist Periodical Report), The Royal Society of Chemistry, London, 1980, Vol. 1.

[3] R. J. Sturgeon, in ref. 2, p. 126.

[4] 'Macromolecules, An Introduction to Polymer Science', ed. F. A. Bovey and F. H. Winslow, Academic Press, New York, 1979.

time, they will have been adopted by reporters in production of further Parts II and readers should endeavour to acquaint themselves with the new rules.

The publication in 1979 of 'Enzyme Nomenclature 1978',[5] is of significance, being a revision of the 1972 recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature.

It is gratifying to see that the now well known format of the publication[5] based on introductory and explanatory text, followed by extensive lists has been retained, and in some instances improved. The main part of the text is concerned with the classification and nomenclature of enzymes, a very useful, but frequently overlooked part which advises workers how, in fact, to name an enzyme. This is followed by the enzyme list in which all of the enzymes are placed in six divisions and sub-classes according to their reaction type: oxidoreductases, transferases, hydrolyases, lyases, isomerases, and ligases. The reaction catalysed by each enzyme is presented along with its recommended name, synonyms, systematic name, comments which delineate the activity, and references. The references to the 'Enzyme List' and the 'Index to the Enzyme List' complete the main part of the book. The index contains in alphabetical order all enzyme names, the print being coded according to whether the name is the recommended name, together with the recommended nomenclature numbers. In addition, this list contains the entries which have been deleted since the 1972 recommendations. A minor but welcome change is to be found in the style of the references, in which the Enzyme Commission number is now included.

It would behove chemists and biochemists, before submitting their papers to the scrutiny of referees of the scientific journals, to pay more attention to the nomenclature of the enzymes upon which they report by checking in 'Enzyme Nomenclature' whether they are using the correct name and whether the name has been deleted. Journal editors and referees should require authors to cite at least once the EC number and recommended and systematic names of all enzymes they mention in a paper. So often it is difficult for a reader to be absolutely certain as to which enzyme an author is discussing, a problem simply overcome by such citation.

In the 1972 recommendations, one of the chapters was devoted to multiple enzyme forms and enzyme precursors, dealing with the definitions and nomenclature of isoenzymes and multiple forms of enzymes. Unfortunately, this section does not appear in the current (1978) edition, and since it deals with an area of enzymology which remains confusing to many authors of scientific papers, the omission is regretted. This nevertheless highlights the fact that more than one protein structure/amino-acid sequence can possess the same enzyme activity. Furthermore, it is now evident that a single enzyme may act upon more than one substrate although its naming may suggest (because it was based on the originally discovered activity/substrate) that it is active on only one substrate. A further possibility is that an enzyme may have been named on the basis of its action upon an artificial substrate, whereas the normal physiological substrate, as yet to be identified, is different. Some enzymes, currently classified as hydrolases, are now known to possess, additionally, transferase activities. To these

[5] 'Enzyme Nomenclature 1978', Academic Press, New York and London, 1979.

phenomenona, for which there is now ample evidence, the Commission might address itself with urgency; the opportunity to do so is currently golden and delay will inevitably produce a number of unofficial nomenclature systems.

Since this is a book on nomenclature, it is a pity that 'Enzyme Nomenclature' does not necessarily use nomenclature currently accepted on an official basis elsewhere. A good example is the area of glycoside hydrolases hydrolysing *O*-glycosyl compounds where the recommended nomenclature is sometimes based on polysaccharide structure of the substrate, and sometimes on a trivial name non-indicative of substrate structure. Furthermore, *exo*- and *endo*-acting polysaccharide hydrolases are not necessarily distinguished, and in a few instances a singular enzyme activity is covered by more than one EC number.

A particular enzyme nomenclature problem which continues to arise, particularly in papers of clinical chemical origin, is the use of the term 'isoamylase' for isoenzymes of α-amylase (EC 3.2.1.1) – a confusion with the true use of the term isoamylase (EC 3.2.1:68) an enzyme with an action pattern different from that of α-amylase. This gross misnomer stems from the sloppy use of the term 'amylase' for α-amylase, which has been commented on before. In this report, the use of 'amylase' by authors, has been taken by reporters as meaning α-amylase and not β-amylase (EC 3.2.1.2). The use of the term 'isoamylase' has been strictly reserved by reporters for isoamylase (EC 3.2.1.68).

3 Significant Advances in Macromolecular Carbohydrate Chemistry

Recent noteworthy advances and trends in macromolecular carbohydrate chemistry include the following:

Studies on the chelation of amylose and cellulose chains have used models based on intra- and extra-cyclic acetals of 2-cyclohexyloxytetrahydropyran that contain secondary hydroxy-group with the correct covalent disposition. The structures of these models were confirmed by n.m.r. spectroscopy and the results reported so far indicate that certain chelations are dominant.

Considerable interest has been expressed in the use of quinones and their derivatives during the alkaline pulping of woods. Modifications of these treatments have shown that the delignification rate can be accelerated and that the rate of depolymerization of the polysaccharides can be decelerated giving an increased pulp yield of a better quality.

The location of inter-residue cyclic acetals has been determined following the oxidation by periodate ion of heteropolysaccharides. These acetals protected hydroxy-groups during methylation analysis. Helix–helix aggregations and intermolecular associations through double helices have been demonstrated by vacuum-u.v.–circular dichroism spectra of agarose films.

The above-mentioned increase in publication of biochemical journals is reflected in the increasing number of citations in Part II of this book, particularly in the field of glycoproteins.

The rapid growth in the science of enzymology is continuing, as is demonstrated by the increasing number of enzymes listed in the 'Enzyme Nomen-

clature' editions over the years. The first report of the Enzyme Commission's 'Enzyme Nomenclature' in 1961 cited some 712 enzymes. By the time of the first publication of 'Enzyme Nomenclature' in 1964, the number had risen to 875. The second 'Enzyme Nomenclature' publication in 1972 listed 1770 and the present version lists 2122 enzymes.

Researchers are giving their attention in renewed sustained efforts to effect enzyme-catalysed conversions of natural carbohydrates such as cellulose and starch into sources of energy, *e.g.*, ethanol.

Enzyme technology *etc.* is still required to maximize the desired yield rather than loss of potential product as carbon dioxide. A new bacterial neuraminidase (from *Arthrobacter ureafaciens*) has been isolated which is able to hydrolyse the neuraminosyl bond of the ganglioside G_{M1}. This is the first report of an enzyme with this activity.

Gangliosides from the starfish (*Asterina pectinifera*) are unique in containing neuraminic acid residues in the inner core of the glycolipid.

It is now possible to use ^1H n.m.r. spectroscopy to assign the anomeric configuration of the carbohydrate moieties of glycosphingolipids.

Considerable interest has been shown in the structure and occurrence of cord factors. Amongst the biological properties examined was their effect on mitochondria.

Conventional fermentative production of carbohydrates has received considerable attention, particularly with respect to the use of xanthan as an adjunct to oil-well drilling. Biochemists and chemists are becoming increasingly aware of the potential of immobilizing whole microbial cells for the large-scale production of a wide variety of molecules.

Recent volumes of *Carbohydrate Research* have been issued in honour of various persons with international reputations in carbohydrate chemistry and their achievements, *viz.* Professor Kurt Heyns and his work on carbohydrate Amadori rearrangements in relation to proteins,[6] and Professor Roy L. Whistler and his work on, *inter alia*, industrial polysaccharides.[7]

4 Conclusions and Readership

From the scientific viewpoint, the value of 'Carbohydrate Chemistry, Part II' is assured, judging from the good reviews previous Volumes have had in the Press. From the sales viewpoint, in one sense the more important since without sales the Royal Society of Chemistry cannot justify and maintain an output, the uptake of this 'Carbohydrate Chemistry' series still has a long way to go. Thus readers are encouraged to make others aware as much as possible of the value of the series, as part of a self-preservation exercise.

[6] H. Paulsen, *Carbohydr. Res.*, 1979, **68**, 173.
[7] J. N. BeMiller, *Carbohydr. Res.*, 1979, **70**, 179.

2

General Methods

BY R. J. STURGEON

1 Gas–Liquid Chromatography

The neutral sugar components in human serum glycoproteins have been identified and determined by hydrolysis of the glycoprotein with ion-exchange resin followed by g.l.c. of the alditol acetates.[1] Hydrolysis conditions were optimized for cleavage of glycosidic bonds with only minimal destruction of L-fucose, D-mannose, and D-galactose. Tritium-labelled alditol acetates, derived from monosaccharides released by acid hydrolysis of glycoproteins and lipopolysaccharides, have been subjected to g.l.c. analysis.[2] By trapping the gas effluent on silicone-coated glass beads, and determining the level of radioactivity, 0.2 nmoles of monosaccharide have been detected with an accuracy of ±10–15%.

A modified method for the analysis of sugars as the *O*-trimethylsilyl derivatives of methyl glycosides has been used to give reproducible fingerprints of the carbohydrates of *Streptococcus mutans*.[3]

After methanolysis of glycoproteins, re-*N*-acetylation of amino-sugars has been performed after the *O*-trimethylsilylation step, directly on gas chromatographic columns, by injecting acetic anhydride together with the sample.[4] Quantitative *N*-acetylation occurs whereas the *O*-trimethylsilyl ethers remain intact. Amino-sugars have been treated with *O*-methylhydroxylamine before acetylation to produce *O*-acetyl-*O*-methyloximes.[5] These derivatives give single peaks on g.l.c. analysis.

The analysis of aldoses as their dimethyldithiotrimethylsilyl ethers has been achieved at the μmole level.[6]

Mannitol is frequently used as an internal standard in the g.l.c. analysis of carbohydrates. However, the existence of this polyol in the urine of healthy adults precludes its use and methyl α-D-glucopyranoside can be used in its place.[7]

Aldoses containing acetamido-, amino-, deoxy-, and thio-groups have been examined by g.l.c. of their peracetylated aldononitrile derivatives.[8] Chemical

[1] C. W. Gehrke, T. P. Waalkes, E. Borek, W. F. Swartz, T. F. Cole, K. C. Kuo, M. Abeloff, D. S. Ettinger, N. Rosenshein, and R. C. Young, *J. Chromatogr.*, 1979, **162**, 507.
[2] P. Prehm and A. Scheid, *J. Chromatogr.*, 1978, **166**, 461.
[3] H. A. S. Aluyi and D. B. Drucker, *J. Chromatogr.*, 1979, **178**, 209.
[4] A. A. Akhrem, G. V. Avvakumov, D. V. Sviridov, and O. A. Strelchyonok, *J. Chromatogr.*, 1978, **166**, 123.
[5] T. P. Mawhinney, M. S. Feather, J. R. Martinez, and G. J. Barbero, *Carbohydr. Res.*, 1979, **75**, C21.
[6] S. Honda, N. Yamauchi, and K. Kakehi, *J. Chromatogr.*, 1979, **169**, 287.
[7] M. Flaker and W. G. Gunn, *Clin. Chim. Acta*, 1979, **96**, 265.
[8] F. R. Seymour, E. C. M. Chen, and S. H. Bishop, *Carbohydr. Res.*, 1979, **73**, 19.

ionization m.s. was used to determine the molecular weights of these derivatives together with the number of aldehydic and alcoholic groups in the parent aldose. Electron impact m.s. indicated the nature and position of functional groups present in the parent aldose.

Sugar alcohols and reducing sugars, added to agar culture media for the purpose of identifying specific Gram-negative bacteria, have been determined by g.l.c. as their alditol acetates and aldononitrile acetates, respectively.[9]

The aldose content of vegetable fibres has been determined by a modification of the alditol acetate procedure.[10]

2 Column and Ion-exchange Chromatography

An apparatus has been designed for the fractionation of small quantities of acidic glycoproteins by stepwise centrifugal elution from columns of DEAE-cellulose.[11] The equipment is inexpensive and the method can be adapted for the routine simultaneous processing of multiple samples.

Study of separation processes for oligomers of D-glucose on polyacrylamide gels has shown that the partition coefficient depends on both the temperature of operation and steric factors associated with oligosaccharides having different glycosidic linkages.[12]

A method based on gel filtration, used in conjunction with specific automated analyses, has permitted the separation of high and low molecular weight urinary material.[13] The potential diagnostic use of the method, based on the elution profiles obtained, was outlined. The method has been applied to a study of some of the inherited disorders of metabolism.[14]

An accelerated, improved single-column procedure for the amino-acid analyses of basement membrane and collagen-like proteins has been reported.[15] The proteins were hydrolysed with methanesulphonic acid to release the amino-acids and amino-sugars. By using a two-column system on a commercially available analyser, amino-acids and the α- and β-anomers of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose have been separated in the 100–1000 pmol range.[16] The separation of the anomeric forms of the amino-sugars is critically dependent on pH. In a method used for glycoprotein analysis, amino-sugars and several L-cysteine derivatives have been resolved on a single-column amino-acid analyser.[17]

Monosaccharides, derived from polysaccharides, glycolipids, and glycoproteins, have been converted into the corresponding 1-amino-1-deoxyglycitols by

[9] G. J. Manius, T. M. Y. Liu, and L. F. L. Wen, *Anal. Biochem.*, 1979, **99**, 365.
[10] R. R. Selvendran, J. F. March, and S. G. Ring, *Anal. Biochem.*, 1979, **96**, 282.
[11] C. W. Ramey, C. F. A. Culling, P. E. Reid, W. L. Dunn, and M. G. Clay, *J. Chromatogr.*, 1979, **164**, 382.
[12] A. Heyraud and M. Rinaudo, *J. Chromatogr.*, 1978, **166**, 149.
[13] C. A. White and J. F. Kennedy, *Clin. Chim. Acta*, 1979, **95**, 369.
[14] C. A. White and J. F. Kennedy, *Clin. Chim. Acta*, 1979, **95**, 381.
[15] J. P. Vergnes and I. L. Freeman, *Anal. Biochem.*, 1979, **99**, 427.
[16] U. Del Valle and J. E. Shively, *Anal. Biochem.*, 1979, **96**, 77.
[17] M. Fauconnet and J. Rochemont, *Anal. Biochem.*, 1978, **91**, 403.

treatment with ammonia and sodium cyanoborohydride, prior to determination by an amino-acid analyser.[18]

Ion-exchange chromatography, incorporating borate buffers, has been used to separate neutral monosaccharides and uronic acids.[19] The method has been applied to the characterization of the hydrolysis products from wood.

3 Thin-layer Chromatography

A modified screening method using t.l.c. has been reported for the rapid diagnosis of glycoprotein storage disorders that are based on the secretion of excess oligosaccharides.[20] The method has been used in the diagnosis of mannosidosis, G_{M_1} gangliosidosis, genetic hyperglucosaminoglycanuria (mucopolysaccharidoses type I and type III), aspartylglycosaminuria, and fucosidosis.

A systematic identification scheme, based on paper chromatography and t.l.c., acidic and enzymic hydrolysis, and the reaction of carbohydrates with several location reagents, has been applied to the analysis of oligosaccharides present in the urine and faeces of sick children and in the diets they are fed.[21] The identity and origin of these oligosaccharides is described and their relevance to the diagnosis and treatment of children with suspected disorders of carbohydrate metabolism is discussed.

A system for the screening and identification of reducing sugars in urine using one-dimensional t.l.c. on silica gel has been developed.[22] Colour was developed by the aniline–diphenylamine method.

Woven glass fibre, impregnated with polysilicic acid, which has a separating potential similar to that of thin layers of silica gel, has been used as a chromatographic support and sorbent because solvents migrate approximately twice as fast as on t.l.c. plates.[23] The use of this fibre has allowed the separation of D-[^{11}C]-glucose and D-[^{11}C]fructose in a time comparable to the half-life of [^{11}C] (20.4 min).

A new solvent system has been developed for the improved separation by t.l.c. of malto- and isomalto-oligosaccharides.[24]

4 High-pressure Liquid Chromatography

Reverse-phase chromatography on a high-performance support with a covalently bonded non-polar phase has proved effective in the separation of mixtures of acetylated D-gluco-oligosaccharides.[25] Benzyloxime perbenzoyl derivatives of mono- and di-saccharides have been separated by h.p.l.c. on a normal-phase

[18] S. Hara, H. Ikegami, A. Shono, T. Mega, T. Ikenaka, and Y. Matsushima, *Anal. Biochem.*, 1979, **97**, 166.
[19] M. H. Simatupang, *J. Chromatogr.*, 1979, **178**, 588.
[20] A. C. Sewell, *Clin. Chim. Acta*, 1979, **92**, 411.
[21] R. S. Ersser, *Clin. Chim. Acta*, 1979, **97**, 225.
[22] C. Auray-Blais, R. Giguerre, P. Draper, D. Schapcott, and B. Lemieux, *Clin. Biochem.*, 1978, **11**, 235.
[23] S. C. Jones, *J. Chromatogr.*, 1978, **166**, 587.
[24] K. Kanaya, S. Chiba, and T. Shimomura, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 1947.
[25] G. B. Wells and R. L. Lester, *Anal. Biochem.*, 1979, **97**, 184.

microparticulate column.[26] Each sugar furnished a single, quantifiable derivative. The method may be of use in the analysis of sugars in biological fluids. Mixtures of disaccharides, monosaccharides, amino-sugars, and alditols have been fractionated by h.p.l.c. of their perbenzoylated derivatives.[27] The corresponding 4-nitrobenzoates were found to be less useful for routine analysis.

The analysis by h.p.l.c. of mixtures of D-glucose, D-fructose, and D-mannose has been reported using inexpensive, easily available, and mechanically stable unmodified silica as the stationary phase.[28]

The content of L-fucose and D-mannose in glycoproteins has been determined by h.p.l.c. of the perbenzoyl-D-[^{3}H]fucitol and -D-[^{3}H]mannitol derivatives.[29] Since D-galactitol and D-glucitol cannot be separated from D-mannitol using this system, samples containing these polyols must be subjected to electrophoresis prior to h.p.l.c.

The direct quantitative comparison of monosaccharides and amino-acids derived from glycoproteins, by h.p.l.c. has been reported.[30] Stepwise elution systems have been developed for the ion-exchange liquid chromatographic separation of these components.

5 Analytical Methods

Glycoproteins, after electrophoresis in polyacrylamide or agarose gels, have been visualized after incubation with fluorescein-labelled concanavalin A.[31] The method has been used to detect less than 100 nanograms of hexose bound to fibrinogen. Glycoproteins have been detected in polyacrylamide gels using sequential treatment which includes treatment with thymol and staining with alcoholic solutions of H_2SO_4.[32] Colours are produced in proportion to the carbohydrate content of the glycoprotein. A laser light scattering technique has been used for the measurement of the concentration of glycoproteins when complexed with lectins.[33] A high degree of sensitivity has allowed less than 25 pmol glycoprotein or 4.5 pmol lectin to be detected.

Methods have been reported for the ultrastructural localization of complex carbohydrates, including glycoproteins, in rat macrophages and monocytes, by employing the reactivity of vicinal glycols to periodate ions and the presence of carboxy- and sulphate ester-groups.[34]

A five-fold increase in sensitivity in the detection of aldopentoses on paper chromatograms has been achieved by using a spray reagent composed of diphenylboronic acid and 2-amino-ethanol prior to spraying with a 4-anisidine phosphate reagent.[35]

[26] R. M. Thompson, *J. Chromatogr.*, 1978, **166**, 201.
[27] C. A. White, J. F. Kennedy, and B. T. Golding, *Carbohydr. Res.*, 1979, **76**, 1.
[28] H. van Olst and G. E. H. Joosten, *J. Liq. Chromatogr.*, 1979, **2**, 111.
[29] P. F. Daniel, *J. Chromatogr.*, 1979, **176**, 260.
[30] M. M. Tikhomirov, A. Y. Khorlin, W. Voelter, and H. Bauer, *J. Chromatogr.*, 1978, **167**, 197.
[31] M. Furlan, B. A. Perret, and E. A. Beck, *Anal. Biochem.*, 1979, **96**, 208.
[32] D. Racusen, *Anal. Biochem.*, 1979, **99**, 474.
[33] E. Köttigen, C. Bauer, and W. Gerok, *Anal. Biochem.*, 1979, **96**, 391.
[34] P. L. Sannes, M. Eguchi, and S. S. Spicer, *J. Histochem. Cytochem.*, 1979, **27**, 1180.
[35] G. J. Niemann, *J. Chromatogr.*, 1979, **170**, 227.

The hexuronic acid content of vegetable fibre has been determined by a modification of the carbazole method.[10]

Carbohydrates have been detected colorimetrically and fluorimetrically on t.l.c. plates using a 2-aminobenzene sulphonic acid reagent.[36] The limits of detection of the fluorescent sugars are 0.05 nmol for monosaccharides and 5.0 nmol for 2-acetamido-2-deoxy-D-glucose. Carbohydrates containing a hydroxy- or amino-group at C-2 at the reducing end react with taurine producing fluorescent compounds.[37] The intensity of fluorescence decreases with increasing degree of polymerization of the oligosaccharides. No reaction was observed with 2-deoxy sugars or glycosides.

D-Xylose in serum and urine has been determined colorimetrically using a phloroglucinol reagent.[38] The method does not require prior protein precipitation and may be useful in the assessment of D-xylose malabsorption in infants.

The total carbohydrate content of rat liver has been determined in a single-step procedure using glucoamylase and D-glucose oxidase.[39] This is a modification of an earlier two-step system for the measurement of rat-liver glycogen.

A systematic investigation of a titrimetric determination of reducing sugars with copper(II) sulphate using the Lane and Eynon method has been reported.[40]

The effect of the concentration of tetraborate on the Morgan–Elson reaction for the determination of 2-acetamido-2-deoxy-D-hexoses has been evaluated.[41] Use of optimal concentrations of tetraborate for chromogen formation lower than those previously advocated is suggested. The *N*-acetylation of 2-amino-2-deoxy-D-hexoses has been shown to be temperature dependent.[42] Conditions have been chosen for the selective *N*-acetylation of 2-amino-2-deoxy-D-galactose and not 2-amino-2-deoxy-D-glucose, and have been applied to an assay of mixtures of these two amino-sugars.

2-Amino-2-deoxy-D-glucose 6-phosphate has been determined after *N*-acetylation, by the Morgan–Elson procedure.[43] The method has been used in a study of L-glutamine : D-fructose 6-phosphate aminotransferase [EC 2.6.1.16] and 2-amino-2-deoxy-D-glucose 6-phosphate aldo-ketoisomerase [EC 5.3.1.10] activities.

Amino-sugars have been detected in the pmol range after condensation with 2-phthalaldehyde in the presence of 2-mercaptoethanol.[44] Amino-acids also react yielding fluorescence intensities similar to those of amino-sugars. The products of acid hydrolysis of glycoproteins have been treated with 1-dimethylaminonaphthalene 5-sulphonyl chloride (dansyl chloride).[45] The dansyl amino-

[36] F. Iinuma, Y. Hiraga, T. Kinoshita, and M. Watanabe, *Chem. Pharm. Bull. (Jpn.)*, 1979, **27**, 1268.
[37] T. Kato and T. Kinoshita, *Chem. Pharm. Bull. (Jpn.)*, 1978, **26**, 1291.
[38] T. J. Eberts, R. H. B. Sample, M. R. Glick, and G. H. Ellis, *Clin. Chem.*, 1979, **25**, 1440.
[39] J. A. Johnson and R. M. Fusaro, *Anal. Biochem.*, 1979, **98**, 47.
[40] T. H. Khan, *Analyst (London)*, 1979, **104**, 261.
[41] M. Oguchi and M. S. Oguchi, *Anal. Biochem.*, 1979, **98**, 433.
[42] W. G. Wagner, *Anal. Biochem.*, 1979, **94**, 394.
[43] E. Enghofer and H. Kress, *Carbohydr. Res.*, 1979, **76**, 233.
[44] S. F. Carroll and D. R. Nelson, *Anal. Biochem.*, 1979, **98**, 190.
[45] D. C. Farwell and A. S. Dion, *Anal. Biochem.*, 1979, **95**, 533.

sugars were isolated by thin layer electrophoresis and t.l.c. and measured fluorimetrically.

A sensitive fluorimetric assay for 2-amino-2-deoxy-D-hexoses with increased sensitivity over the Elson–Morgan and Dische–Borenfreund indole methods has been reported.[46] The 2,5-anhydrohexoses produced by nitrous acid deamination of heparin, heparan sulphate, and 2-amino-2-deoxy-D-hexoses have been treated with 3,5-diaminobenzoic acid to give stable derivatives. Neither neutral sugars nor acetamido sugars interfere in the reaction. In a modification of an earlier procedure, equivalent colour complexes were formed by the interaction of 3-methyl 2-benzothiazoline and the 2,5-anhydrohexoses produced by deamination of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose.[47]

The thiobarbituric acid method has been applied to the measurement of the 3-deoxyoctulosonic acid content of bacterial lipopolysaccharides.[48] However, interpretation of the results should be regarded with caution when neuraminic acid is also present in the samples.

Neuraminic acid, free or glycosidically bound, has been treated with sodium periodate and the liberated formaldehyde then treated with methyl 3-benzothiazolinone 2-hydrazone, giving a method for the determination of this monosaccharide.[49]

A method has been developed for the measurement of D-glucuronic acid in urine based on a reaction with tetraborate in the presence of sulphuric acid and 3-hydroxydiphenyl.[50] Both free and bound acids can be determined more accurately than by the commonly used decarboxylation and resorcinol methods.

Conditions have been established for the measurement of D-glucose using the hexokinase–D-glucose 6-phosphate dehydrogenase method.[51] This has enabled a new approach to be made in kinetic analyses in which the dependencies upon experimental variables are much closer to equilibrium methods than to the more common kinetic methods.

Immobilized D-glucose oxidase has been used for the determination of serum samples containing D-glucose in a commercially available continuous flow analyser.[52] The method appears to be a convenient and economical alternative to the use of soluble enzyme reagents. The D-glucose oxidase–4-aminophenazone-phenol procedure for the determination of D-glucose has been evaluated for use in an autoanalyser.[53] The method compared well with two other available routine methods using the same autoanalyser.

Detergents have been incorporated into the reagents of the D-glucose oxidase assay to prevent their precipitations when used in the polarographic determination of D-glucose.[54]

[46] J. E. Scott, *Biochem. J.*, 1979, **183**, 91.
[47] R. L. Smith and E. Gilkerson, *Anal. Biochem.*, 1979, **98**, 478.
[48] S. S. Kuwahara and M. C. Snetting, *Anal. Biochem.*, 1979, **100**, 118.
[49] Y. Massamiri, M. Beijean, G. Durand, J. Feger, M. Pays, and J. Agneray, *Anal. Biochem.*, 1979, **91**, 618.
[50] D. Rubin, *Clin. Chem.*, 1979, **25**, 1867.
[51] G. E. Meeling, H. L. Pardue, J. E. Thompson, and R. A. Smith, *Clin. Chem.*, 1979, **25**, 1581.
[52] R. Chirillo, G. Caenaro, B. Pavan, and A. Pin, *Clin. Chem.*, 1979, **25**, 1744.
[53] G. V. Purcell, D. B. Behenna, and P. R. Walsh, *Clin. Chem.*, 1979, **25**, 1844.
[54] L. L. Bajema, W. Lee, A. M. Zebelman, and M. A. Kenny, *Clin. Chem.*, 1979, **25**, 127.

D-Glucose oxidase and peroxidase, when adsorbed on to concanavalin A–Sepharose® or to *Lens culinaris* lectin-Sepharose have been used in an analytical system for the estimation of D-glucose.[55]

Whole cells of *Pseudomonas fluorescens* have been immobilized in collagen membranes and used in conjunction with an oxygen electrode for the determination of D-glucose in microbial cultures.[56]

An enzyme thermistor containing co-immobilized D-glucose oxidase and catalase has been used for the measurement of D-glucose in the outflow from a column reactor containing immobilized β-D-galactosidase acting on a solution of lactose.[57]

D-Glucose oxidase has been immobilized on porous glass and the product used in column form for the determination of D-glucose.[58] The liberated hydrogen peroxide was determined colorimetrically with peroxidase and a hydrogen acceptor.

An automated kinetic assay for the determination of D-glucose in blood has employed D-glucose dehydrogenase [EC 1.1.1.47] in the presence of mutarotase and nicotinamide adenine dinucleotide as hydrogen acceptor.[59] The technique is highly specific and can be applied to blood and other body fluids.

D-Fructose in seminal plasma has been estimated from the initial rate of oxidation of NADH in the presence of D-glucitol dehydrogenase [EC 1.1.1.14].[60] The sensitivity and specificity of the method have been compared with existing colorimetric methods.

An isotachophoretic method has been developed for the separation and determination of 5–100 nmol quantities of iodate, periodate, and formate ions arising from the periodate oxidation of carbohydrates.[61] The carboxylic acid fragments arising from the periodate oxidation of oligosaccharides, oxidation of the resulting dialdehydes, and total acid hydrolysis, have also been determined by use of this technique.[62]

A calorimetric and a pH method have been used to measure the solution stability constants of borate complexes of pentitols and hexitols.[63]

6 Structural Methods

A treatise on Organic Chemistry contains a chapter on polysaccharides which includes details of all the important structural methods.[64]

[55] B. Mattiasson and C. Borrebaeck, *FEBS Lett.*, 1978, **85**, 119.
[56] I. Karube, S. Mitsuda, and S. Suzuki, *Eur. J. Appl. Microbiol. Technol.*, 1979, **7**, 343.
[57] B. Danielsson, B. Mattiasson, R. Karlsson, and F. Winqvist, *Biotechnol. Bioeng.*, 1979, **21**, 1749.
[58] J. Endo, M. Tabata, S. Okada, and T. Murachi, *Clin. Chim. Acta*, 1979, **95**, 411.
[59] C. P. Price and K. Spencer, *Ann. Clin. Biochem.*, 1979, **16**, 100.
[60] R. A. Anderson, J. M. Reddy, C. Oswald, and L. J. D. Zaneveld, *Clin. Chem.*, 1979, **25**, 1780.
[61] S. Oka, M. Hirotsune, and S. Shigeta, *Anal. Biochem.*, 1979, **98**, 417.
[62] S. Honda, H. Wakasa, M. Terao, and K. Kakehi, *J. Chromatogr.*, 1979, **177**, 109.
[63] W. J. Evans, E. J. McCourtney, and W. B. Carney, *Anal. Biochem.*, 1979, **95**, 383.
[64] J. F. Kennedy and C. A. White, 'Comprehensive Organic Chemistry', ed. W. D. Ollis, Pergamon, Oxford, 1979, Vol. 5, p. 764.

I.r. Spectroscopy. – Structures of polysaccharides have been investigated using Fourier-transform i.r. difference spectra.[65] The i.r. absorbance difference spectra of dextrans have been correlated to the type and degree of branching of the polysaccharide which have previously been established by permethylation analysis.

N.m.r. Spectroscopy. – A correlation of the structures of polysaccharides with their ^{1}H n.m.r. spectra using dextrans as model compounds has been reported.[66] The ^{1}H n.m.r. spectra at 100 MHz are less discriminating regarding the structure of the polysaccharide than are the ^{13}C n.m.r. spectra at similar field strengths. Dextrans, linear (1→6)-linked α-D-glucans with (1→2)-linked α-D-glucosyl branches, are readily distinguished from the corresponding dextrans having 1,3- and 1,4-branches. Although the latter two classes cannot be distinguished by ^{1}H n.m.r. spectroscopy alone, the use of this technique together with Fourier-transform i.r. difference spectroscopy provides complementary structural information.

The ^{13}C n.m.r. spectra of seven hetero-oligosaccharides isolated from blood-group substance H have been recorded.[67] The data obtained confirmed their structures.

Characteristic differences in the ^{13}C n.m.r. chemical shifts of the mono- and di-*O*-methyl derivatives of methyl 2-acetamido-2-deoxy-D-glucopyranoside have been observed and should be of value in the structural analysis of carbohydrate chains containing these residues.[68]

Differences in chemical shifts have been observed for stereoisomeric pairs of acetalic methyl groups of pyruvic acid and related acetals linked to the 3,4- and 4,6-positions, respectively, of the anomeric methyl D-galactopyranosides, and the corresponding acetals linked to the 4,6-positions of the anomeric methyl D-glucopyranoside.[69] This has made possible the unequivocal determination of the stereochemistry of the pyruvic acid acetals in algal and bacterial polysaccharides. Combined g.l.c.–m.s. has been used to identify 4,6-*O*-(1-carboxyethylidene)-D-galactose in the carrageenan from *Petrocelis middendorfii*.[70]

Methylation Analysis. – Information on the m.s. of permethylated disaccharides has been recorded.[71] Fragmentation mechanisms for the electron impact m.s. of twenty-two permethylated aldohexopyanosyl (1→*x*) aldoses, where $x = 1$–6, have been reported.[72] Using high resolution measurements, data on metastable ions were obtained.

[65] F. R. Seymour and R. L. Julian, *Carbohydr. Res.*, 1979, **74**, 63.
[66] F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydr. Res.*, 1979, **74**, 77.
[67] A. S. Shashkov, N. P. Arbatsky, V. A. Derevitskaya, and N. K. Kochetkov, *Carbohydr. Res.*, 1979, **72**, 218.
[68] A. S. Shashkov, A. J. Evstigneev, and V. A. Derevitskaya, *Carbohydr. Res.*, 1979, **72**, 215.
[69] P. J. Garegg, B. Lindberg, and I. Kvarnström, *Carbohydr. Res.*, 1979, **77**, 71.
[70] V. L. Di Ninno, R. A. Bell, and E. L. McCandless, *Biomed. Mass Spec.*, 1978, **5**, 671.
[71] E. C. De Jong, W. Heerma, B. C. G. Dujardin, J. Haverkamp, and J. F. G. Vliegenthart in 'Recent Developments in Mass Spectrometry in Biochemistry and Medicine', ed. A. Frigerio, Plenum, Oxford, 1978, Vol. 1.
[72] E. G. De Jong, W. Heerma, J. Haverkamp, and J. P. Kamerling, *Biomed. Mass Spec.*, 1979, **6**, 72.

Negative chemical ionization m.s. of oligosaccharides has been carried out and the utility of the spectra in the structural elucidation of underivatized carbohydrates demonstrated.[73] Electron impact m.s. of methyl *O*-methyl glucopyranosiduronamides has been reported.[74]

A modified liquid phase, based on OV-225 and high molecular weight polyethylene glycol, and exhibiting a low level of bleeding from columns, has been used in the g.l.c. separation of methylated alditol acetates.[75]

It has been predicted from the stability of partially methylated methyl α-D-glucopyranosides towards trifluoroacetolysis, that the glycosidic bonds of oligo- and poly-saccharides containing hexopyranosyl residues should be stable in mixtures of trifluoroacetic acid and trifluoroacetic anhydride, provided no hexose residue is more than disubstituted.[76]

In the acetolysis and hydrolysis procedure for the cleavage of permethylated oligosaccharides from glycoproteins, no *N*-demethylation of methylated 2-amino-2-deoxyhexitols has been observed during the acetolysis step.[77] Upon acetylation, this yields a 1-*O*-acetyl-2-(*N*-methylacetamido)-2-deoxyhexitol and not, as suggested previously, a 2-acetamido-2-deoxyhexitol derivative.

Miscellaneous Methods. – An accurate identification of carbohydrates by mass spectrometry has been reported for biological fluids after fractionation by h.p.l.c. of their perbenzoyl *O*-benzyloxime derivatives.[78]

Chemical ionization m.s. has been applied to the identification of amino-sugars in bacterial lipopolysaccharides.[79] Some 2-amino-2-deoxy-sugars can be differentiated by both electron impact and chemical ionization m.s.

Silver oxide adsorbed on to celite (Fetizon reagent) has been used to effect the oxidation of aldoses from the C-1 position in a series of degradation steps which cease when the 2-*O*-substituted aldose is formed.[80] The method has been applied to the determination of the substituent position in mono-*O*-methyl-aldoses.

The hydroxy- and oxo-compounds derived from di- and tri-saccharides after the sequence of periodate oxidation, sodium borohydride reduction, and acid hydrolysis have been identified by g.l.c. of their *O*-trimethylsilyl ether derivatives, thus giving useful information for linkage analysis.[81]

Aldehydes formed as the products of the periodate oxidation of oligosaccharides have been determined after separation by g.l.c. of their trimethylsilyl ethers of the dithioacetals.[82,83] Data on the types and molar proportions

[73] A. G. Ganguly, N. F. Cappuccino, H. Fujiwara, and A. K. Bose, *J. Chem. Soc., Chem. Commun.*, 1979, 148.
[74] V. Mihálov, V. Kováčik, and P. Kováč, *Carbohydr. Res.*, 1979, **70**, 239.
[75] A. A. Akhrem, G. V. Aviakumov, and O. A. Strel'chyonok, *J. Chromatogr.*, 1979, **176**, 207.
[76] L. E. Franzen and S. Svensson, *Carbohydr. Res.*, 1979, **73**, 309.
[77] M. Caroff and L. Szabo, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 410.
[78] R. M. Thompson and D. A. Cory, *Biomed. Mass Spec.*, 1979, **6**, 117.
[79] D. V. Bowser, R. G. Teece, and S. M. Somani, *Biomed. Mass Spec.*, 1978, **5**, 627.
[80] S. Morgenlie, *Carbohydr. Res.*, 1979, **73**, 315.
[81] H. Yamaguchi and T. Mukumoto, *J. Biochem. (Jpn.)*, 1977, **82**, 1673.
[82] S. Honda, K. Takeda, and K. Kakehi, *Carbohydr. Res.*, 1979, **73**, 135.
[83] S. Honda, Y. Takai, and K. Kakehi, *Anal. Chim. Acta*, 1979, **105**, 153.

of the aldehydes formed, provide information on the nature of the interglycosidic linkages. The method has been applied to an investigation of non-dialysable urinary carbohydrates.[84] Prevention of over oxidation of reducing oligosaccharides by sodium periodate has been achieved by their conversion into 1,5-anhydroalditol derivatives, by sequential acetylation, hydrobrominolysis, and reduction with lithium aluminium hydride.[85] The products of complete oxidation may then be determined by g.l.c. of the dithioacetal derivatives.

Cyclic hemiacetals, formed following periodate oxidation of some polysaccharides, lead to protection of hydroxy groups during methylation, and their positions can be located by analysis of the resulting di- and mono-*O*-methyl ethers.[86] Such derivatives were not observed on methylation analysis of native and periodate-oxidized and borohydride-treated glycans. Inter-residue hemiacetals were identified in all oxidized glycans between aldehyde groups at C-2 or C-3 of oxidized residues and hydroxy-groups at C-3 or C-2 of adjacent unoxidized residues.

The reducing end of oligosaccharides have been coupled to 2-aminopyridine by reductive amination and the fluorescent products subjected to two-dimensional electrophoresis.[87] In the first direction, oligosaccharide derivatives were found to migrate according to their degree of polymerization,[88] and in the second direction according to the structure of their borate complexes. Linkage analysis of 2-aminopyridine oligosaccharide derivatives has been established by permethylation, partial acid hydrolysis, and then re-methylation with trideuteriomethyl iodide.[89]

The bibliography of crystal structures of carbohydrates has been updated to cover publications from 1975–77.[90–92]

[84] S. Honda, S. Okuyama, Y. Kishi, S. Suzuki, and K. Kakehi, *J. Chromatogr.*, 1979, **164**, 9.
[85] S. Honda, K. Kakehi, and Y. Kubono, *Carbohydr. Res.*, 1979, **75**, 61.
[86] L. S. Forsberg and J. H. Pazur, *Carbohydr. Res.*, 1979, **75**, 129.
[87] S. Hase, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Jpn.)*, 1979, **85**, 989.
[88] S. Hase, S. Hara and Y. Matsushima, *J. Biochem. (Jpn.)*, 1979, **85**, 217.
[89] S. Hase, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Jpn.)*, 1979, **95**, 995.
[90] J. M. Sundaralingam, *Adv. Carbohydr. Chem. Biochem.*, 1977, **34**, 345.
[91] P. R. Sundararajan and R. H. Marchessault, *Adv. Carbohydr. Chem. Biochem.*, 1978, **35**, 377.
[92] P. R. Sundararajan, *Adv. Carbohydr. Chem. Biochem.*, 1979, **36**, 315.

3

Plant and Algal Polysaccharides

BY I. M. MORRISON

1 Introduction

A number of review articles and book chapters have appeared during the year under report. A section is devoted to plant and algal polysaccharides in the chapter on polysaccharides in a series entitled 'Comprehensive Organic Chemistry'.[1] The dielectric properties and d.c. conduction of cellulose are reviewed in a volume devoted to applied fibre science as well as the inter- and intra-crystalline reactions in cellulose fibres.[2] Another book is devoted to some aspects of the properties of biological macromolecules and polyelectrolytes in solution.[3] Information of relevance to this chapter can be found in a volume on complex carbohydrates, part of the series on 'Methods in Enzymology'.[4] The bibliography of the crystal structure of polysaccharides has been updated to the end of 1975[5] and the hemicelluloses of grasses and cereals have been reviewed with respect to isolation, structural features, and occurrence within the plantstuff.[6]

2 Starches

The ^{13}C n.m.r. spectra of the previously reported comb-like derivatives prepared by orthoester- and Helferich-type condensations of D-glucose on to amylose have been recorded.[7] It was known from monomeric studies that the orthoester method favoured the β-D-glycosidic bond, whereas the Helferich method favoured the α-D-glycosidic bond. The spectra confirmed that 4,6-disubstitution occurred on the D-glucose residues of amylose and that a degree of polymer linearity existed but the spectra also indicated that extensive, if not exclusive, β-D-glucosylation occurred with both condensation methods.

[1] J. F. Kennedy and C. A. White, 'Comprehensive Organic Chemistry', Pergamon, Oxford, 1979, Vol. 5, p. 775.
[2] J. E. Algie and S. P. Rowland, 'Applied Fibre Science', ed. F. Happey, Academic Press, London, 1979, Vol. 2.
[3] H. Eisenberg, 'Biological Macromolecules and Polyelectrolytes in Solution', Oxford University Press, London, 1976.
[4] V. Ginsburg, 'Complex Carbohydrates Pt. C., Methods in Enzymology', Academic Press, New York, 1978, Vol. 50.
[5] P. R. Sundararajan and R. H. Marchessault, in 'Advances in Carbohydrate Chemistry and Biochemistry', ed. R. S. Tipson and D. Horton, Academic Press, New York, 1978, Vol. 35, p. 377.
[6] K. C. B. Wilkie, 'Advances in Carbohydrate Chemistry and Biochemistry', ed. R. S. Tipson and D. Horton, Academic Press, New York, 1979, Vol. 36, p. 265.
[7] F. R. Seymoor, R. D. Knapp, T. E. Nelson, and B. Pfannemüller, *Carbohydr. Res.*, 1979, **70**, 125.

Differential thermal analysis has been applied to an examination of the thermal behaviour of starch and foodstuffs.[8] Potato starch showed an endotherm at *ca.* 65 °C which was not influenced by either the rate of heating, or the concentration and the initial amount of starch. Similar analyses, applied to intact potato, Indian lotus, taro, and sweet potato, were distinct from each other but were similar to the starch from each plant except that the isotherm was shifted by 3–7 °C to the higher temperature region.[9]

The absorption and circular dichroism spectra of the blue colour produced by tri-iodide ions in amylose have been characterized.[10] Four fundamental Raman lines were observed for I_3^- bound to amyloses which were not dependent on the DP in the range 20–100 or the excitation wavelength. Concanavalin A does not react with amylose but does react with the amylose–iodine complex although the complex can be dispersed by shaking.[11] The complex is non-specific as it cannot be displaced by concanavalin A-specific sugars.

A simplified determination of amylose in milled rice has involved an initial dispersion in dilute alkali.[12] The amylose–iodine complex at pH 7.5 was estimated from the absorption at 630 nm. Ultrafiltration was considered to be a suitable method for a hydrodynamic study of the amylose–iodine complex formation.[13] No partially complexed amylose molecules were present in the permeate and so it was considered that complex formation took place instantaneously in a whole molecule and not successively in different zones of the molecule. The conformation of amylose in aqueous solution is entirely that of loose and extended helices. The formation of the iodine complex is visualized as a trapping of iodine atoms by the contraction of the loose helical macromolecule into tight and stiff helices.

The specific volumes of the crystalline and amorphous portions of starch have been determined.[14] All the values were corrected by extrapolation to zero moisture content. The amorphous portion gave values very similar to those for cellulose, being *ca.* 0.673 for potato, waxy corn, and waxy rice starch and *ca.* 0.684 for sweet potato, tapioca, corn, and wheat starch.

Digital oscillator densimeter techniques have been used to determine the density of dispersions of starch, glycogen, dextrin, amylopectin, and amylose in water at 40 °C but at different concentrations.[15]

The conformation of amylose in aqueous solution was determined by light-scattering sedimentation–equilibrium measurements and was found to be dependent on the molecular weight.[16] When the molecular weight was outside the 'dissolving gap' ($6.5 \times 10^3 < M_r < 1.6 \times 10^5$) the molecule behaved as a

[8] K. Takahashi, K. Shirai, K. Wada, and A. Kawamura, *J. Agric. Chem. Soc. (Jpn.)*, 1978, **52**, 201.
[9] K. Takahashi, K. Shirai, K. Wada, and A. Kawamura, *J. Agric. Chem. Soc. (Jpn.)*, 1978, **52**, 441.
[10] T. Handa and H. Yajima, *Biopolymers*, 1979, **18**, 873.
[11] S. V. Bhide and N. R. Kale, *Carbohydr. Res.*, 1979, **68**, 161.
[12] C. M. Sowbhagya and K. R. Bhattacharya, *Starch*, 1979, **31**, 159.
[13] Q. T. Nguyen, P. Aptel, and J. Neel, *Biopolymers*, 1976, **15**, 2097.
[14] S. Nara, *Starch*, 1979, **31**, 73.
[15] H. M. El Saied, *Starch*, 1979, **31**, 84.
[16] M. Kodama, H. Noda, and T. Kamata, *Biopolymers*, 1978, **17**, 985.

random coil while inside the range the molecules aggregated to a rigid coil. The conformation of the rigid coil was that of a double helix and resembled β-type (retrograded) amylose.

When potato starch granules were heated with a limited amount of water (water : starch < 1.5) two endothermic transitions were observed on differential scanning calorimetry.[17] The lower endothermic transition always occurred at 66 °C and was the only transition observed when excess water was present. The temperature of the higher transition was inversely proportional to the water content and decreased in intensity with increasing water content. The appearance of the higher endothermic transition allowed the stoicheiometry for full hydration of 14 water molecules per hexose unit to be determined.

Synthetic amyloses have been prepared from maltopentaose and D-glucose 1-phosphate by the action of potato phosphorylase.[18] The products were characterized by the analysis of the products of β-amylolysis and were used as standards in the determination of the molecular weight of starches by gel chromatography.

To elucidate the most probable types of chelation that exist in the chains of amylose and cellulose, studies have been undertaken on molecular models which represent, in part, the maltose and cellobiose entities in the polymers.[19] These models were some intra- and extra-cyclic acetals of the 2-cyclohexyloxytetrahydropyran type, which contained primary or secondary hydroxy-groups whose covalent disposition, relative to the acetal function, were analogous to those of OH-2, OH-3′, OH-6, and OH-6′ of amylose and cellulose. Most of the diastereoisomers, containing at least two asymmetrical carbon atoms were separated by gel permeation chromatography and their structures were confirmed by ^{1}H and ^{13}C n.m.r. spectroscopy. It was possible, by i.r. spectroscopy, to observe absorption bands corresponding to chelation in rings containing 5, 6, 7, or 8 members.[20] In maltose, methyl β-maltoside, and amylose chelation was observed between OH(2) and OH(3′) and possibly between OH(2) and O(1).

A Raman spectroscopic study of the amylose–monoglyceride complex, using monostearin as the model compound, indicated that the hydrocarbon conformation inside the amylose helix was ordered as if in a crystalline state.[21] Three helical turns existed for each hydrocarbon chain.

The average chain length of corn starch has been shown to be reduced by γ-irradiation as well as by acidic hydrolysis, the extent being dependent on the dose rate.[22] There was no evidence for the formation of dialdehydes by ring cleavage of the D-glucose residues.

The degrees of branching of commercial D-glucose syrups and D-glucose syrups prepared by reverse osmosis have been investigated.[23] The results of

[17] J. W. Donovan, *Biopolymers*, 1979, **18**, 263.
[18] W. Praznik and R. Ebermann, *Starch*, 1979, **31**, 288.
[19] F. Lafuma and C. Quiveron, *Can. J. Chem.*, 1978, **56**, 2065.
[20] F. Lafuma and C. Quiveron, *Can. J. Chem.*, 1978, **56**, 2076.
[21] T. L.-G. Carlson, K. Larsson, N. Dinh-Nguyen, and N. Krog, *Starch*, 1979, **31**, 222.
[22] Y. Ghali, S. Gabr, N. Ibrahim, and H. Aziz, *Starch*, 1979, **31**, 372.
[23] G. G. Birch, R. A. J. Foyle, and Y. M. Gallali, *J. Sci. Food Agric.*, 1979, **30**, 85.

methylation and enzymatic degradation analyses showed no significant differences.

The acidic and thermal stabilities of unmodified pea starch are destroyed on preparing the acetate or monophosphate derivatives.[24] These properties are similar to those of corn starch. The thermal stability was not affected on acetylation of a cross-linked pea starch. In this paper, a unique purification step was outlined that removed damaged starch granules along with pea hull fragments. A higher concentration of oval or irregularly shaped granules were found in the starch from *Vicia faba* than corn starch.[25] The granules also had a higher amylose content, gelatinization temperature range, and water-binding capacity, but a lower swelling power and solubility than either wheat or corn starch. Starches have been prepared from finger millet (*Eleusine coracana*) and Foxtail millet (*Setaria italica*) and were characterized by their low solubility.[26] The mature starches were susceptible to α-amylase action. The amylose contents varied between 15.5 and 17.5%, but successive leaching with water and alkali gave amylose components with different molecular weights and β-amylolysis limits. The results suggested that the amylose fraction was heterogeneous.

Recent studies on oat starch showed that the iodine affinity, solubility, and λ_{max} of the iodine complex were all lower than for other cereal starches.[27] The concentration of fractions between the size of amylose and amylopectin was higher than in wheat and the β-amylolysis limit was lower, indicating that the starch was more highly branched.

Flours, prepared from the five legumes, navy, pinto, faba, mung bean, and lentil all had similar starch and moisture contents but the starch content was lower than that of wheat flour.[28] The legume starch granules are oblong and do not have such a wide variation in size as wheat flour granules.[29] The range of amylose contents was lower than that for wheat starches. The acetylated wheat starch derivative had a higher molecular weight than the legume starches. A method for the separation of the amylose and amylopectin components of cotton leaves, but which should be applicable to other plants, has been reported.[30] After extraction with aqueous dimethylsulphoxide, the amylose component was separated from the amylopectin and cell-wall particles by chromatography on macroporous agarose (Sepharose 2B), while the amylopectin was purified by its interaction with concanavalin A.

It is now generally accepted that the digestion of starch by many mammalian species involves, firstly, the action of pancreatic α-amylase to give oligosaccharide end-products which are hydrolysed further to D-glucose by the enzymes of the small intestine brush border. The enzymology of these reactions has been reviewed[31] with respect to both nutritional and clinical implications.

[24] F. W. Comer and M. K. Fry, *Cereal Chem.*, 1978, **55**, 818.
[25] K. Lorenz, *Starch*, 1979, **31**, 181.
[26] D. B. Wankhede, A. Shehnaz, and M. R. R. Rao, *Starch*, 1979, **31**, 153.
[27] D. Paton, *Starch*, 1979, **31**, 184.
[28] O. Naivikul and B. L. D'Appolonia, *Cereal Chem.*, 1978, **55**, 913.
[29] O. Naivikul and B. L. D. D'Appolonia, *Cereal Chem.*, 1979, **56**, 24.
[30] C. W. Chang, *Plant Physiol.*, 1979, **64**, 833.
[31] D. J. Manners, 'Polysaccharides in Food', ed. J. M. V. Blanshard and J. R. Mitchell, Butterworths, London, 1979, p. 75.

The controlled hydrolysis of corn starch with resin-bound α-amylase has yielded a modified starch of high molecular weight in yields of *ca.* 93%.[32] The amylose component was hydrolysed preferentially and due to the *exo* nature of the enzyme, oligosaccharides of DP 1–8 were isolated. The modified starch had a lower tendency to retrograde. The hydrolysis of gelatinized modified and unmodified starch with porcine pancreatic α-amylase has been studied.[33] Modification of the starch with hydroxypropyl and acetate groups reduced the extent of digestion, acetylation being better than hydroxypropylation. Cross-linking of the unmodified starch with phosphate groups also lowered the extent of digestion. The ability of native and dioxan-extracted starch from potato and maize to bind Ca^{2+} ions influenced their digestibility *in vitro* with pancreatic α-amylase.[34] With maize starch, the digestibility was improved on extraction but lowered by Ca^{2+} ions, whereas the digestibility of potato starch was improved with both native and extracted starch.

Scanning electron microscopy showed that numerous pin holes could be observed on the surface of starch granules when attacked by various amylases.[35] It was also noticed that these pores penetrated into the inner layers of the granules during enzymic action and in some granules, a terraced or step-shaped appearance in their inner portions was observed. These internal characteristics are probably indicative of a layered internal structure in the granules. The other observations from scanning electron microscopy were the presence of striated structures on the surface of starch granules from banana, lily, and lotus which had been attacked by pancreatin.

Rapid heating to 200 °C and rapid cooling of starch with a 20% moisture content resulted in a product which could be gelatinized at room temperature.[36] The thermally modified starch was a better substrate than the native starch for saccharification with D-glucoamylase.

The action of Q-enzyme on a model amylodextrin which contained a single-branch linkage has been studied.[37] It was concluded that the enzymatic process for synthesizing the branch linkage in amylopectin is a random action of the Q-enzyme on a complex, possibly a double helix, formed between two (1→4)-linked α-D-glucan chains. The action pattern predicts a novel arrangement of the unit chains in amylopectin.

The intestinal surface membrane from rats contains both (1→4)-α- and (1→6)-α-D-glucosidase (-glucanase) activities and these enzymes have been given the trivial names of 'sucrase' and 'isomaltase', respectively.[38] The concurrent action of the sucrase and isomaltase active sites of the hybrid sucrase–isomaltase enzyme complex has been studied on the hexasaccharide (1). Hydro-

[32] B. T. Hofreiter, K. L. Smiley, J. A. Boundy, C. L. Swanson, and R. J. Fecht, *Cereal Chem.*, 1978, **55**, 995.
[33] M. Wootton and M. A. Chaudhry, *Starch*, 1979, **31**, 224.
[34] G. Sukan, M. W. Kearsley, and G. G. Birch, *Starch*, 1979, **31**, 125.
[35] H. Fuwa, Y. Sugimoto, T. Takaya, and Z. Nikuni, *Carbohydr. Res.*, 1979, **70**, 233.
[36] A. Sroczynski, M. Boruch, and T. Pierzgalski, *Starch*, 1979, **31**, 129.
[37] D. Borovsky, E. E. Smith, W. J. Whelan, D. French, and S. Kikumoto, *Arch. Biochem. Biophys.*, 1979, **198**, 627.
[38] G. M. Gray, B. C. Lally, and K. A. Conklin, *J. Biol. Chem.*, 1979, **254**, 6038.

lysis occurred by sequential removal of D-glucose residues from the non-reducing terminal end of the molecule through the pentasaccharide (2), tetrasaccharide (3), maltotriose, maltose, and finally D-glucose. The free isomaltase monomer could hydrolyse each step outlined, but displayed maximal specificity for the hydrolysis of (3) to maltotriose. The free sucrase was active against all 1,4-linked residues but could not hydrolyse (3) to maltotriose. Instead of acting synergystically, the two α-D-glucosidase sites appear to cleave α-limit dextrins by their complementary specificity.

α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 → 4)-α-D-Glc*p*

1

↓ →

6

α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 → 4)-D-Glc*p*

(1)

α-D-Glc*p*-(1 → 4)-α-D-Glc*p*

1

↓ →

6

α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 → 4)-D-Glc*p*

(2)

α-D-Glc*p*

1

↓

6

α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 → 4)-D-Glc*p* → Maltotriose → Maltose → D-Glucose

(3)

The retrogradation of amylose in solutions of enzymatically liquified starch depended on the degree of starch hydrolysis and on the manner of preparing the solution.[39] Even at DE values of 20–30%, retrogradation occurred in solutions of $<5\%$ and $>50\%$ concentration.

The subcellular localization of the starch biosynthetic and degradative enzymes of spinach leaves was carried out by measuring the distribution of the enzymes in a crude chloroplast pellet and in separated components of a protoplast lysate.[40] The enzymes which were involved in the syntheses of starch were detected in the chloroplasts, whereas some of the enzymes involved in the degradation of starch were mainly in the soluble protein fraction but were also found in the chloroplast. The digestion pattern of the amylase on amylopectin as substrate indicated that the enzyme was an α-amylase from its *endo*-lytic activity, but displayed properties unlike the typical α-amylase isolated from endosperm tissue. A time-sequence analysis of the starch digestion pattern in germinating

[39] M. Boruch and T. Pierzgalski, *Starch*, 1979, **31**, 149.

[40] T. W. Okita, E. Greenberg, D. N. Kuhn, and J. Preiss, *Plant Physiol.*, 1979, **64**, 187.

rice showed that activity, in the initial stage, was localized in the epithelium septum between the scutellum and the endosperm.[41] The breakdown of starch in endosperm tissue began thereafter. Amylase activity could only be detected in the aleurone layer after two days.

α-D-Glucopyranose 1,2-cyclic phosphate is a potent inhibitor of phosphorylase-catalysed starch elongation in potato. The affinity of the enzyme for the cyclic ester is *ca.* thirty times that of D-glucose 1-phosphate. Under conditions where elongation has been retarded by a factor of three, UDP-D-glucose, ADP-D-glucose, D-glucose 6-phosphate, and D-glucose 2-phosphate cause a rate reduction of less than 10%.[42]

3 Fructans

Methylation analysis of the D-fructose polymers from cocksfoot (*Dactylis glomerata*) indicated that both (2→1)-β-D-(inulin) and (2→6)-β-D-(levan) types of linkages were present.[43] Chemical analysis failed to detect any low molecular weight oligosaccharides during fructan synthesis in the leaves of cocksfoot (*D. glomerata*).[44] These results suggest that direct transfer of D-fructose to existing fructan polymers takes place. As the percentage dry weight of individual onion bulbs increased, the D-fructan content also increased, with a higher proportion of molecules with higher molecular weight being present.[45] The percentage dry matter and fructan content increased from outer to inner layer of the bulbs.

4 Cycloamyloses

Cycloamyloses have been separated by h.p.l.c. on a μ-Bondapak-carbohydrate column using acetonitrile–water mixtures as eluant.[46] The molecular dynamics of the inclusion complexes formed between cyclohexa-amylose and some aromatic amino-acids and dipeptides have been studied by ^{13}C n.m.r. spectroscopy.[47] The forces binding the complexes were found to be weak. The c.d. spectra of cyclohepta-amylose which had been complexed with 2-substituted naphthalenes were measured at various concentrations of cyclohepta-amylase and temperatures between 10–70 °C.[48] The complex with 2-naphthoxyacetic acid showed 1:1 stoicheiometry. The molar ellipticity and thermodynamic parameters were determined and enthalpy and entropy ranges calculated. The correlation was explained by a cyclohepta-amylose guest molecule interaction where the guest molecule was highly solvated. The induced c.d. spectra of cyclohepta-amylose complexes with substituted benzenes confirmed that an axial inclusion

[41] K. Okamoto and T. Akazawa, *Plant Physiol.*, 1979, **63**, 336.
[42] F. C. Kokesh, R. K. Stephenson, and Y. Kakuda, *Biochim. Biophys. Acta*, 1977, **483**, 258.
[43] C. J. Pollock, M. A. Hall, and D. P. Roberts, *J. Chromatogr.*, 1979, **171**, 411.
[44] C. J. Pollock, *Phytochemistry*, 1979, **18**, 777.
[45] B. Darbyshire and R. J. Henry, *J. Sci. Food Agric.*, 1979, **30**, 1035.
[46] B. Zsadon, K. H. Otta, F. Tüdös, and J. Szejtli, *J. Chromatogr.*, 1979, **172**, 490.
[47] Y. Inoue, Y. Katôno, and R. Chûjo, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1692.
[48] K. Harada, *Bull. Chem. Soc. (Jpn.)*, 1979, **52**, 1807.

was favoured, whereby the long axis of the substituted benzenes is parallel to the axis of the cyclohepta-amylose cavity.[49]

The binding forces contributing to the association of cyclohexa- and cyclohepta-amyloses with alcohols in aqueous solution have been determined from a spectrophotometric examination of the inhibitory effect of the various alcohols on the association of the cycloamyloses with Azo dyes.[50] Hydrophobic and van der Waals interactions were found to be of primary importance in complex formation. As the bulkiness of the alcohol increased, the stability of the cyclohexa-amylose adduct decreased, whereas the stability of the cyclohepta-amylose adduct was enhanced owing to the larger cavity in the cyclohepta-amylose molecule allowing better contact of the alcohol within the cavity. Inclusion complexes of poly-(cyclohepta-amylose) with two separate fluorescent probes have been used as models to measure the pressure effects upon ligand-protein complexes.[51] The data permitted the calculation of compressibility curves, which were in broad agreement with those of liquid aliphatic and aromatic hydrocarbons in the low-pressure range, but indicated a reduced compressibility at higher pressures. Metastable crystals of cyclohepta-amylose complexes with propan-1-ol have been prepared and their structure has been discussed in terms of the membrane diffusion model proposed previously by these authors.[52]

5 Cellulose

Single crystals of cellulose II have been grown from dilute aqueous solutions of cellulose acetate by deacetylation and consequent precipitation.[53] The crystals had a ribbon-like lamellar appearance and the best results were obtained using methylamine, at 90 °C, as the deacetylating agent. It has been suggested that the transformation from cellulose I to cellulose II during mercerization is the result of a progressive shift of the sheets of cellulose chains within the crystallites of a microfibril from the quarter-staggered relationship in cellulose I to the complete correspondence found in cellulose II.[54] This idea agrees with the observed changes in lateral disorder, cell dimensions, swelling, and *X*-ray diffraction reflections of cellulose fibres. Such a shift may take place in the transformation of native celluloses with antiparallel structures as well as those with parallel polarity.

The dielectric constant and dielectric loss for moist cellulose fibres was measured over a frequency band, a temperature range, and relative humidity range.[55] The dielectric constant increased with increasing frequency and temperature owing to an increase in the rotation and polarization of the flexible part of the fibre. As the relative humidity was increased, the dielectric constant

[49] H. Shimizu, A. Kaito, and M. Hatano, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2678.
[50] Y. Matsui and K. Mochida, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2808.
[51] P. M. Torgerson, H. G. Drickamer, and G. Weber, *Biochemistry*, 1979, **18**, 3079.
[52] K. H. Jogun and J. J. Stezowski, *Nature*, 1979, **278**, 667.
[53] A. Buleon and H. Chanzy, *J. Polym. Sci., Polym. Phys. Ed.*, 1978, **16**, 833.
[54] M. Takai and J. R. Colvin, *J. Polym. Sci., Polym. Chem. Ed.*, 1978, **16**, 1335.
[55] S. Boutros and A. A. Hanna, *J. Polym. Sci., Polym. Chem. Ed.*, 1978, **16**, 89.

and dielectric loss both increased. This may have been due to the presence of the polar water molecules; the freeing of polar groups; or the freeing of ions in the fibre molecule.

The ^{13}C n.m.r. spectra of peracetylated cello-oligosaccharides have been recorded and compared with that of cellulose acetate.[56] All of the signals from cellulose acetate were only observed in the spectra of cellotetraose and cellopentaose peracetates. Some signals were not observed in the spectra of cellobiose and cellotriose peracetates.

A bioengineering approach has been used for the separation of cellodextrins.[57] Cellulose was hydrolysed with HCl and a combination of columns of Sephedex G15 and Dowex AG50W × 4 allowed a one step desalting and separation of the hydrolysate using water as the eluant. The column was stable and did not require to be regenerated after every run. Up to 3 g of cellodextrins could be produced per day.

A method for the gel permeation chromatographic analysis of the molecular weight distribution of wood pulp holocellulose as the carbanilate derivative has been applied to red maple (*Acer rubrum*) and loblolly pine (*Pinus taeda*).[58] Either the chlorine–ethanolamine or acid–chlorite method could be used to prepare the holocellulose and the derivative was obtained by heating at 80 °C with phenylisocyanate in pyridine. Higher temperatures caused depolymerization.

Scanning electron microscopy of cellulose xerogels indicated that a membraneous phase existed with individual pores being present which were separated by thin walls.[59] These pores had a short diameter of 50–90 μm and a long diameter of 80–300 μm. The cellulose xerogels were distinct from agarose xerogels but were similar to chitin xerogels which had been chemically prepared from chitosan by *N*-acetylation.

Dye fixation on modified celluloses depended on the pH, temperature, time of thermofixation, type and concentration of the dye used.[60] Diffusion data have been obtained for helium, nitrogen, oxygen, and carbon dioxide gases through membranes of the acetate derivative of cellulose.[61] The thermodynamic data have been evaluated and the mechanism of diffusion was discussed.

To assist with the statistics of cross-linking, the relationship between relative reactivity and accessibility of the hydroxy groups in cellulose have been determined.[62] When epichlorhydrin was used as the cross-linking agent, the reactivity was in the order OH-3 > OH-6 > OH-2.

Considerable interest has been shown in the use of anthraquinone in wood pulping operations. The optimal yield of pulp and pulp viscosity at a given Kappa number was obtained with 0.25 M-NaOH.[63] The rate of delignification was

[56] B. Capon, D. S. Rycroft, and J. W. Thomson, *Carbohydr. Res.*, 1979, **70**, 145.
[57] A. Huebner, M. R. Ladisch, and G. T. Tsao, *Biotechnol. Bioeng.*, 1978, **20**, 1669.
[58] L. R. Schroeder and F. C. Haigh, *TAPPI*, 1979, **62**, 103.
[59] S. Hirano, R. Yamaguchi, N. Matsuda, and H. Takeuchi, *Int. J. Biochem.*, 1978, **9**, 501.
[60] A. Hebeish, A. Waly, E. El-Alfy, N. Abou-Zeid, A. T. El-Aref, and M. H. El-Rafie, *Cell. Chem. Technol.*, 1979, **13**, 327.
[61] V. M. Patel, K. C. Patel, and R. D. Patel, *Makromol. Chem.*, 1978, **179**, 2531.
[62] P. Luby, L. Kuniak, and C. Fanter, *Makromol. Chem.*, 1979, **180**, 2379.
[63] J. Basta and O. Samuelson, *Sven. Papperstidn.*, 1979, **82**, 9.

increased by the addition of anthraquinone (38 mg l^{-1}). Reducing end groups of hydrocelluloses were stabilized more effectively against degradation during alkaline cooking by the presence of anthraquinone 2-sulphate than by anthraquinone itself.[64] This is probably due both to the better solubility and oxidation potential of the sulphate. Neither anthraquinone or naphthaquinone had any effect on the cooking of wood meal with sodium hydrogen carbonate in the presence of oxygen, but the presence of amines did retard the rate.[65] Pretreatment of wood meal in the presence of oxygen with anthraquinone gave an increased yield of pulp on NaOH cooking. The rates of delignification and depolymerization were increased as well as the stabilization of the carbohydrate towards an endwise β-alkoxy elimination reaction. The presence of magnesium hydroxide retarded the rate of depolymerization and delignification.[66] During the pulping of the tracheid cell wall from the sapwood of *Pinus sylvestris* by alkali in the presence of anthraquinone, the rate of delignification of the secondary wall and middle lamella was increased, with the middle lamella being degraded more rapidly than the secondary wall.[67]

Low molecular weight products from the alkaline degradation of cellulose have been identified by g.c.–m.s. of their trifluoroacetate derivatives.[68] Both the residual aqueous phase and the floating oily product were examined and found to contain essentially the same products. These included unsaturated, aliphatic and alicyclic hydrocarbons, aldehydes, ketones, alcohols, and furans. The synthesis of molecules with more than six carbon atoms also occurred.

Reductic acid, separated as its lead salt, has been isolated from the pyrolysis products of cellulose by reaction at 450 °C in the presence of oxygen.[69] Amylose, maltose, and D-glucose also gave reductones. A mass spectrometer with a data system was required to study the products of the direct pyrolysis of cellulose and cellulose which had been treated with a fire-retardant.[70] The amount of products evolved at any point during, or throughout, the entire pyrolysis could be analysed.

A philosophical discussion of cellulose as a vast and renewable resource and potential source of food has been published.[71] The properties of cellulose and the uses to which it can be put, as well as a discussion on cellulases, which can convert the potential value of the cellulose into reality are presented.

An immunochemical approach has been used to study the cellulolytic complex of *Trichoderma reesei* QM91414.[72] *Endo*-(1 → 4)-β-D-glucanase (CM-cellulase) and cellobiohydrolase (Avicelase) activities were separated on diethylaminoethyl-Sepharose CL 6B. The *endo*-(1 → 4)-β-D-glucanase activity

[64] U. Carlson and O. Samuelson, *Sven. Papperstidn.*, 1979, **82**, 48.
[65] K. Abrahamsson and O. Samuelson, *Sven. Papperstidn.*, 1979, **82**, 105.
[66] J. Basta and O. Samuelson, *Sven. Papperstidn.*, 1979, **82**, 337.
[67] H. Bruun, L. Gädda, and M. Storsjö, *TAPPI*, 1979, **62**, 65
[68] P. M. Molton, R. K. Miller, J. M. Donovan, and T. F. Demmitt, *Carbohydr. Res.*, 1979, **75**, 199.
[69] G. Matsui, H. Obata, K. Shikata, T. Tokuyama, and T. Ueno, *J. Agric. Chem. Soc. (Jpn.)*, 1978, **52**, 69.
[70] W. E. Franklin, *Anal. Chem.*, 1979, **51**, 992.
[71] M. P. Coughlan and M. A. Folan, *Int. J. Biochem.*, 1979, **10**, 103.
[72] L. G. Fägerstam and L. G. Pettersson, *FEBS Lett.*, 1979, **97**, 363.

was located in a single peak, but the cellobiohydrolase activity was associated with two peaks. Each peak showed several components on isoelectric focusing.

The digestion of cellulose by anaerobic micro-organisms from a variety of habitats has been investigated.[73] Of the rumen organisms, *Ruminococcus albus* was the most effective, being able to digest 10 g cellulose per litre producing 1.4 g dry cells, 2.8 g acetic acid, and 2.6 g ethanol. A strain from a compost, similar to *Clostridium thermocellum*, digested 4 g cellulose per day giving 0.8 g dry cells, 0.6 g acetic acid, and 0.9 g ethanol.

In studies on the biosynthesis of cell-wall polysaccharides in cultured carrot cells, D-glucose was only incorporated into the neutral sugar residues, particularly cellulose, whereas *myo*-inositol appeared in the uronic acid and pentose residues.[74] An increase in cellulose content was observed during the thickening of the cell walls of parenchymous tissue of *Discorea dumetorum* tubers during storage.[75]

The $^{18}O:^{16}O$ ratio of the cellulose from two sets of wheat plants, grown under conditions similar in all respects except for a large difference in the $^{18}O:^{16}O$ ratio of the carbon dioxide supplied to them, only differed by a small amount.[76] The difference in ratio of the cellulose was similar to the $^{18}O:^{16}O$ ratio of the water present in the plants. These results indicate that the oxygen of the carbon dioxide undergoes complete exchange with the oxygen of the water in the plant during cellulose synthesis and that the $^{18}O:^{16}O$ ratio of the water within the plant is the primary influence on the $^{18}O:^{16}O$ ratio of cellulose in terrestrial plants.

6 Hemicelluloses

Two different L-arabinans have been isolated from azuki beans (*Phaseolus radiatus*) by alkaline extraction and separated by gel chromatography.[77] No other sugar residues were present and, from structural analysis, the polymers were composed of chains of 1,5-linked L-arabinofuranose residues with branch points at O-2 and/or O-3. These branches were composed of non-reducing terminal L-arabinofuranose residues and there were more $(1 \rightarrow 2)$-linkages than $(1 \rightarrow 3)$-linkages. The polysaccharides differed in their molecular weights and solubilities in aqueous ethanol.

An *endo*-L-arabinanase and two α-L-arabinofuranosidases are produced when *Bacillus subtilis* is grown in a medium containing sugar beet arabinan.[78] The *endo*-L-arabinanase was homogeneous by sodium dodecyl sulphate–polyacrylamide gel electrophoresis, had a molecular weight of 3.2×10^4, and a pH optimum of 6.0. It is able to release residues of L-arabinose from suspension-cultured sycamore cell walls. α-L-Arabinofuranosidase activity was determined

[73] M. Taya, Y. Ito, K. Ohmiya, T. Kobayashi, and S. Shimizu, *J. Agric. Chem. Soc. (Jpn.)*, 1978, **52**, 567.
[74] T. Asamizu and A. Nishi, *Planta*, 1979, **146**, 49.
[75] S. Treche and F. Delpeuch, *C.R. Hebd. Seances Acad. Sci., Ser. D*, 1979, **288**, 67.
[76] L. L. Hood, P. J. Coleman, and D. E. Wilhelms, *Science*, 1979, **204**, 51.
[77] S. Ukai, C. Hara, T. Kiho, and K. Hirose, *Chem. Pharm. Bull.*, 1978, **26**, 1729.
[78] L. Weinstein and P. Albersheim, *Plant. Physiol.*, 1979, **63**, 425.

in 306 species of *Actinomycetales*.[79] *Streptomyces* species produced these enzymes at a faster rate than *Ascomycetes* or *Basidiomycetes* species. Activity appeared in the *Streptomyces* species after 2–4 days and, although the pH of the medium was *ca.* 8.5, the pH optimum of the enzyme was *ca.* 6.0.

An L-arabino-D-galactan, in 2% yield, has been isolated from the fruits of *Dillenia indica*.[80] From the results of partial acid hydrolysis and methylation analyses, when 2,6-di-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-galactose, 2,3,4,6-tetra-*O*-methyl-D-galactose, and 2,3,5-tri-*O*-methyl-L-arabinose residues in the molar ratio 7:3:1:8 were identified, it was concluded that the polysaccharide was a (1→4)-β-D-galactan that contained L-arabinofuranose residues linked at O-3 of some of the D-galactose residues in the main chain.

The occurrence, isolation, chemistry, and physico-chemistry of the plant L-arabino-D-galactans and their protein conjugates have been reviewed.[81] The structural relationship between those found in woods, gum exudates, and plant callus cells, and whole tissue is discussed and the nature of the glycoproteins compared with L-arabinose- and D-galactose-containing cell-wall glycoproteins. The possible biological role of these materials is also considered.

Structural studies have been carried out on the L-arabino-D-galactan–protein which is the major component of the mucilage of *Gladiolus* style.[82] It was isolated by affinity chromatography on the D-galactose-binding lectin from the clam coupled to macroporous agarose. The preparation contained 3% protein and a D-galactose : L-arabinose molar ratio of 6 : 1. Carbohydrate and protein were co-eluted even on chromatography in urea buffers. Ultracentrifugation showed the material to be polydisperse in the molecular weight range $1.5–4.0 \times 10^5$. From the results of methylation, partial acid and enzymatic hydrolysis analyses, the structure was confirmed as that of a (1→3)-D-galactan with (1→6)-D-galactose side chains, some of which contain terminal non-reducing α-L-arabinofuranosyl residues. Removal of these residues reduced the solubility of the complex.

A new water-soluble polysaccharide has been isolated from the seeds of *Cassia multifuga*, and the polysaccharide, on methylation analysis, gave residues of 2,3-di-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-galactose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3-di-*O*-methyl-D-mannose, 2-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, and 2,3,4-tri-*O*-methyl-D-xylose in the molar ratio 2:4:4:2:1:2:1, respectively.[83] Partial acid hydrolysis gave the oligosaccharides (4)-(7) and periodate oxidation studies indicated that 32.4% of the monosaccharide residues were end groups. The polysaccharide was therefore considered to have a highly branched structure.

The alkali-extracted gum from oats contained a β-D-glucan as the major component.[84] Some endogenous D-glucanases survived the alkaline treatment so deactivation of these enzymes increased the yield of the β-D-glucan with a corre-

[79] W. Fleck and M. Passarge, *Z. Allgm. Mikrobiol.*, 1978, **18**, 297.
[80] B. K. Srivastava and C. S. Pande, *Indian J. Chem.*, 1978, **16**, 662.
[81] A. E. Clarke, R. L. Anderson, and B. A. Stone, *Phytochemistry*, 1979, **18**, 521.
[82] P. A. Gleeson and A. E. Clarke, *Biochem. J.*, 1979, **181**, 607.
[83] P. Dubey and P. C. Gupta, *Carbohydr. Res.*, 1979, **72**, 151.
[84] P. J. Wood, I. R. Siddiqui, and D. Paton, *Cereal Chem.*, 1978, **55**, 1038.

α-D-Gal*p*-(1 → 6)-D-Gal (4)

α-D-Gal*p*-(1 → 6)-D-Man (5)

β-D-Gal*p*-(1 → 4)-D-Xyl (6)

β-D-Xyl*p*-(1 → 3)-D-Xyl (7)

sponding increase in the viscosity of the extract. The high viscosity gums had a high limiting viscosity number (17–18 $dL\,g^{-1}$) in both dimethylsulphoxide and 7 M urea. Low concentrations of the dyes Calcofluor White M2R, Tinopol CBS-X, or Congo Red caused preferential precipitation of the β-D-glucan from an aqueous solution of oat gum in the presence of protein, pentosan, and starch.[85] The presence of salt suppressed the precipitation while higher temperatures (>25 °C) dissolved the precipitate. The precipitation seemed to be specific for (1 → 4)-β-D-glucose residues.

A (1 → 3)-β-D-glucan occurs in cotton fibres.[86] Its solubility varies with fibre age, a proportion being soluble in cold water, while some cannot even be extracted with alkali. Enzymatic digestion studies indicated that all of the linkages are of the β-configuration, whereas methylation analysis revealed that the predominant linkage is (1 → 3) although a small proportion of (1 → 6)-linkages are also present. The timing of the deposition of the (1 → 3)-β-D-glucan during fibre development coincides closely with the onset of secondary wall-cellulose synthesis.

In the hemicellulosic fraction from rice endosperm cell walls, methylation and Smith degradation analyses as well as cellulase fragmentation studies have confirmed the presence of a mixed (1 → 3), (1 → 4)-β-D-glucan in which blocks of (1 → 3)-β-D-glucose residues occur.[87]

D-Glucose-containing polysaccharides have been isolated from *Colletotrichum trifoli*, *C. destructivuum*, and *C. lindemuthianum*, which elicit browning and phytoalexin production in *Phaseolus vulgaris*.[88] A high molecular weight D-glucan was isolated from each organism and this polysaccharide initiated the browning symptoms at a concentration of 10^{-7} g D-glucose equivalents per hypocotyl.

The molecular weight distribution of sinistrin, a polysaccharide isolated from the bulbs of the red squill and which has been used for renal clearance, covered the range 8.0×10^2 to 1.6×10^4.[89] Viscosity measurements indicated a highly branched molecule in which residues of D-glucose were present at the reducing end of most molecules.

The fine structures of curdlan and paramylon, the (1 → 3)-β-D-glucans from *Alcaligenes faecalis* and *Euglena gracilis*, respectively, have been determined, and it was found that they resembled the native–regenerated cellulose system with curdlan being the regenerated polysaccharide and paramylon the native one.[90]

[85] P. J. Wood and R. G. Fulcher, *Cereal Chem.*, 1978, **55**, 952.
[86] D. Montezinos, C. Kulow, and D. P. Delmer, *Plant Physiol.*, 1979, **63**, 1158.
[87] N. Shibuya and A. Misaki, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 2267.
[88] A. J. Anderson, *Phytopathology*, 1978, **68**, 189.
[89] E. Nitsch, W. Iwanov, and K. Lederer, *Carbohydr. Res.*, 1979, **72**, 1.
[90] R. H. Marchessault and Y. Deslandes, *Carbohydr. Res.*, 1979, **75**, 231.

From the negative birefringence of annealed fibres and positive birefringence of paramylon granules, a tangential disposition of these chains in the granules may be concluded.

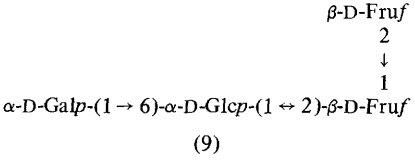
A new α-D-glucan, elaborated by *Elsinoe leucospila*, has a structure based on the repeating sequence (8).[91]

$$\ldots [\rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow]_n$$

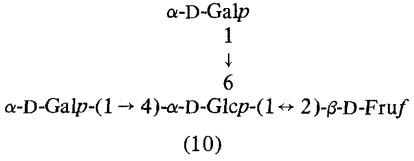
(8)

The D-glucan produced by *Botrytis cinerea*, when grown on grape berries, has a structure based on a backbone of (1 → 3)-β-D-glucopyranose residues with (1 → 6)-β-D-glucopyranose side chains.[92] The D-glucan is not produced from cellulose, but only from simple hexoses.

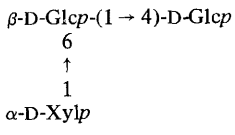
Two novel tetrasaccharides, (9) and (10), have been isolated and characterized from cotton seed.[93]



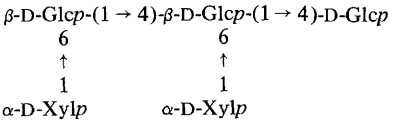
(9)



(10)



(11)



(12)

The oligosaccharides (11) and (12) have been isolated from the degradation products obtained from cellulase treatment of the L-arabino-D-xylo-D-glucan from tobacco leaves.[94] Their structures were determined from the results of methylation analysis both before and after reduction with borohydride ion as well as by ^{1}H and ^{13}C n.m.r. spectroscopies. Present in the hemicellulose fraction from the endosperm cell walls of rice was a D-galacto-D-xylo-D-glucan.[87] This polysaccharide was based on a (1 → 4)-β-D-glucan backbone to which were attached residues of D-xylose and D-galactose at O-6.

The modes of action of β-D-glucanases of *Penicillium notatum* on galacto-xylo-D-glucans from various sources have provided a means of establishing their structure.[95] The main chains are based on (1 → 4)-β-D-glucans although an occasional (1 → 3)-linkage may also be present. The side chains are nearly equimolar

[91] A. Misaki, Y. Tsumuraya, and S. Takaya, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 491.
[92] D. Dubourdieu, B. Fournet, A. Bertrand, and P. Ribéreau-Gayon, *C.R. Hebd. Seances Acad. Sci., Ser. D*, 1978, **286**, 229.
[93] K. Kato, M. Abe, K. Ishiguro, and Y. Ueno, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 293.
[94] M. Mori, S. Eda, and K. Kato, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 145.
[95] A. Dali-Youcef, P. Le Dizet, and J.-E. Courtois, *Phytochemistry*, 1979, **18**, 1949.

amounts of β-D-xylopyranosyl and *O*-β-D-galactopyranosyl-(1 → 2)-β-D-xylopyranosyl units linked at O-6 of some of the main chain D-glucose residues. The regions with disaccharide substitution were inaccessible to the enzyme, supposedly confirming the irregularity of this substitution.

Auxin only induced growth in segments of rice coleoptile when the cell wall was rich in non-cellulosic D-glucose residues.[96] The effect decreased with age and with the total amount of non-cellulosic polysaccharide present. A decrease in the relative amount of non-cellulosic D-glucose and an increase in the content of D-xylose residues were found in the growth cycle of cell walls of rice coleoptiles irrespective of the culture conditions.[97]

D-Galacto-D-mannans, which differed in their average molecular weight, polydispersity, and D-mannose : D-galactose molar ratio, have been isolated from seven legumes.[98] The Mark–Houwink relationship between intrinsic viscosity and average molecular weights held for these D-galacto-D-mannans despite the variation in the D-mannose : D-galactose molar ratio. Estimates were made of the polydispersity of the polysaccharides from both the sedimentation coefficient distribution and also from the diffusion coefficient ratios, D_M/D_A, and the two methods gave similar results.

The apparent viscosities of aqueous solutions of guar and locust bean D-galacto-D-mannans were measured over a range of shear rates both alone and in the presence of D-glucose, sucrose, or D-glucose syrups.[99] For all of the solutions, the variation of the relative viscosity with shear rate fitted a power law equation. The addition of the sugar solutions had no effect on the non-Newtonian behaviour of the gum solutions. The presence of casein had no effect on the relationship between relative viscosity and shear rate for the two D-galacto-D-mannans but its presence had a marked effect on κ-carrageenan.[100]

The location of inter-residue cyclic acetals, formed following the oxidation of guar D-galacto-D-mannan and other heteroglycans by the periodate ion, has been determined by methylation analysis of the oxidized glycans.[101] The formation of cyclic hemiacetals led to the protection of hydroxy-groups during methylation in dimethylsulphoxide and their positions were located by the analysis of the resulting mono- and di-methyl ethers. These derivatives were not observed in either the native glycans or the glycans treated sequentionally with periodate and borohydride ions. Inter-residue hemiacetals were identified in all glycans and were formed between aldehydo-groups at C-2 or C-3 of the oxidized residues and the hydroxy-groups at C-3 or C-2 of the adjacent unoxidized residues. The selective removal of six *O*-substituents from the oxidized residues resulted in a decreased ability of the latter to form inter-residue hemiacetals. An analysis of the types and proportions of the methyl ethers resulting from inter-residue hemiacetal formation could yield further structural information on the glycan.

[96] I. Zarra and Y. Masuda, *Plant Cell. Physiol.*, 1979, **20**, 1125.
[97] I. Zarra and Y. Masuda, *Plant Cell. Physiol.*, 1979, **20**, 1117.
[98] W. R. Sharman, E. L. Richards, and G. N. Malcolm, *Biopolymers*, 1979, **17**, 2817.
[99] A. M. Elfak, G. Pass, and G. O. Phillips, *J. Sci. Food Agric.*, 1979, **30**, 439.
[100] A. M. Elfak, G. Pass, and G. O. Phillips, *J. Sci. Food Agric.*, 1979, **30**, 994.
[101] L. S. Forsberg and J. H. Pazur, *Carbohydr. Res.*, 1979, **75**, 129.

A new D-galacto-D-mannan, isolated from the seeds of *Ipomoea fistulosa* possessed a D-galactose:D-mannose molar ratio of 3:10.[102] The results of methylation and periodate-ion oxidation analyses confirmed that the polysaccharide was based on a main chain of (1→4)-D-mannose residues to which were attached single residues of D-galactose at O-6. Five oligosaccharides were found in the hydrolysis products of the polysaccharide from the seeds of *Caisas-Grandis*.[103] There were three disaccharides, a trisaccharide, and a tetrasaccharide, whose structure was confirmed by methylation, periodate ion oxidation, and Smith degradation analyses. The tri- and tetra-saccharides had the unusual structures (13) and (14).

β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-D-Man

(13)

β-D-Man*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-D-Man

(14)

A number of enzymic complexes containing β-D-mannanase activity were purified by affinity chromatography on D-gluco-D-mannan-derivatized AH-Sepharose.[104] Isoelectric focusing gave multiple protein bands all of which showed β-D-mannanase activity, while a single band was obtained on sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The purified enzymes differed in their physical properties and had wide variations in their ability to hydrolyse highly substituted D-galacto-D-mannans.

A series of papers has appeared on the gelation of Konjac D-mannan. On reaction with either sodium hydroxide or sodium carbonate, an induction period occurred before gelation due to the requirement for deacetylation.[105] The rate of the induced reaction was of the form $V = 4.8[OH^-]^{0.61}$ where V is the reaction rate (min^{-1}).[106] The power value of 0.61 suggested that the rate of deacetylation was proportional to the concentration of hydroxide ion and that gelation of the deacetylated molecules was inhibited by the hydroxide ion. The degree of deactylation, calculated from the Arrhenius equation, increased with a decrease in the concentration of D-mannan and an increase in the concentration of hydroxide ion, but was not influenced by temperature or the nature of the cation (Na^+ or K^+).[107] Deacetylation was an induced reaction which was essential for gelation and occurred after the equilibrium of the deacetylation and peptization reactions by hydroxide ion.

To clarify the macromolecular and chemical properties of Konjac D-mannan, light scattering and viscosity measurements have been carried out on partially

[102] O. C. D. Gupta, R. Gupta, V. P. Srivastava, and P. C. Gupta, *Carbohydr. Res.*, 1979, **73**, 145.
[103] S. Bose and H. C. Srivastava, *Indian J. Chem.*, 1979, **16**, 966.
[104] B. V. McCleary, *Phytochemistry*, 1979, **18**, 757.
[105] K. Maekaji, *J. Agric. Chem. Soc. (Jpn.)*, 1978, **52**, 251.
[106] K. Maekaji, *J. Agric. Chem. Soc. (Jpn.)*, 1978, **52**, 485.
[107] K. Maekaji, *J. Agric. Chem. Soc. (Jpn.)*, 1978, **52**, 513.

methylated derivatives.[108] The weight average molecular weight was found to be $1.0–1.2 \times 10^6$ and it was suggested that a random coil configuration applied. The intrinsic viscosity was related to the molecular weight by a simple mathematical relationship, which meant that the molecular weight could easily be determined from viscosity measurements.

The ^{13}C n.m.r. spectra of D-gluco-D-manno-oligosaccharides have been recorded.[109] Considerable structural heterogeneity was shown by this method, mainly with respect to the sequence of linkages in the oligosaccharides.

Four partially acetylated D-gluco-D-mannans have been isolated from the leaves of *Aloe vera*.[110] The polysaccharides differed in the ratio of D-glucose to D-mannose as well as in their acetyl content. The results of methylation and periodate-ion oxidation analyses showed that the polymers were all linear with only $(1 \rightarrow 4)$ linkages present.

The hemicellulosic fraction of the seed endosperm of groundnuts (*Arachis hypogea*) contains a $(1 \rightarrow 4)$-β-D-gluco-D-mannan.[111] The homogeneity of the polymer was confirmed by electrophoresis, sedimentation, and sugar analysis. Hydrolysis of the methylated polysaccharide yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and -D-mannose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,6-tri-*O*-methyl-D-mannose in the molar ratio 1.6 : 21.2 : 5.6, respectively.

High-resolution electron microscopy of fractions from potassium hydroxide extracts of sprucewood holocellulose showed the presence of D-galacto-D-gluco-D-mannan and 4-*O*-methyl-D-glucurono-D-xylan in the form of microfibrils with different diameters and flexibility.[112] The supramolecular elements of the D-mannans from the 5% and 24% KOH extracts were thin fibrils. The longer D-mannan fibril obtained from the 24% KOH extract had a higher molecular weight. Lignin was sometimes present in the fractions.

Methyl glycosides of D-xylo-oligosaccharides (linear and branched) and 4-*O*-methyl-D-glucuronic acid-containing oligosaccharides, closely reflecting the main structural features of native D-xylans, were studied by thermal analysis and pyrolysis–g.c.[113] The number of monomeric units in the oligomers markedly affected the course of active thermal decomposition. Although the thermal stability increased with increasing numbers of monomeric residues, the ratio of 2-furaldehyde to 2-hydroxy-2-penteno-1,5-lactone produced remained constant. Considerable differences between reducing sugars and glycosides were observed during the course of thermal decomposition and other major differences were also observed when an ionic dehydrating catalyst was added to the pyrolysis sample. The results suggested that the 4-*O*-methyl-D-glucopyranosyl uronic acid linkage was the most thermally stable in the acetylated 4-*O*-methyl-D-glucurono-D-xylan, and acetyl groups do not significantly accelerate the thermal decomposition of the polysaccharide.

[108] N. Kishida, S. Okimasu, and T. Kamata, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 1645.
[109] T. Usui, T. Mizuno, K. Kato, M. Tomoda, and G. Miyajima, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 863.
[110] D. C. Gowda, B. Neelisiddiah, and Y. V. Anjaneyalu, *Carbohydr. Res.*, 1979, **72**, 201.
[111] D. B. Wankhede, R. N. Tharanathan, and M. R. R. Raghavendra Rao, *Carbohydr. Res.*, 1979, **74**, 207.
[112] D. Fengel, *Cellul. Chem. Technol.*, 1979, **13**, 279.
[113] M. Košík, V. Reiser, and P. Kováč, *Carbohydr. Res.*, 1979, **70**, 199.

The kinetics of the hydrolysis of D-xylo-oligosaccharides, ranging from di- to penta-saccharide, in dilute sulphuric acid have been studied.[114] The rate of hydrolysis of the glycosidic bond in 4-*O*-β-D-xylopyranosyl-D-xylose was similar to that of the same bond at the reducing end of the other oligosaccharides and was 1.8 times the rate of each internal bond. The yield of D-xylose from each oligosaccharide could be calculated from this ratio and from the empirical equation which describes the rate constant for the above xylobiose.

The hemicellulose component of the endosperm cell walls of rice is mainly composed of L-arabino-D-xylan.[115] This L-arabino-D-xylan is based on the usual (1 → 4)-β-D-xylan backbone to which are attached L-arabinose residues at O-3 of 78% of the D-xylose residues.[87]

Alkaline extraction of the husks of *Ispaghula* has yielded a highly branched acidic L-arabino-D-xylan.[116] The polysaccharide is unusual in containing both (1 → 3)- and (1 → 4)-D-xylose residues in the main chain with the majority of residues further substituted at O-2 or O-3 with residues of L-arabinose, D-xylose, or D-galactopyranosyluronic acid-(1 → 2)-L-rhamnose. Another acidic polysaccharide, based on a (1 → 4)-D-xylan main chain has been isolated from the seeds of *Ocimum basilicum*.[117] The polysaccharide contained D-xylose, L-arabinose, L-rhamnose, and D-galacturonic acid in the molar ratio 15 : 9 : 7 : 12 and the branch points were present at both O-2 and O-3 of the D-xylose residues in the main chain. Two oligosaccharide sequences, (15) and (16), were isolated from the side chains.

D-Gal*p*A-1 → L-Rha*p*-(1 → 4 or 5)-L-Ara-1 → D-Xyl*p*-(1 → 3)-L-Rha

(15)

α-D-Gal*p*A
1
↓
3 or 2
D-Gal*p*A-(1 → 4)-D-Xyl*p*-(1 → 2 or 3)-L-Ara

(16)

Exhaustive extraction of the milled-wood lignin fraction from beechwood was unable to remove small amounts of carbohydrate.[118] N.m.r. spectroscopy of model compounds as well as the acetylated milled-wood lignin confirmed that the carbohydrate was a xylan and was covalently bonded to the lignin. Both milled-wood lignin and lignin obtained after exhaustive cellulase treatment of Douglas fir and red alder sapwood contained covalently bound lignin.[119] The method of pulping, duration of pulping, type of polysaccharide, and method of isolation all affected the lignin–carbohydrate bond. During studies on the etherification

[114] Y. Kamiyama and Y. Sakai, *Carbohydr. Res.*, 1979, **73**, 151.
[115] N. Shibuya and T. Iwasaki, *Agric. Biol. Chem. (Jpn.)*, 1979, **42**, 2259.
[116] J. F. Kennedy, J. S. Sandhu, and D. A. T. Southgate, *Carbohydr. Res.*, 1979, **75**, 265.
[117] Y. V. Anjaneyalu and D. C. Gowda, *Carbohydr. Res.*, 1979, **75**, 251.
[118] K. Lundquist, R. Simonson, and K. Tingsvik, *Sven. Papperstidn.*, 1979, **82**, 272.
[119] W. G. Glasser and C. A. Barnett, *TAPPI*, 1979, **62**, 101.

reactions of vanillyl and veratryl alcohols, it was observed that lignin–carbohydrate bonds could be formed, subsequent to lignification, through esterification of the *p*-hydroxybenzyl alcohol groups in the lignin polymer.[120]

The carbohydrates present in groundnuts have been reviewed.[121] As well as starch and cellulose, the more abundant polysaccharides were D-gluco-D-mannans and highly-branched D-xylans. Higher levels of non-starchy polysaccharides were present in legume flours than in wheat flour.[122] The distribution of monosaccharide residues and consequently the polysaccharides present varied considerably between the different types of legume although D-xylans were present in each species examined except field bean.

7 Pectins

The effect of the degree of polymerization of D-galacturonic acid oligosaccharides (DP 2–5) and D-galacturonans on their c.d. spectra has been examined in aqueous solution.[123] The chiroptic properties were correlated with the degree of polymerization in terms of the optical superposition of monomeric unit increments. The interpretation of the results led to the conclusion that the conformation of D-galacturonans in solution is close to that of a helical structure.

Above the critical concentration of pectin (0.02 g l^{-1}) in 0.1 M-NaCl, the addition of an increased amount of Ca^{2+} ion resulted in an apparent increase in molecular size owing to the formation of intermolecular cross-linkages.[124] Below the critical concentration, any increase in the apparent molecular weight was slight and due to a stretching of the polymer chains with expansion to a rod-like shape although the amount of cross-linking did increase with time. When the concentration was as low as 0.004 g l^{-1}, no increase in molecular weight was observed and the chains contracted owing to intramolecular cross-linking.

Grapefruit pectic polysaccharides have been shown to contain D-galacturonic acid residues (78%), and amongst the neutral sugar residues found were D-galactose, D-glucose, D-xylose, L-arabinose, and D-mannose of which D-galactose accounted for about half. Oxidation of the complex with D-galactose oxidase followed by reduction with potassium borotritide gave a radio-labelled product in which about 90% of the incorporated tritium was located in D-galactose residues.[125]

D-Galacturonic acid oligosaccharides, with degrees of polymerization from 2 to 9 have been isolated in 54.8% yield by ion-exchange chromatography from an enzymic digest of D-galacturonan.[126] The trisaccharide was the major product. Improved purification could be gained by t.l.c. on cellulose-coated plates.

[120] J. A. Hemmingson, *Aust. J. Chem.*, 1979, **32**, 225.
[121] R. N. Tharanathan, D. B. Wankhede, and M. R. R. Rao, *J. Sci. Food Agric.*, 1979, **30**, 1077.
[122] O. Naivikul and B. L. D'Appolonia, *Cereal Chem.*, 1979, **56**, 45.
[123] S. Bystrický, R. Kohn, and T. Sticzay, *Collect. Czech. Chem. Commun.*, 1979, **44**, 167.
[124] A. Kawabata, S. Sawayama, and T. Kamata, *J. Agric. Chem. Soc. (Jpn.)*, 1979, **53**, 61.
[125] M. M. Baig and J. J. Cerda, *Anal. Biochem.*, 1979, **98**, 429.
[126] Y. K. Liu and B. S. Luh, *J. Chromatogr.*, 1978, **151**, 39.

The recovery and degradation of sugars from potato pectin fractions by hydrolysis with 2 M trifluoroacetic acid has indicated that between 61 and 96% of each sugar residue was recovered, depending on the monosaccharide residue.[127] Lower recoveries were obtained from intact onion cell walls.

Various commercial pectins and pectinesterase-treated pectins have been fractionated according to their degree of esterification in the range 6–70%.[128] These fractions were tested for their ability to destabilize the 'cloud' in orange juice. The juice was clarified by fractions with degrees of esterification between 6 and 14%, but not at all by those with degrees of esterification greater than 21%.

A new dialdose dianhydride derivative has been obtained from an acid hydrolysate of the water-soluble pectic fraction of wobaku wood by successive treatment with methanolic hydrogen chloride and acetic anhydride–pyridine.[129] The compound was the 1,2 : 1′,2′ dianhydride of 3,4-di-*O*-acetyl-β-L-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-D-galactopyranosyluronate. This is the first report of a dianhydride containing two different sugar residues.

The role of secondary alcoholic groups in the degradation of D-galacturonan by the *endo*-D-galacturonanase of *Aspergillus niger* has been investigated using *O*-acetyl derivatives of pectic acid.[130] The initial rate depended on the numbers of free hydroxy-groups on C-2 and C-3, with substitution giving an enhanced value for the Michaelis constant. The rate of degradation decreased in the presence of acetyl groups according to the relationship for competitive inhibition, and it was concluded that the lowered rate of reaction and extent of degradation was due to the lowered affinity of the enzyme for the substrate caused by modifying the groups which are required for enzyme–substrate binding.

From the rates of hydrolysis of D-galacturonic acid oligosaccharides from pure heptamer to trimer, as well as mixed higher oligomers, it was suggested that the active site of the *endo*-D-galacturonanase from *Aspergillus niger* could accommodate at least seven units.[131] The release of end units from larger oligomers also suggested that the interaction was with four sugar units from the non-reducing side of the bond to be hydrolysed and three sugar units from the reducing side.

The content of D-galacturonic acid, D-galactose, and L-arabinose residues of tomato cell walls declined during ripening.[132] A water-soluble D-galacturonan found in isolated walls probably arises from the action of D-galacturonanase *in vivo*. This polysaccharide and that from the walls of mature green fruit, after incubation with galacturonanase, contained fewer neutral sugar residues than intact walls. The loss of D-galactose and L-arabinose residues on ripening was not related to the loss of D-galacturonan.

[127] A. T. Mankarios, C. F. G. Jones, M. C. Jarvis, D. R. Threlfall, and J. Friend, *Phytochemistry*, 1979, **18**, 419.
[128] R. A. Baker, *J. Agric. Food Chem.*, 1979, **27**, 1387.
[129] T. Fujiwara and K. Arai, *Carbohydr. Res.*, 1979, **69**, 97.
[130] L. Rexová-Benková, M. Mračková, O. Luknár, and R. Kohn, *Collect. Czech. Chem. Commun.*, 1977, **42**, 3204.
[131] J. H. McClendon, *Phytochemistry*, 1979, **18**, 765.
[132] K. C. Gross and S. J. Wallner, *Plant Physiol.*, 1979, **63**, 117.

The predominant change that took place in the cell walls of apples and pears on ripening was a dissolution of the middle lamella.[133] This change could be brought about in unripe pears by application of solutions of D-galacturonanase and cellulase, while the change in unripe apples could be initiated by D-galacturonanase alone. Ripe strawberries, artificially inoculated with various fungi, contained *endo*-D-galacturonanase and pectinesterase activities in their culture filtrates, whereas uninfected strawberries had *exo*-D-galacturonanase and pectinesterase activities.[134] No other pectin-degrading enzymes were detected in either sound or infected berries. Since the disintegration could be substantially reduced by inhibition of the D-galacturonanase activity, it was concluded that the D-galacturonanases secreted by the fungi were primarily responsible for the breakdown in sulphited strawberries.

Sodium pectate can be cross-linked with epichlorhydrin in an alkaline medium.[135] Different degrees of cross-linking can be obtained by varying the concentration of epichlorhydrin. These materials could be used for the separation of the two pectinesterase isoenzymes from oranges. Both components had a 7–8 fold increase in activity and could saponify 93% of the pectin.

The D-galacturonanase activity secreted by an aggressive strain of *Colletotrichum lagenarium* can be separated into two proteins.[136] These proteins differ in their molecular weight and in their mode of action on the hydrolysis of D-galacturonans.

The anti-inflammatory drugs benzydamine and ketoprofen formed coprecipitates with pectin.[137] A Langmuir-type of binding isotherm was obtained on equilibrium dialysis and this slow dissolution of the precipitate suggested that binding may help to reduce the adverse effects of these drugs to the stomach on oral administration.

8 Acidic Gums

The acidic polysaccharide from peach gum and macromolecular products obtained from it by mild acidic hydrolysis have been characterized by their molecular weight and composition.[138] The single-ion activity coefficient of Ca^{2+} ions bound to the polysaccharide and to the corresponding aldobiouronic acids was measured and the c.d. spectra of both K^+ and Ca^{2+} salts were recorded. Ca^{2+} ions were bound to the carboxy-groups by electrostatic bonds in disperse solutions. There is a close arrangement of uronic acid residues which does not undergo alteration with a stepwise degradation of the macromolecules. It was concluded that the main chain of the polysaccharide is substituted in long segments with uronic acid residues at least on each second D-galactose unit. The

[133] R. Benarie, N. Kislev, and C. Frenkel, *Plant Physiol.*, 1979, **64**, 197.
[134] S. A. Archer, *J. Sci. Food Agric.*, 1979, **30**, 692.
[135] F. M. Rombouts, A. K. Wissenburg, and W. Pilnik, *J. Chromatogr.*, 1979, **168**, 151.
[136] J.-P. Barthe and A.-M. Rème-Delga, *C.R. Hebd. Seances Acad. Sci., Ser. D*, 1979, **288**, 63.
[137] Y. Takahashi, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, 1978, **26**, 3836.
[138] R. Kohn, J. Rosik, J. Kubala, and A. Malovikova, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2517.

enzymic hydrolysis of peach gum polysaccharide has yielded the oligosaccharides (17)–(20), whose structures were confirmed by g.c.–m.s. of their partially methylated alditol acetates.[139,140]

β-D-Gal*p*-(1 → 6)-D-Gal (17)

α-D-Man*p*-(1 → 3)-D-Gal (18)

β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 6)-D-Gal (19)

β-D-Gal*p*-(1 → 6)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 6)-D-Gal (20)

The Hofman degradation has been applied to mangle gum, and a variety of fragments were obtained on borohydride ion reduction and acidic hydrolysis of the product.[141] The same fragments were obtained on the Barry degradation of the Hofman-degraded product. The results supported the earlier conclusions about the structure of the gum.

An analytical study of eight *Acacia* gums (four each from the *Gummiferae* and *Vulgares* series) has confirmed the main chemotaxonomic differences between the species that have been previously reported.[142]

During the methylation analysis of *Combretum nigricans* gum by the Hakomori procedure, losses of D-galacturonic acid and L-rhamnose residues were observed, which did not occur in the Haworth or a modified-Haworth procedure.[143] However, during the Haworth procedure, *C. nigricans* gum was degraded to a greater extent than the gum from *Acacia seyal*.

A new acidic polysaccharide, isolated from the bark of *Pterospermum acerifoli*, contains D-galacturonic acid, D-galactose, and L-rhamnose in the molar ratio 5 : 3 : 3.[144] The oligosaccharide (21) was isolated from a partial acid hydrolysate and its structure was confirmed by methylation and periodate oxidation analyses.

The polysaccharide of Junsai mucilage (*Brasenia schreberi*) contained D-galactose, D-glucuronic acid, L-fucose, D-mannose, L-rhamnose, D-xylose, and L-arabinose in decreasing order of magnitude.[145] The actual composition varied

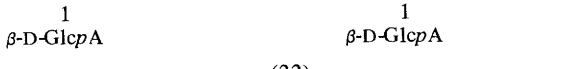
α-D-Gal*p*A-(1 → 2)-L-Rha*p*-(1 → 4)-D-Gal (21)

[139] A. Kardošová, J. Rosik, and J. Kubala, *Collect. Czech. Chem. Commun.*, 1978, **43**, 3428.
[140] A. Kardošová, J. Rosik, J. Kubala, and V. Kováčik, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2250.
[141] M. Sarkar and C. V. N. Rao, *Indian J. Chem.*, 1978, **16**, 369.
[142] D. M. W. Anderson and J. G. K. Farquhar, *Phytochemistry*, 1979, **18**, 609.
[143] D. M. W. Anderson and A. Stefani, *Anal. Chim. Acta*, 1979, **105**, 147.
[144] P. Bishnoi and P. C. Gupta, *J. Chem. Soc., Perkin Trans. 2*, 1979, 1680.
[145] M. Kakuta and A. Misaki, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 993.

between different samples. From the results of methylation analysis, the precise structure was difficult to establish but it was found to be highly branched.

Partial acid hydrolysis of paniculatan, the mucous polysaccharide from the inner bark of *Hydrangea paniculata*, led to the isolation of five acidic oligosaccharides.[146] The structure of each oligosaccharide was established by methylation analysis although each has previously been isolated from other sources. A possible structure of the polysaccharide was proposed. The polysaccharide had a molecular weight of 5.46×10^5.[147] There were *O*-acetyl groups present on 40% of the L-rhamnose residues. Complete deacetylation of the polysaccharide in 0.1 M alkali led to a reduced molecular weight of 4.2×10^4.

A mucilage, Abelmoschus-mucilage M, isolated from the roots of *Abelmoschus maniliot*, appeared homogeneous. The polysaccharide contained L-rhamnose, D-galacturonic acid, and D-glucuronic acid in the molar ratio 1.2 : 1.0 : 1.0 and had a molecular weight of 2.53×10^4.[148] Reduction of the carboxy-groups followed by methylation analysis suggested that the main chain comprised (1 → 2)-L-rhamnose and (1 → 4)-D-galacturonic acid residues. These latter residues were substituted at O-3 by D-glucuronic acid residues. These results were confirmed by the isolation, *inter alia*, of the oligosaccharide (22) from a partial acid hydrolysate of the polysaccharide.[149]



(22)

The mucous polysaccharide from *Lilium longiflorum* was homogeneous, contained D-mannose and D-glucose residues in the molar ratio 5 : 2 and had a molecular weight of 2.63×10^5.[150] From the results of methylation and periodate ion oxidation analyses and the products of partial acid hydrolysis studies, it was concluded that the main chain was composed of a (1 → 4)-β-D-glycan with ten residues per non-reducing terminal group. The branch points were located on O-2 or O-3 of the D-mannose residues. The polysaccharide isolated from the mucous of *L. maculatum* was qualitatively similar to that from *L. longiflorum*.[151]

The water-soluble extract from *Opuntia ficus-indica* contained a number of polysaccharides.[152] Four acidic components were present, each of which contained residues of D-galacturonic acid, L-arabinose, L-rhamnose, D-galactose, and D-xylose in a variety of proportions. Other studies on the same mucilage

[146] M. Tomoda and N. Satoh, *Chem. Pharm. Bull.*, 1977, **25**, 2910.
[147] M. Tomoda, N. Satoh, and G. Masumura, *Chem. Pharm. Bull.*, 1978, **26**, 3215.
[148] M. Tomoda and Y. Suzuki, *Chem. Pharm. Bull.*, 1977, **25**, 3061.
[149] M. Tomoda, Y. Suzuki, and N. Satoh, *Chem. Pharm. Bull.*, 1979, **27**, 1651.
[150] M. Tomoda, N. Satoh, and C. Ohmori, *Chem. Pharm. Bull.*, 1978, **26**, 2768.
[151] M. Tomoda and C. Odaka, *Chem. Pharm. Bull.*, 1978, **26**, 3373.
[152] B. S. Paulsen and P. S. Lund, *Phytochemistry*, 1979, **18**, 569.

suggested that the core contained D-galacturonic acid, L-rhamnose, and D-galactose residues with the pentose sugars being present on the periphery.[153]

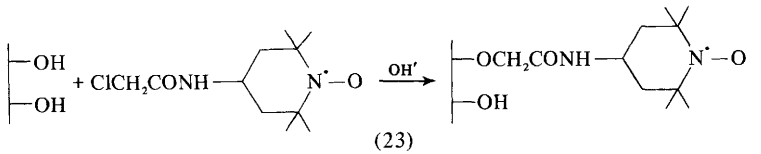
The mucilages in callus cultures of higher plants have been investigated particularly with regard to their monosaccharide composition.[154]

In studies on the effect of polysaccharides as ice-cream stabilizers, the gum from *Khaya grandifoliola* was most effective.[155] The viscosity effect was low but the polysaccharide had a wide range of desirable effects.

9 Algal Polysaccharides

The vacuum-u.v. c.d. spectra of solid films of agarose have been measured and show a positive band near 180 nm and a larger negative band about 152 nm.[156] The positive band remains accessible in aqueous solution and has been used to characterize the changes in molecular conformation and interactions during sol–gel transitions. The temperature profile of the vacuum-u.v. c.d. spectra shows sharp discontinuous changes around melting and setting points of the gel. These changes were interpreted in terms of co-operative intermolecular associations through double helices and also show pronounced hysteresis, which was considered as evidence of helix–helix aggregation.

The rates of tumbling and exchange of free nitroxides in aqueous solutions are unaffected by high concentrations of agarose gels.[157] Low-level covalent attachment of this spin-label to the agarose gels by means of a stable acetamido-ether linkage (23) caused considerable diminution of their rate of reorientation. Dissolution of this material to form a gel, and melting and setting of the gel, caused further changes in the e.s.r. spectrum.



(23)

Studies on the interaction between agarose and the *endo*-D-galacturonanase from *Aspergillus niger* has shown it to be of an ion-exchange nature which did not involve biospecific interactions.[158]

The sequence and composition of uronate residues in intact alginate samples have been determined by high resolution ^{1}H n.m.r. spectroscopy.[159] Problems encountered owing to the viscosity of the samples were overcome by controlled and slight depolymerization before n.m.r. at 90 °C. The D-mannuronic acid : L-guluronic acid molar ratio was obtained from the intensities of the signals from

[153] D. McGarvie and H. Parolis, *Carbohydr. Res.*, 1979, **69**, 171.
[154] C. K. Kokate and S. S. Radwan, *Phytochemistry*, 1979, **18**, 662.
[155] J. I. L. Cottrell, G. Pass, and G. O. Phillips, *J. Sci. Food Agric.*, 1979, **30**, 1085.
[156] J. N. Liang, E. S. Stevens, E. R. Morris, and D. A. Rees, *Biopolymers*, 1979, **18**, 327.
[157] J. D. Aplin and L. D. Hall, *Carbohydr. Res.*, 1979, **75**, 17.
[158] J.-F. Thibault, *C.R. Hebd. Seances Acad. Sci., Ser. D*, 1978, **287**, 1433.
[159] H. Grasdalen, B. Larsen, and O. Smidsrød, *Carbohydr. Res.*, 1979, **68**, 23.

the anomeric protons. A sequence-dependent deshielding of H-5 of the L-guluronic acid residues made it possible to determine the fractions of the four possible doublets of nearest neighbours along the chain. The results were consistent with ^{13}C n.m.r. spectroscopic data. Significant deviation from results obtained by chemical analysis appeared only in samples containing a large fraction of mixed doublets.

An enzyme preparation from the marine brown alga *Pelvetia canaliculata* contained both alginate 5-epimerase and alginate lyase activities.[160] Ca^{2+} ions activated both enzymes, but Mn^{2+} ions only inhibited lyase activity. Increasing concentrations of substrate D-mannuronanate removed the inhibition of lyase activity by Mn^{2+} ions.

The storage D-glucans of the algae *Chlorella* and *Prototheca* species are identical in that they consist of a linear polymer, akin to amylose, and a branched amylopectin component.[161] The branched D-glucans from the above two species are markedly different from that formed by the alga *Cyanidium caldarium* which exists in hot springs. The amylopectin component of the hot springs alga was more highly branched.

The osmotic coefficients of Na^+, K^+, and Ca^{2+} counter-ions have been determined in aqueous solutions of κ-, ι- and λ-carrageenans and the results were correlated with those calculated from the limiting law of Manning.[162] An ordered secondary structure exists in κ- and ι-carrageenans, whose stability can be discussed as a function of temperature, ionic strength, and nature of the counter-ion.

The viscosity and other physical properties of two carrageenan–water gels have been compared with those of an agar gel.[163] The carregeenan gels had a smaller viscosity gradient and a higher viscosity at a given concentration. The structure of the gels were also different as revealed by microscopic analysis; those from the carrageenans were coarse and spongy, while that from agar contained closely intertwined fibrous molecules.

Carrageenans from male and female gametophytic plants of *Rhodoglossum californicum*, *Chondrus crispus*, *Gigartina pistillata*, *Irideae cordata*, and another unidentified *Gigartina* species have been fractionated into KCl-soluble and -insoluble components.[164] An anti-(κ-carrageenan) antibody was used to analyse the components immunochemically. The KCl-insoluble fractions were highly reactive κ-types whereas the soluble fractions were less reactive. These latter fractions showed anti-(λ-reactivity). These data suggest that the KCl-soluble carrageenans contain either λ- or μ-carrageenan as no increase in immunological activity to anti-(κ-carrageenan) antibody was observed on treatment with alkali.

A χ-carrageenanase from *Pseudomonas carrageenovora* has been isolated and purified and its identity has been confirmed by an inspection of the products

[160] J. Madgwick, A. Haug, and B. Larsen, *Botanica Marina*, 1978, **21**, 1.
[161] J. F. Fredrick, *Phytochemistry*, 1979, **18**, 1823.
[162] M. Rinaudo, A. Karimian, and M. Milas, *Biopolymers*, 1979, **18**, 1673.
[163] S. Sawayama, A. Kawabata, M. Okabe, and J. Sugi, *J. Agric. Chem. Soc. (Jpn.)*, 1978, **52**, 409.
[164] V. L. Di Ninno and E. L. McCandless, *Carbohydr. Res.*, 1979, **72**, 157.

obtained by its enzymic action.[165] The limit digest was neo-carrabiose 4-sulphate.

Addition of carrageenan to flours from soft wheats gave a product that was more akin to the flour from hard wheats in its bread-making quality.[166]

The non-Newtonian behaviour of the viscosity of κ-carrageenan solutions at various shear rates increased on addition of D-glucose or sucrose.[167] The results were explained in terms of changes in the solute–solute interactions.

The structure of the laminaran from *Eisenia bicyclis* has been determined by methylation and periodate ion oxidation analyses as well as by ^{1}H and ^{13}C n.m.r. spectroscopies.[168] The polysaccharide possessed a branched structure with both (1→3)- and (1→6)-β-D-glucopyranose residues being present in the ratio of 3 : 2.

(1→3)-β-D-Glucans were specific activators of the prophenoloxidase from crayfish serum.[169] Laminaran chains, terminated at the reducing end with a D-glucose residue (G-type chains) were more potent activators than the laminaran chains terminated at the reducing end with a D-mannitol residue (M-type chains).

The extracellular polysaccharide of *Porphyridium cruentum* contained D-xylose, D-glucose, D- and L-galactose, 3-*O*-methyl-D-xylose, 3(or 4)-*O*-methyl-D-galactose, D-glucuronic acid, 2-*O*-methyl-D-glucuronic acid, and a 2-*O*-methyl hexose in the molar ratio 3 : 1 : 2.5 : 0.13 : 0.13 : 0.8 : 0.2 : 0.13, whereas that from *P. aerugineum* contained the first six sugar residues in the molar ratio 1.7 : 1.0 : 1.1 : 0.3 : 0.6 : 0.5, respectively, as well as 2,4-di-*O*-methyl-D-galactose.[170] Each polysaccharide contained, in addition, 10% half-ester sulphate and *ca.* 0.5% protein. Structural studies showed, *inter alia*, that the D-glucuronic acid residues were linked to O-3 of the D-galactose residues and that 2-*O*-methyl-D-glucuronic acid residues were linked to O-4 of the L-galactose residues. The polysaccharide from *P. cruentum* had a molecular weight of 4×10^6, and that from *P. aerugineum* had a molecular weight of 5×10^6.

The sulphated heteropolysaccharide from the brown alga, *Dyctyota dichotoma*, contains residues of D-glucuronic acid, D-galactose, D-mannose, D-xylose, and L-fucose.[171] Evidence, from chemical analyses, was provided that the polysaccharide is partially sulphated and that the sugar residues are (1→4)-linked except L-fucose which is (1→2)-linked. The carbohydrates of the brown seaweed *Padina pavonia* contained a sulphated heteropolysaccharide that contained residues of D-glucuronic acid, L-fucose, D-glucose, D-mannose, and D-xylose as well as a D-mannan–protein complex.[172]

The red alga *Laurencia spectabilis* contains a glycoproteineous species which, on purification, was found to contain 92% carbohydrate and 8% protein.[173] The

[165] M. W. McLean and F. B. Williamson, *Eur. J. Biochem.*, 1979, **93**, 553.
[166] F. R. Huebner and J. S. Wall, *Cereal Chem.*, 1979, **56**, 68.
[167] A. M. Elfak, G. Pass, and G. O. Phillips, *J. Sci. Food Agric.*, 1979, **30**, 724.
[168] T. Usui, T. Toryama, and T. Mizuno, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 603.
[169] K. Söderhäll and T. Unestam, *Can. J. Microbiol.*, 1979, **25**, 406.
[170] E. Percival and R. A. J. Foyle, *Carbohydr. Res.*, 1979, **72**, 165.
[171] M. M. D. Hussein, S. T. Fouad, and A. F. Abdel-Fattah, *Carbohydr. Res.*, 1979, **72**, 177.
[172] A. F. Abdel-Fattah and M. Edrees, *Phytochemistry*, 1977, **16**, 939.
[173] G. J. Court and I. E. P. Taylor, *Phytochemistry*, 1979, **18**, 411.

carbohydrate portion mainly consisted of D-galactose and uronic acid residues and there was no ester sulphate present.

The polysaccharides from the heterocyst and spore envelopes of the blue-green alga *Anabaena cylindrica* have a backbone, which consists of the repeating units (24) to which are attached residues of D-glucose, D-xylose, D-galactose, and D-mannose as side chains.[174] The main chain contained between 128 and 150 sugar residues. After treatment of the polymer with various glycoside hydrolases, the degraded product gave, on treatment with *endo*-(1 → 3)-β-D-glucanase, the trisaccharide (25) and the pentasaccharide (26).

—β-D-Man*p*-(1 → 3)-β-D-Glc*p*-(1 → 3)-β-D-Glc*p*-(1 → 3)-β-D-Glc*p*-(1 → 3)—

(24)

β-D-Man*p*-(1 → 3)-β-D-Glc
4
↑
1
D-Glc*p*

(25)

β-D-Glc*p*-(1 → 3)-β-D-Man*p*-(1 → 3)-β-D-Glc
4 4
↑ ↑
1 1
D-Xyl*p* D-Glc*p*

(26)

The use of ^{13}C n.m.r. spectroscopy, and methylation and Smith degradation analyses have confirmed the structure of the (1 → 2)-β-D-mannopyranan from *Crithidia deanei*.[175] It was assumed that the polymer arises from the protozoon and not a bacterial endosymbiont, since a related organism *C. fasciculata* also contains a D-mannan. The protozoon *Herpetomonas samuelpessoai* also contains a (1 → 2)-β-D-mannopyranan as well as a branched chain D-glucurono-D-xylan.[176] Since injection of cells or flagellar suspensions from *H. samuelpessoai* cause protection against infection with *Trypanosoma cruzi*, the immunological protection may be due to these polysaccharides or glycoproteins. The surface coat of *T. congolense* has been characterized as a glycoprotein.[177]

At high N:P ratios in the growth medium, the marine diatom *Chactoceros affinis* produces large amounts of extracellular polysaccharide, whereas *Skeletonema costatum*, grown under similar conditions, produces only very small amounts of extracellular polysaccharide.[178]

[174] L. Cardemil and C. P. Wolk, *J. Biol. Chem.*, 1979, **254**, 736.
[175] J. O. Previato, L. Mendonça-Previato, and P. A. J. Gorin, *Carbohydr. Res.*, 1979, **70**, 172.
[176] L. Mendonça-Previato, P. A. J. Gorin, and J. O. Previato, *Biochemistry*, 1979, **18**, 149.
[177] L. Rovis, A. F. Barbet, and R. O. Williams, *Nature (London)*, 1978, **271**, 654.
[178] S. Myklestad, *J. Exp. Mar. Biol. Ecol.*, 1977, **29**, 161.

4

Microbial Polysaccharides

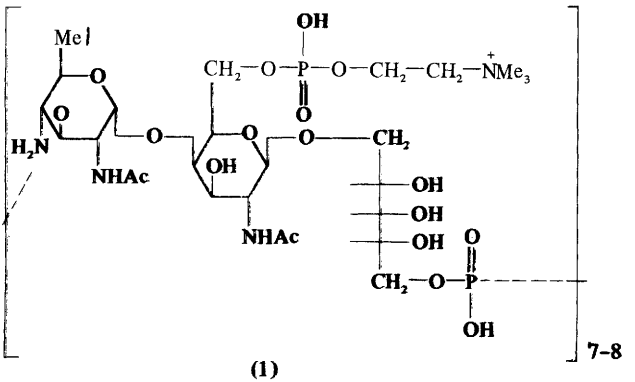
BY R. J. STURGEON

1 Teichoic Acids

The ^{13}C n.m.r. spectra of a number of ribitol teichoic acids substituted with glycosyl residues, and of their dephosphorylated repeating units, have been recorded.[1] Identical spectra are obtained for teichoic acids substituted with 2-*O*- and 4-*O*-glycosyl residues, so it is not possible to differentiate between these positional isomers using this technique.

Investigation of the cell-wall composition and associated properties of strains of *Staphylococcus aureus*, which are resistant to methicillin, has not revealed evidence for any unusual wall polymers.[2] The ribitol teichoic acid isolated from *Staphylococcus hyicus* contains 2-acetamido-2-deoxy-D-glucosyl residues.[3] Interaction of the teichoic acid with concanavalin A, and its susceptibility to α-, but not to β-D-2-acetamido-2-deoxyglucosidase, showed that the amino-sugar is in the α-configuration.

The cell walls of *Streptococcus pneumoniae* contain a teichoic acid (C polysaccharide) composed of 2-acetamido-2-deoxy-D-galactose, 2-acetamido-4-amino-2,4,6-trideoxy-D-hexose, ribitol phosphate, choline phosphate, and D-glucose.[4] From the results of periodate oxidation and methylation analysis, together with an examination of the ^{13}C n.m.r. spectra, the structure (1) has been proposed. It is speculated that the C-3 hydroxy-group of the chain terminal diaminotrideoxy-D-hexose unit is glycosylated with isomaltose.



(1)

[1] E. Tarelli and J. Coley, *Carbohydr. Res.*, 1979, **75**, 31.
[2] B. J. Wilkinson, K. J. Dorian, and L. D. Sabath, *J. Bacteriol.*, 1978, **136**, 976.
[3] A. Osland, A. Grov, and P. Oeding, *Acta Pathol. Microbiol. Scand.*, 1979, **87B**, 165.
[4] I. R. Poxton, E. Tarelli, and J. Baddiley, *Biochem. J.*, 1978, **175**, 1033.

Pneumococcal cell-wall teichoic acid, rather than peptidoglycan, is considered to be responsible for the activation of the alternative complement pathway.[5] This contrasts with group A streptococci in which the greatest activity was found associated with a heat labile protein of the cell membrane.

The addition of a pulse of phosphate to a phosphate-limited chemostat culture of *Bacillus subtilis* has been found to lead to the synthesis of teichoic acid and the consequent development of bacterial ability to bind phage SP50 within about one generation line after the addition of the pulse.[6] The time taken for newly synthesized wall material to become maximally exposed at the surface of the cells was directly related to their growth rate and corresponded to about three-quarters of a generation time. The newly synthesized material does not become exposed exclusively at any single highly localized zone, but appears near the middle and along the cylindrical length of the bacteria at newly formed polar caps. The regulation of teichoic acid synthesis during phosphate limitation in *B. subtilis* has been shown to be due to the inactivation of the enzyme catalysing the formation of P^1-2-acetamido-2-deoxy-D-glucopyranosyl P^2-polyisoprenyl pyrophosphate.[7] Conditions have been described for the continuous culture of a derivative of *Staphylococcus aureus* H, with L-cysteine as the sole amino-acid and under various nutrient limitations.[8] The proportion of ribitol teichoic acid present in the wall and the extent to which it is substituted with 2-acetamido-2-deoxy-D-glucose varies on growth of the organism under different conditions, as does the extent of cross-linking of the peptidoglycan. Neither the derivative nor the original strain H produced teichuronic acid when grown under phosphate limitation.

Phosphate-repressible phosphodiesterases, isolated from *Bacillus subtilis*, have been reported to be responsible for the depolymerization of teichoic acids.[9]

Lipoteichoic acid from *Streptococcus mutans* has been purified by a combination of gel filtration, hydrophobic chromatography, and adsorption on to phospholipid vesicles.[10]

Plasma membranes from *Staphylococcus aureus*, purified on columns of immobilized human immunoglobulin G, have been characterized as lipoteichoic acids, composed of D-glucosylglycerol teichoic acid containing ester-linked L-alanine with pentadecanoic acid as the major fatty acid.[11] Growth conditions for the production of extracellular lipoteichoic acid by *Streptococcus mutans* have been investigated.[12] The greatest levels were produced by slow-growing organisms. A lipoteichoic acid fraction and a presumed deacylated form were

[5] J. A. Winkelstein and A. Tomasz, *J. Immunol.*, 1978, **120**, 174.
[6] A. J. Anderson, R. S. Green, A. J. Sturman, and A. R. Archibald, *J. Bacteriol.*, 1978, **136**, 886.
[7] L. Glaser and A. Loewy, *J. Biol. Chem.*, 1979, **254**, 2184.
[8] B. C. Dobson and A. R. Archibald, *Arch. Mikrobiol.*, 1978, **119**, 295.
[9] W. D. Grant, *FEMS Microbiol. Lett.*, 1979, **6**, 301.
[10] L. J. Silvestri, R. A. Craig, L. O. Ingram, E. M. Hoffman, and A. S. Bleiweis, *Infect. Immun.*, 1978, **22**, 107.
[11] P. Aasjord and A. Grov, *Acta Pathol. Microbiol. Scand.*, 1978, **86**, 131.
[12] N. A. Jacques, L. Hardy, K. W. Knox, and A. J. Wicken, *Infect. Immun.*, 1979, **25**, 75.

separated by column chromatography. The effect of growth rate on lipid and lipoteichoic acid composition in *Streptococcus faecium* has been reported.[13]

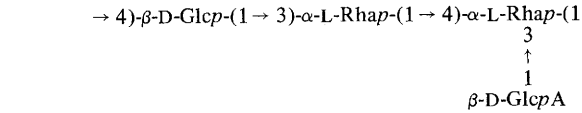
Lipoteichoic acids from *Bacillus subtilis* 168 and its *gta* mutants have been purified and shown to contain glycerol and phosphorus in equimolar amounts, together with D-glucose and fatty acids.[14] The D-glucosyl residues have the α-configuration on this partially glycosylated molecule. Antibodies to the lipoteichoic acid were directed against poly(glycerolphosphate) and not against the D-glucosyl residues.

Human erythrocyte membranes possess a single population of specific binding sites for lipoteichoic acids of group A streptococci, located almost exclusively at the surface of the membranes.[15]

Parotid saliva has been examined for antibodies (immunoglobulin A) reacting with lipoteichoic acids and peptidoglycans of whole cells of *Streptococcus mutans* using an enzyme-linked immunoadsorbent assay.[16] The significance of lipoteichoic acid may vary between different subjects and for different serotypes of *S. mutans*.

D-Glucosylphosphatidylglycerol, but not di-D-glucosyldiacylglycerol (the head group of lipoteichoic acids), has been shown to inhibit the extracellular autolysin of *Staphylococcus aureus*.[12] This suggests a requirement for the phosphate esters of the lipoteichoic acid molecule, together with the fatty acid ester group, for inhibition.[17]

Scanning electron microscopy has revealed concentric circular structures on newly exposed surfaces of the walls and isolated cross-walls of *Staphylococcus epidermidis*.[18] Extraction of the walls with trichloracetic acid removes most of the phosphorus and about half of the 2-acetamido-2-deoxy-D-glucosyl residues, and destroys this type of structure. Hence, teichoic acid and possibly peptidoglycan (since these polymers are covalently linked) may be arranged circularly on the surface of the wall.



(2)

The primary structure (2) of the teichuronic acid of *Bacillus megaterium* M46 has been elucidated, using a combination of partial acidic hydrolysis, enzymic hydrolysis, periodate oxidation, and Smith degradation studies.[19] The

[13] D. Carson, R. A. Pieringer, and L. Daneo-Moore, *Biochim. Biophys. Acta*, 1979, **575**, 225.
[14] S. Thaniyavarn, R. J. Doyle, H. L. T. Mobley, and U. N. Streips, *Carbohydr. Res.*, 1979, **73**, 203.
[15] T. H. Chiang, M. L. Alkan, and E. H. Beachey, *Infect. Immun.*, 1979, **26**, 316.
[16] D. Bratthall, A. Carlen, K. W. Knox, and A. J. Wicken, *Acta Pathol. Microbiol. Scand.*, 1979, **87C**, 251.
[17] H. Suginaka, M. Shimatani, Y. Ohno, and I. Yano, *FEMS Microbiol. Lett.*, 1979, **5**, 353.
[18] K. Amako and A. Umeda, *J. Gen. Microbiol.*, 1979, **113**, 421.
[19] R. J. Ivatt and C. Gilvarg, *J. Biol. Chem.*, 1979, **254**, 2759.

teichuronic acids of *B. megaterium* are unusual in that they resemble capsular polysaccharides in their size (mol. wt. 5×10^5) and composition, yet are structurally and functionally integral components of the cell wall.

The proton-decoupled ^{15}N n.m.r. spectra of ^{15}N-labelled intact cells, isolated cell walls, and cell-wall digests have been described.[20] Each of five Gram-positive bacteria studied displayed a unique set of cell wall ^{15}N resonances, which reflected variations in the primary structure of peptidoglycans and in the amounts of teichoic acid and teichuronic acid in the cell wall. Specific assignments of cell wall ^{15}N resonances assigned to teichoic acid D-alanyl residues, teichuronic acid and acetamido groups, and to peptidoglycan acetamido, peptide amido and free amino-groups, have been made on the basis of a combination of specific isotope labelling and dilution experiments, and from the results of cell wall fractionation.

In *Micrococcus varians* all three glycerol phosphate residues in the linkage unit are derived from CDP-glycerol and are therefore not transferred from a lipoteichoic acid carried.[21]

2 Peptidoglycans

A range of helical conformations for the glycan chains of bacterial peptidoglycans has been proposed on the basis of *X*-ray diffraction studies of cell walls and peptidoglycans from several bacterial species.[22] These studies support the view that the peptide conformation has proportions of the residues in the helical and hydrogen-bonded β-sheet conformations.[23] The balance between the two conformations is presumably related to the mean separation of the glycan chains, which varies with the water content by a factor of approximately two.

The pentapeptide chains of peptidoglycans have a sequence of amino-acids terminated with D-Ala-D-Ala at positions 4 and 5. Possible conformations of D-Ala-D-Ala and its analogues L-Ala-D-Ala, D-Ala-L-Ala, D-butyryl-L-Ala, D-Ala-D-butyric acid, D-Val-D-Ala, and D-Ala-D-Val have been analysed by theoretical methods.[24] From theoretical studies it is predicted that L-Ala or D-Val at the 4 or 5 position of the pentapeptide group of the peptidoglycan will reduce the cross-linking in peptidoglycan biosynthesis, whereas the effect of D-butyric acid will be marked at the 4 position and moderate at the 5 position. This is in agreement with experimental results.

Possible conformations of the disaccharide–peptide sub-units of the peptidoglycans of *Staphylococcus aureus* and *Micrococcus luteus* have been studied using an energy-minimization procedure.[25] Contrary to earlier reports the favoured conformation of the disaccharide 2-acetamido-2-deoxy-β-D-glucosyl-

[20] A. Lapidot and C. S. Irving, *Biochemistry*, 1979, **18**, 704.
[21] F. M. Roberts, H. A. I. McArthur, I. C. Hancock, and J. Baddiley, *FEBS Lett.*, 1979, **97**, 211.
[22] R. E. Burge, A. G. Fowler, and D. A. Reaveley, *J. Mol. Biol.*, 1977, **117**, 927.
[23] R. E. Burge, R. Adams, H. H. M. Balyuzi, and D. A. Reaveley, *J. Mol. Biol.*, 1979, **117**, 955.
[24] R. Virudachalam and V. S. R. Rao, *Biopolymers*, 1978, **17**, 2751.
[25] R. Virudachalam and V. S. R. Rao, *Biopolymers*, 1979, **18**, 571.

(1 → 4)-*N*-acetylmuramic acid is different from that of cellulose or chitin. Three types of conformation, two compact and one extended, are postulated with all three being stabilized by intramolecular hydrogen bonds. Two different models were proposed for the three-dimensional arrangement of peptidoglycan in the cell wall.

X-Ray crystallographic studies of the binding of the trisaccharide β-MurNAc-(1 → 4)-β-D-Glc*p*NAc-(1 → 4)-MurNAc to sub-sites B, C, and D of lysozyme indicate no distortion of sugar residues at sub-site D.[26]

The specificity of antisera to oligoglycylalbumin and to L-alanyl-tetraglycyl-albumin has been used to demonstrate serological differentiation of some bacterial strains on the basis of the primary structure of their cell-wall peptidoglycan.[27]

Numerous synthetic glycopeptide analogues of peptidoglycans have been analysed by m.s.[28] The immunoadjuvant activity of a number of synthetic glycopeptide analogues of peptidoglycans appears to be dependent on the type of substitution of the γ-carboxy-group of the D-isoglutamine residue.[29]

The peptidoglycan of *Acetobacterium woodii* contains D-ornithyl residues, which function as interpeptide bridges between the γ-carboxy-group of D-glutamic acid and the carboxy-group of the terminal D-alanyl residue of an adjacent peptide sub-unit.[30] The usual D-alanyl residue in position 1 of the peptide sub-unit is replaced by an L-seryl residue. The primary site of action of the antibiotic amphomycin has been demonstrated to involve inhibition of the phospho-*N*-acetylmuramylpentapeptide translocase, an enzyme involved in the first step of a lipid cycle of peptidoglycan synthesis.[31]

Although *Bacillus cereus* 569 is known to be resistant to lysis by lysozyme, because of the presence in the peptidoglycan of residues of deacetylated 2-amino-2-deoxy-D-glucose, the action of lysozyme on isolated cell walls has been reported to release some free reducing groups, indicating a limited breakage of the polysaccharide chains of the peptidoglycan.[32] The action of the enzyme modifies the peptidoglycan and makes it more susceptible to autolysin(s) and also enhances the rate of septum separation.

A carboxypeptidase–transpeptidase, which incorporates free diaminopimelic acid into previously formed cell walls, has been isolated from *Bacillus megaterium*.[33] Membranes from *B. megaterium* have been solubilized and then reconstituted in a form capable of synthesizing peptidoglycan.[34] Using this reconstituted system, an assay has been developed for a factor necessary for

[26] J. A. Kelly, A. R. Sielecki, B. D. Sykes, M. N. G. James, and D. C. Phillips, *Nature (London)*, 1979, **282**, 875.
[27] P. H. Seidl and K. H. Schleifer, *Mol. Immunol.*, 1979, **16**, 385.
[28] E. Nebelin and B. C. Das, *FEBS Lett.*, 1979, **107**, 254.
[29] K. Mašek, M. Zadral, J. Ježek, and V. Krchňák, *Experientia*, 1979, **35**, 1397.
[30] O. Kandler and S. Schoberth, *Arch. Mikrobiol.*, 1979, **120**, 181.
[31] H. Tanaka, R. Oiwa, S. Matsukura, and S. Omura, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 902.
[32] D. Westmacott and H. R. Perkins, *J. Gen. Microbiol.*, 1979, **115**, 1.
[33] H. Das Gupta and D. P. Fan, *J. Biol. Chem.*, 1979, **254**, 5672.
[34] A. Taku and D. P. Fan, *J. Biol. Chem.*, 1979, **254**, 3991.

peptidoglycan synthesis. The factor may be a polymerase, which assembles the disaccharide–pentapeptide sub-units of the peptidoglycan into polymers.

A peptidoglycan complex, possessing a linear, non-crosslinked structure composed of disaccharide–pentapeptide units, has been isolated from a penicillin-treated mutant of *Brevibacterium divaricatum* requiring biotin for growth.[35] The peptidoglycan fragments appear to be synthesized after incubation of the cells with penicillin.

A peptidoglycan, of different type from that of other species of *Cellulomonas*, has been isolated from *C. cartalyticum*.[36] L-Lysine replaces ornithine and the interpeptide bridge consists of D-aspartyl-D-serine. The same peptidoglycan type has been identified in *Arthrobacter luteus*, *Brevibacterium liticum*, and *Corynebacterium manihot*.

The peptidoglycan isolated from the photosynthetic organelles of *Cyanophora paradoxa* contains 2-acetamido-2-deoxy-D-glucose, *N*-acetylmuramic acid, L-alanine, L-glutamic acid, and diaminopimelic acid (1 : 1 : 1.6 : 1 : 1) and may be present as a lipoprotein–peptidoglycan complex.[37] Close similarities to the structures of other Gram-negative bacteria and cyanobacteria were observed.

A preferential orientation of the glycan chains perpendicular to the axis of the cell has been observed in an electron microscopic study of the sacculus of *Escherichia coli*.[38] Selective partial hydrolysis of inter-peptide bridges was achieved with an *endo*-peptidase.

The regulation of peptidoglycan synthesis in *rel* A^+ and *rel* A^- strains of *Escherichia coli* during diauxic growth on D-glucose and lactose has been reported.[39] In both strains, the biosynthesis of peptidoglycan, lipid intermediates, and nucleotide precursors abruptly halted at the onset of diauxic lag from D-glucose to lactose, with the concomitant accumulation of guanosine 5′-diphosphate and guanosine 3′-diphosphate. This is consistent with the proposal that the accumulation is involved in the inhibition of the incorporation of disaccharide–pentapeptide into peptidoglycan and in regulating nucleotide precursor synthesis.

Lanthionine, a monosulphated analogue of diaminopimelic acid, has been isolated from the peptidoglycan of *Fusobacterium nucleatum*, which contained all the other normal constituents, with the exception of diaminopimelic acid.[40]

Glycopeptides have been isolated from the cell walls of *Lactobacillus plantarum*.[41] Arthritogenic and immunoadjuvant activities are associated with peptidoglycan fractions having an average glycan chain length of more than five disaccharide units. The peptidoglycan of *L. vaccinostercus* contains *meso*-diaminopimelic acid, an unusual component in heterofermentative lactobacilli.[42]

[35] D. Keglević, B. Ladešić, J. Tomašić, Z. Valinger, and R. Naumski, *Biochim. Biophys. Acta*, 1979, **585**, 273.
[36] E. Stackebrandt, F. Fiedler, and O. Kandler, *Arch. Mikrobiol.*, 1978, **117**, 115.
[37] A. Aitken and R. Y. Stanier, *J. Gen. Microbiol.*, 1979, **112**, 219.
[38] R. W. H. Verwer, N. Nanninga, W. Keck, and U. Schwarz, *J. Bacteriol.*, 1978, **136**, 723.
[39] E. E. Ishiguro, *Can. J. Microbiol.*, 1978, **25**, 1206.
[40] E. W. Vasstrand, T. Hofstad, C. Endresen, and H. B. Jensen, *Infect. Immun.*, 1979, **25**, 775.
[41] T. Koga, K. Maeda, K. Onoue, K. Kato, and S. Kotani, *Molec. Immunol.*, 1979, **16**, 153.
[42] S. Okada, Y. Suzuki, and M. Kozaki, *J. Gen. Appl. Microbiol.*, 1979, **25**, 215.

The turnover of cell walls during myxospore formation in *Myxococcus xanthus* has been studied by measuring the incorporation and release of *meso*-diamino[^{14}C]pimelic acid.[43] Following addition of glycerol to induce myxospore formation, there is a greatly increased rate of incorporation, resulting in substantial replacement of existing wall with newly synthesized wall.

None of the representatives of four genera of methanogenic bacteria grown in pure culture was found to contain typical peptidoglycan as a cell-wall polymer.[44] However, each of the four genera was found to contain different cell-wall polymers, indicating a very early divergence, not only of the methanogens from all other prokaryotes, but also of the various genera of the methanogens from one another.

The appearance of soluble peptidoglycan fragments and concurrent turnover of the glycan and peptide region of peptidoglycan from growing Gonococci indicate that both glycan-splitting and peptidoglycan amidase activities are involved.[45] If released during gonococcal infections, these or similar soluble peptidoglycan fragments might influence the consequences of host–gonococcus interactions.

Muramic acid has been detected in the cell walls of the anomalous alga *Prochloron* species, which although prokaryotic, shares several factors with the Chlorophyta.[46]

Lipoprotein, covalently linked to peptidoglycan, has been found absent from exponential phase cultures, but present in stationary phase cultures of *Proteus mirabilis*.[47] The overall peptidoglycan structure did not change during transition from logarithmic to stationary growth. The covalently linked lipoprotein is not as essential for cell-wall stability in this organism as it apparently is in *Escherichia coli*. It is possible that there is a higher degree of hydrophobicity in the rigid layer, owing to the presence of *O*-acetyl groups on some of the muramic acid residues, and that this suffices for the association of the outer-membrane components to the peptidoglycan later in exponentially growing cells.

Comparison of the structural features in normal peptidoglycan and the peptidoglycan of the unstable sphaeroplast L-form of *Proteus mirabilis* confirms earlier reports of a continuation of the peptide cross-linking reaction during L-form growth and peptidoglycan synthesis in the presence of benzylpenicillin.[48] However, the peptidoglycans formed, in the presence or absence of the antibiotic, were found to differ in the degree of *O*-acetylation of their muramic acid residues.

The mechanism of peptidoglycan synthesis in *Pseudomonas aeruginosa* appears to be similar to that of *Escherichia coli*, although evidence has suggested that in the former the DD-carboxypeptidase and transpeptidase systems may have somewhat different specificity restrictions from those in *E. coli*.[49] Some

[43] H. Dawson and M. V. Jones, *J. Gen. Microbiol.*, 1979, **112**, 143.
[44] O. Kandler, *Naturwissenschaften*, 1979, **66**, 95.
[45] R. S. Rosenthal, *Infec. Immun.*, 1979, **24**, 869.
[46] D. J. W. Moriarty, *Arch. Mikrobiol.*, 1979, **120**, 191.
[47] J. Gmeiner, *Arch. Mikrobiol.*, 1979, **121**, 177.
[48] H. H. Martin and J. Gmeiner, *Eur. J. Biochem.*, 1979, **95**, 487.
[49] D. Mirelman and Y. Nuchamowitz, *Eur. J. Biochem.*, 1979, **94**, 541.

similarities in the effects of β-lactam antibiotics on the biosynthesis of peptidoglycan in the two bacteria were also observed.[50]

A combined ^{15}N- and ^{13}C-n.m.r. spectroscopic study of the chain segmental motion of the peptidoglycan-pentaglycine chain of [^{15}N]glycine- and [2-^{13}C]-glycine-labelled *Staphylococcus aureus* cells and isolated cell walls has been reported.[51] Main-chain motions of the pentaglycine bridge have been interpreted in terms of flexible pentaglycine chains attached to immobile glycan strands, whose packing arrangements can vary as a function of temperature and growth conditions. Although cell surface components of *Staphylococcus aureus*, including teichoic acid, peptidoglycan, and protein A, have all been shown to be capable of activating the classical complement pathway, only peptidoglycan consumed complement *via* the alternative pathway.[52] Using a quantitative immunofluorescence assay, the peptidoglycan was shown to bind to the C-3 component of complement in both pathways and in the absence of immunoglobulins G and A antibodies.

Peptidoglycan synthesis in *Staphylococcus aureus* is inhibited by 3-amino-3-deoxy-D-glucose.[53] The primary mode of action appears to be the prevention of formation of 2-amino-2-deoxy-D-glucose 6-phosphate from D-fructose 6-phosphate.

The physical state of the membrane lipid in *Staphylococcus aureus*, as measured by fluorescence and e.s.r. spectroscopy, has been shown to affect the activity of the phospho-*N*-acetylmuramylpentapeptide transferase (E.C. 2.7.8.13).[54] A correction to conclusions from earlier studies on the biosynthesis of the peptidoglycan of *Staphylococcus aureus*[55] has been reported.[56]

The occurrence of 2-amino-2-deoxy-D-glucosyl residues in the cell-wall peptidoglycan of group A *Streptococcus pyogenes* type 4 has been confirmed.[57]

The ultrastructural localization of peptidoglycan in streptococcal cell walls has been explored using an indirect immunoferritin technique.[58] The ferritin particles were bound predominantly to filamentous structures, which were produced both from surfaces of isolated peptidoglycan fragments and from isolated walls. The results contradict proposed models of the streptococcal wall in which the peptidoglycan is thought to form the innermost layer of the wall. The alternative view is of a mosaic structure in which peptidoglycan forms a network of peptidoglycan–polysaccharide complexes.

[50] D. Mirelman and Y. Nuchamowitz, *Eur. J. Biochem.*, 1979, **94**, 549.
[51] A. Lapidot and C. S. Irving, *Biochemistry*, 1979, **18**, 1788.
[52] H. A. Verbrugh, W. C. Van Dijk, R. Peters, M. E. Van Der Tol, and J. Verhoef, *Immunology*, 1979, **37**, 615.
[53] H. Tanaka, S. Shimizu, R. Oiwa, Y. Iwai, and S. Omura, *J. Biochem. (Jpn.)*, 1979, **86**, 155.
[54] W. A. Weppner and F. C. Neuhaus, *Biochim. Biophys. Acta*, 1979, **552**, 418.
[55] W. A. Weppner and F. C. Neuhaus, *J. Biol. Chem.*, 1978, **253**, 472.
[56] W. A. Weppner and F. C. Neuhaus, *J. Biol. Chem.*, 1979, **254**, 569.
[57] R. S. Rosenberg and R. W. Wheat, *Microbios Lett.*, 1978, **7**, 117.
[58] M. Wagner and M. Ryc, *J. Gen. Microbiol.*, 1978, **108**, 283.

3 Lipopolysaccharides

A radioimmunoassay for bacterial lipopolysaccharide has been developed.[59] The lipopolysaccharide could be derivatized and radiolabelled with ^{125}I without apparent loss in its biophysical, immunological, or biological activities.

From studies of the interactions between lipopolysaccharide and phosphatidyl ethanolamine in molecular monolayers at air–water interfaces, it was concluded that each lipopolysaccharide molecule is surrounded by approximately sixteen phosphatidyl ethanolamine molecules.[60] The hydrocarbon chains of lipopolysaccharides can undergo a reversible thermal order–disorder transition, as shown by thermal phase-transition studies.[61] The ordered conformation of lipopolysaccharides interpreted from wide angle *X*-ray studies is considered less developed than that in normal phospholipid bilayers.

Pulse-chase studies with D-galactose on suspensions of *Alteromonas haloplanktis* have been used to follow the progress of newly synthesized lipopolysaccharide through the three outermost layers of the cell wall.[62] The lipopolysaccharide moves through the periplasmic space, through the outer membrane, and spreads over the outer surface of the organism; its presence in each of the three layers was confirmed by polyacrylamide gel electrophoresis.[63]

The lipopolysaccharide of *Fusobacterium nucleatum* has been coupled to horse radish peroxidase and used for locating the lipopolysaccharide binding sites on erythrocytes.[64] Fluorescence analysis of dansylated derivatives of various lipopolysaccharides has been used to define the nature of metal binding sites of lipopolysaccharides.[65] A high affinity binding site for Ca^{2+} and Mg^{2+}, which appears to be formed by the 3-deoxy-D-*manno*-octulosonate trisaccharide unit, is involved in the assembly and maintenance of the normal structural organization of the outer membrane. Lipopolysaccharide–protein interactions, as well as protein–protein interactions, have been shown to be significant in maintaining ghost structures in strains of *Escherichia coli*.[66]

The recognition of unopsonized bacteria by mouse peritoneal macrophages is thought to involve capsular- and lipo-polysaccharides.[67]

Bacterial lipopolysaccharides have been degraded by enzymic preparations from *Helix pomatia*, resulting in an extensive loss of anticomplementary activity.[68] The lipid A moiety of the lipopolysaccharide appeared to be the main site of attack with little evidence for the degradation of the polysaccharide component.

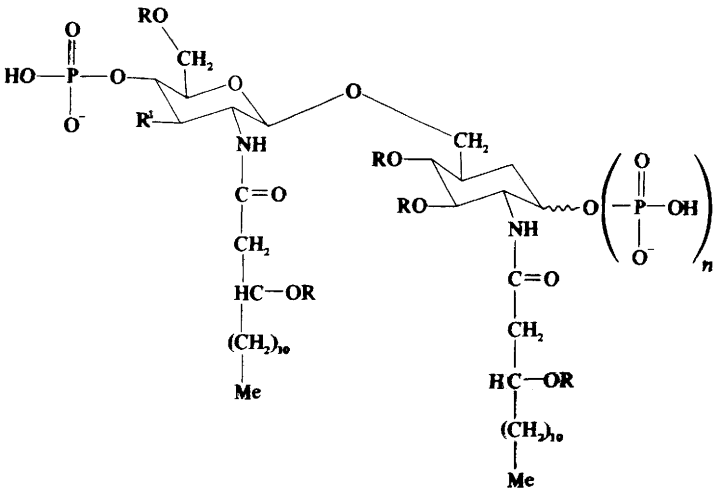
[59] J. A. Gutowski and D. M. Jacobs, *Immunol. Commun.*, 1979, **8**, 347.
[60] V. A. Fried and L. I. Rothfield, *Biochim. Biophys. Acta*, 1978, **514**, 69.
[61] G. Emmerling, U. Hemming, and T. Gulik-Krzywicki, *Eur. J. Biochem.*, 1977, **78**, 503.
[62] R. A. MacLeod and M. Goodbody, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 326.
[63] J. M. Di Rienzo, C. F. Deneke, and R. A. MacLeod, *J. Bacteriol.*, 1978, **136**, 148.
[64] H. Nygren and G. Dahlen, *J. Immunol. Methods*, 1979, **25**, 355.
[65] M. Schindler and M. J. Osborn, *Biochemistry*, 1979, **18**, 4425.
[66] R. T. Irvin, J. Lam, and J. W. Costerton, *Can. J. Microbiol.*, 1979, **25**, 436.
[67] N. B. Freimer, H. M. Ogmundsdottir, C. C. Blackwell, I. W. Sutherland, L. Graham, and D. M. Weir, *Acta Pathol. Microbiol. Scand.*, 1978, **86B**, 53.
[68] J. N. Saddler, R. Parton, and A. C. Wardlaw, *Experimentia*, 1979, **35**, 494.

Two fatty acyl amidases, amidases I and II, purified from *Dictyostelium discoideum*, act upon the disaccharide (3, R = R^1 = H, n = 1), which has been isolated from the lipopolysaccharide of a heptose-less mutant of *Escherichia coli* K-12.[69] Amidase I removes the 3-hydroxymyristoyl unit, which is esterified to the amino-group of the 2-amino-2-deoxy-D-glucosyl residue adjacent to the C-1 phosphate of the disaccharide. The product is then acted upon by amidase II, which removes the remaining 3-hydroxymyristoyl unit from the amino-group on the distal 2-amino-2-deoxy-D-glucosyl residue.

Amino-sugars derived from bacterial lipopolysaccharides have been identified by g.l.c.–electron impact-m.s. and g.l.c.–chemical ionization-m.s.[70]

The absence of amino-sugars from the lipopolysaccharide (mol. wt. 8.5×10^4) isolated from *Acholeplasma oculi* has been reported.[71] Neutral sugars (L-fucose, D-galactose, D-glucose, 1:12:3) were detected but the lipopolysaccharide elicited no antibody response in rabbits.

Treatment of the lipopolysaccharide of a mutant of *Escherichia coli*, containing no heptose, with alkali released two phosphorylated components, LPS I and II, both consisting of a substituted 2-amino-2-deoxy-D-glucosyl disaccharide, but differing in the nature of the phosphate groups present at the glycosyl position.[72] Their structures (3) were determined. LPS I contained two phospho-



n = 1 or 2
R = fatty acyl residues or H (acyl groups are myristic, lauric, and β-hydroxymyristic acids)
R^1 = 3 moles of 3-deoxy-D-*manno*-2-octulosonic acid

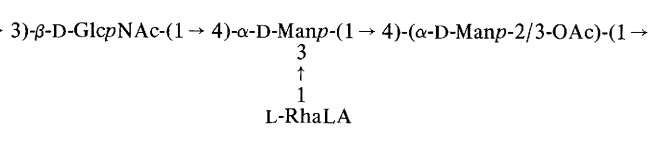
(3)

[69] M. R. Rosner, R. C. Verret, and H. G. Khorana, *J. Biol. Chem.*, 1979, **254**, 5926.
[70] D. V. Bowser, R. G. Teece, and S. M. Somani, *Biomed. Mass Spectr.*, 1978, **5**, 627.
[71] A. J. N. Al-Shammari and P. F. Smith, *J. Bacteriol.*, 1979, **139**, 356.
[72] M. R. Rosner, J. Y. Tang, I. Barzilay, and H. G. Khorana, *J. Biol. Chem.*, 1979, **254**, 5906.

monoester groups, one at the 4′-hydroxy-position and one at the glycosyl position. The remaining hydroxy-groups were substituted with lauryl, myristoyl, and 3-hydroxymyristoyl groups although the exact assignment of the different fatty acyl groups to specific hydroxy-groups in the amino-sugar rinks was not determined. Further information on the nature of the phosphate linkages has been obtained from ^{31}P n.m.r. spectroscopic studies on the original lipopolysaccharide and on two products of alkaline treatment.[73]

The structure of the K-13 antigenic polysaccharide of *Escherichia coli* consists of a repeating sequence of 3-linked D-ribofuranosyl residues and 7-linked 3-deoxy-D-*manno*-octulosonic acid (KDO) residues, approximately 50% of which are *O*-acetylated at C-4 or C-5.[74] The serological specificity of this polysaccharide is expressed through KDO and its *O*-acetylated derivative.

The polysaccharide chain of the cell wall lipopolysaccharide of *Escherichia coli* O58 contains D-mannose, 2-acetamido-2-deoxy-D-mannose, 3-*O*-[(1′*R*)-carboxyethyl)]-L-rhamnose (rhamnolytic acid), and *O*-acetyl groups in the molar ratios of 2:1:1:1.[75] Structural investigations show that this polysaccharide, which contains a substituted trisaccharide repeating unit, has an identical structure (4) to that of *Shigella dysenteriae* type 5.



L-RhaLA = rhamnolytic acid

(4)

The O8- and O9-specific lipopolysaccharides of *Escherichia coli* lose their serological activity upon mild acid treatment to liberate their polysaccharide (mannan) moieties.[76] Restoration of the activity was achieved by substitution of one or two stearoyl groups per polysaccharide chain. The D-mannans obtained by *in vitro* biosynthesis were serologically active only when bound to the membrane-associated hydrophobic carrier.

After removal of lipid A from the lipopolysaccharide of *Fusobacterium nucleatum*, the resultant high molecular weight fraction, which was devoid of phosphate and 3-deoxy-D-*manno*-octulosonic acid, was composed of L-*glycero*-D-*manno*-heptosyl-, 2-amino-2-deoxy-D-glucosyl-, and D-glucosyl-residues.[77] Serological evidence has shown that this fraction contains the O-antigenic side-chains of the lipopolysaccharide.

Alterations in the cell envelope of *Pseudomonas aeruginosa* resistant to polymyxin are associated with the alteration of the outer membrane through a

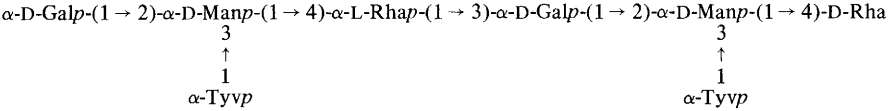
[73] M. R. Rosner, H. G. Khorana, and A. C. Satterthwait, *J. Biol. Chem.*, 1979, **254**, 5918.
[74] W. F. Vann and K. Jann, *Infect. Immun.*, 1979, **25**, 85.
[75] B. A. Dmitrev, Y. A. Knirel, N. K. Kochetkov, B. Jann, and K. Jann, *Eur. J. Biochem.*, 1977, **79**, 111.
[76] K. Jann, B. Jann, V. Winter, and C. Wolf-Ullisch, *J. Gen. Microbiol.*, 1979, **110**, 203.
[77] T. Hofstad and G. Frederiksen, *Eur. J. Biochem.*, 1979, **94**, 59.

diminution of lipopolysaccharide and outer membrane proteins.[78] Analysis of the lipopolysaccharides of *P. aeruginosa* PAO and three mutants selected for resistance to a virulent lipopolysaccharide-specific bacteriophage shows alterations in both O-specific side-chains and core regions.[79] In a typical lipopolysaccharide, isolated from the walls of *P. cepacia*, phosphate, L-rhamnose, D-glucose, heptose, and amino-sugars were found but there was no deoxyoctulosonic acid.[80] The lipid A component of the lipopolysaccharide of *P. diminuta* has been reported to contain 2,3-diamino-2,3-dideoxy-D-glucose instead of 2-amino-2-deoxy-D-glucose.[81] Some *Rhodopseudomonas* species also contain this unusual amino-sugar.

The lipopolysaccharides of three strains each of *Rhizobium leguminosarum*, *R. phaseoli*, and *R. trifolii* have been purified and partially characterized.[82] The composition and immunodominant structures of the lipopolysaccharides were found to vary as much among strains of a single *Rhizobium* species as among the different species of *Rhizobium*. No obvious correlation between the nodulation group to which a *Rhizobium* species belongs and the chemical composition or immunochemistry of the lipopolysaccharides was observed. A component of the lipopolysaccharide of *R. trifolii*, previously designated as 'heptose I' is not D-*glycero*-D-*manno*-heptose but a 3-*O*-methylheptose.[83] Heterogeneity of the lipopolysaccharide moiety of the intact rhizobium of free-living and bacterial forms of *R. leguminosarum* has been reported.[84]

A low molecular weight polypeptide exhibiting high affinity for lipopolysaccharides has been isolated from the cell surface of a heptose-less mutant of *Salmonella minnesota*.[85] Antiserum adsorption studies reveal that this protein also represents a common antigen of the Enterobacteriaceae.

Antibodies against the synthetic disaccharide 3-*O*-α-abequosyl-L-rhamnose, representative of *Salmonella* O-antigen 8, have been used in the diagnosis of *Salmonella* bacteria by indirect immunofluorescence and co-agglutination using sensitized protein A-containing staphylococci.[86] The octasaccharide (5), the



Tyv*p* = tyvelopyranosyl residue

(5)

[78] H. E. Gilleland and R. D. Lyle, *J. Bacteriol.*, 1979, **138**, 839.
[79] A. M. Kropinski, L. C. Chan, and F. H. Milazzo, *Can. J. Microbiol.*, 1979, **25**, 390.
[80] J. M. Manniello, H. Heymann, and F. W. Adair, *J. Gen. Microbiol.*, 1979, **112**, 397.
[81] S. G. Wilkinson and D. P. Taylor, *J. Gen. Microbiol.*, 1978, **109**, 367.
[82] R. W. Carlson, R. E. Sanders, C. Napoli, and P. Albersheim, *Plant Physiol.*, 1978, **62**, 912.
[83] R. Russa and Z. Lorkiewicz, *FEMS Microbiol. Lett.*, 1979, **6**, 71.
[84] K. Planqué, J. J. van Nierop, A. Burgers, and S. G. Wilkinson, *J. Gen. Microbiol.*, 1979, **110**, 151.
[85] R. Geyer, C. Galanos, O. Westphal, and J. R. Golecki, *Eur. J. Biochem.*, 1979, **98**, 27.
[86] B. Svenungsson and A. A. Lindberg, *Acta Pathol. Microbiol. Scand.*, 1979, **87B**, 29.

synthesized disaccharide methyl 3-*O*-α-tyvelopyranosyl-β-D-mannopyranoside, and methyl α-tyveloside inhibit the precipitation of *S. typhi* T2 alkali-treated lipopolysaccharide by O-factor 9 antibodies.[87] From the relative effectiveness of the oligosaccharides in inhibiting the precipitation reaction it appears that the 3-*O*-α-tyvelopyranosyl-D-mannopyranosyl structure is immunodominant in the *Salmonella* O9 antigen. 3-*O*-α-Tyvelopyranosyl-D-mannose coupled to bovine serum albumin elicited O-antibodies of higher specificity than those obtained by adsorption of antibacterial immune serum.[88]

The polysaccharide moiety of the lipopolysaccharide of *Schizothrix calcicola* contains 2-amino-2-deoxy-D-glucose, D-galactose, D-glucose, D-mannose, D-xylose, and L-rhamnose.[89] In contrast to many enterobacterial lipopolysaccharides, the major fatty acid component has been identified as 3-hydroxypalmitic acid and not 3-hydroxymyristic acid.

A lipopolysaccharide has been isolated from *Shigella flexneri*, type 2, and some of its structural aspects have been elucidated.[90]

The specific polysaccharide isolated from the lipopolysaccharide of *Shigella newcastle* (*S. flexneri*, type 6) contains L-rhamnose, 2-acetamido-2-deoxy-D-galactose, D-galacturonic acid, and *O*-acetyl groups (in the molar ratio 2 : 1 : 1 : 1).[91] On the basis of ^{1}H and ^{13}C n.m.r. spectroscopy, methylation analysis, partial acid hydrolysis, and chromium oxide oxidation, the structure (6) has been assigned. Both structural and immunochemical evidence indicate that the lipopolysaccharide from *Shigella newcastle* belongs to the 'non-classical' type of somatic antigens with acidic O-specific polysaccharide chains.

The hexose region of the core polysaccharides of lipopolysaccharides isolated from *Shigella flexneri* and some *Escherichia coli* species have a common structure (7).[92]

$$[\rightarrow 4)\text{-}\alpha\text{-Gal}p\text{A-}(1\rightarrow 3)\text{-}\alpha\text{-Gal}p\text{NAc-}(1\rightarrow 2)\text{-}\alpha\text{-L-Rha}p\,3\,\text{Ac-}(1\rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow]_n$$

(6)

$$\alpha\text{-D-Glc}p\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{-}(1\rightarrow$$

$$\begin{array}{c} 3 \\ \uparrow \\ 1 \\ \alpha\text{-D-Glc}p\text{NAc} \end{array}$$

(7)

[87] H. Jörbeck, H. E. Carlsson, S. B. Svenson, A. A. Lindberg, G. Alfredsson, P. J. Garegg, S. Svensson, and N. K. Wallin, *Int. Arch. Allergy Appl. Immunol.*, 1979, **58**, 11.

[88] H. E. Carlsson and A. A. Lindberg, *Acta Pathol. Microbiol. Scand.*, 1978, **86C**, 237.

[89] G. Keleti, J. L. Sykora, E. C. Lippy, and M. A. Shapiro, *Appl. Envir. Microbiol.*, 1979, **38**, 471.

[90] B. A. Dmitriev, V. L. L'Vov, I. L. Hofman, and N. K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1977, **26**, 1484.

[91] B. A. Dmitriev, Y. A. Knirel, O. K. Sheremet, A. A. Shashkov, N. K. Kochetkov, and I. L. Hofman, *Eur. J. Biochem.*, 1979, **98**, 309.

[92] P. E. Jansson, B. Lindberg, A. A. Lindberg, and R. Wollin, *Carbohydr. Res.*, 1979, **68**, 385.

A lipopolysaccharide isolated from *Vibrio cholerae* 4715 (NAG) has been reported to be devoid of 2-amino-2,6-dideoxy-D-glucose (quinovosamine), a characteristic sugar of cholera vibrios, but to contain significant amounts of glycine in the lipid A moiety.[93] The occurrence of odd-numbered fatty acids in the lipopolysaccharides of a number of *Vibrio cholerae* strains has been reported.[94–96]

A high molecular weight heptose-free fraction representing the O-specific side-chain polysaccharide, and a low molecular-weight fraction containing heptose and representing the core polysaccharide region have been isolated from the lipopolysaccharides from *Vibrio cholerae* and *V. el-tor* (Indaba).[97] The core polysaccharide is composed of residues of D-glucose, D-mannose, heptose (D-*glycero*-L-*manno*-heptose and D-*glycero*-L-*gluco*-heptose), 2-amino-2-deoxy-D-glucose, and D-glucuronic acid (in molar ratio 9 : 4 : 5 : 1 : 2 : 5).[98] A branched structure for the polysaccharide has been proposed, with (1 → 2)-D-manno-pyranosyl-, (1 → 4)-D-glucopyranosyluronic acid-, heptopyranosyl-. and 2-amino-2-deoxy-D-glucosyl-residues in the interior part of the molecule with D-glucopyranosyl- and heptopyranosyl-residues as the non-reducing end groups. Mild acid hydrolysis of the lipopolysaccharide released an oligo-saccharide, representing the O-antigen of the lipopolysaccharide and composed of (1 → 2)-D-perosamine (4-amino-4,6-dideoxy-D-mannose) residues.[99] The amino-groups are acylated with 3-hydroxypropionyl residues. Some of the toxic and immunological properties of the lipopolysaccharide and lipid A moiety of *V. el-tor* have been described.[100]

By use of different extraction procedures, two types of lipopolysaccharides have been isolated from a slightly virulent strain of *Yersinia pestis*.[101] Some structural information was obtained from methylation and periodate oxidation studies. The immunodominant moiety of lipid A has been shown to consist of a residue of 2-amino-2-deoxy-D-glucose, *N*-acylated with 3-hydroxytetradecanoic acid.[102] A lipopolysaccharide isolated from *Y. enterolitica* exhibits serological cross-reactions with lipopolysaccharides from *Brucella abortus* and *Vibrio cholerae*.[103] Common antigenic relationships between the lipopolysaccharides of *Escherichia coli*, *Kloechera africana*, and *Salmonella aberdeen* have been demonstrated.[104]

[93] K. Hisatsune, S. Kondo, S. Nozaki, and T. Kawata, *Chem. Pharm. Bull. Jpn.*, 1978, **26**, 3597.
[94] K. Hisatsune, S. Kondo, and T. Kawata, *Chem. Pharm. Bull. Jpn.*, 1978, **26**, 2920.
[95] K. Hisatsune, S. Kondo, T. Kawata, and Y. Kishimoto, *J. Bacteriol.*, 1979, **138**, 288.
[96] K. Hisatsune, S. Kondo, T. Kawata, and Y. Kishimoto, *J. Bacteriol.*, 1979, **138**, 288.
[97] S. Raziuddin, *Biochem. J.*, 1977, **167**, 147.
[98] A. K. Sen, A. K. Mukherjee, B. Guhathakurta, A. Dutta, and D. Sasmal, *Carbohydr. Res.*, 1979, **72**, 191.
[99] J. W. Redmond, *Biochim. Biophys. Acta*, 1979, **584**, 346.
[100] S. Raziuddin, *Immunochemistry*, 1978, **15**, 611.
[101] C. Bordet, M. Bruneteau, and G. Michel, *Eur. J. Biochem.*, 1977, **79**, 443.
[102] V. I. Gorbach, I. N. Krasikova, P. A. Lukyanov, O. Y. Razmakhnina, T. F. Solov'eva, and Y. S. Ovodov, *Eur. J. Biochem.*, 1979, **98**, 83.
[103] R. Sandulache and A. Marx, *Ann. Microbiol.*, 1978, **129B**, 425.
[104] N. Aksoycan, F. Mercangöz, and G. Wells, *Ann. Microbiol.*, 1979, **130B**, 29.

A survey of the composition of lipopolysaccharides and exopolysaccharides produced by *Cystobacter*, *Archangium*, *Sorangium*, and *Stigmatella* species has been reported.[105] 3-*O*-Methyl-D-xylose was detected in several lipopolysaccharides.

UDP-D-glucose, in addition to being required for the synthesis of the core oligosaccharide region of the lipopolysaccharide of *Escherichia coli* and for the synthesis of UDP-D-galactose, is now recognized to be required as a precursor for the D-glucosyl residues of the membrane-derived oligosaccharides.[106] Mutant strains of *E. coli* specifically blocked in the synthesis of UDP-D-glucose were unable to synthesize membrane-derived oligosaccharides. A technique has been described for the selection of hybrid plasmids from *E. coli* and *Salmonella typhimurium*, containing genes for the glycosyl transferases involved in lipopolysaccharide biosynthesis.[107] Plasmids carrying the *rfa* G^+ gene, responsible for the synthesis of UDP-D-glucose:(heptosyl) lipopolysaccharide D-glucosyltransferase, and the *rfa* M^+ gene, responsible for the synthesis of UDP-D-glucose:(D-glucosyl) lipopolysaccharide 1,3-D-glucosyltransferase have been identified. In addition, the identity of a plasmid carrying a gene capable of restoring the activity of UDP-D-galactose:(D-glucosyl)lipopolysaccharide α-1,3-D-galactosyl transferase in an *rfa* H mutant of *Salmonella typhimurium* has been reported.[108] The D-galactosyl transferase was also identified in *E. coli* K12 cells despite the apparent absence of the product of the enzyme-catalysed reaction in the cellular lipopolysaccharide of that organism.

A glucolipid is considered to be an intermediate in the sequence of reactions involved in the biosynthesis of the O-9-D-mannan of *Escherichia coli*.[109] The nature of the lipid, the characterization of the glucosylation reaction, and the sequence of the D-mannosyl transfer reaction have still to be clarified. The capacity of membranes derived from *E. coli* O8 and O9, when grown in the absence of D-glucose, to synthesize D-mannan, is stimulated by lipid-derived material from cells of either strain that had been grown on D-glucose.[110] It is possible that the first stage of the pathways of D-mannan synthesis may be the same, namely the synthesis of a hydrophobic D-glucosyl-D-mannose acceptor group. The incorporation of 2-acetamido-2-deoxy-D-glucose into the core region of the lipopolysaccharide of *E. coli* involves a 2-acetamido-2-deoxy-D-glucosyltransferase that is localized in the inner membrane of the organism.[111] The endogenous acceptor for the enzyme, which is also localized at the inner membrane, appears to be the basic polysaccharide structure of the organism. Subsequent to the incorporation of the amino-sugar, the polymer is assembled into a protein–lipopolysaccharide complex.

Lactose, covalently attached to bovine serum albumin, has been used to elicit antilactose antibodies that are active against *Neisseria gonorrhoeae* lipopolysaccharide.[112] Gonococcal cell-wall polysaccharide is immunogenic in BALB/c

[105] I. W. Sutherland, *J. Gen. Microbiol.*, 1979, **111**, 211.
[106] H. Schulman and E. P. Kennedy, *J. Biol. Chem.*, 1977, **252**, 6299.
[107] E. S. Creeger and L. I. Rothfield, *J. Biol. Chem.*, 1979, **254**, 804.
[108] E. S. Creeger, J. F. Chen, and L. I. Rothfield, *J. Biol. Chem.*, 1979, **254**, 811.
[109] S. Kanegasaki and K. Jann, *Eur. J. Biochem.*, 1979, **95**, 287.
[110] G. Goldemann, S. Kanegasaki, and K. Jann, *FEMS Microbiol. Lett.*, 1979, **5**, 443.
[111] R. Stein and E. C. Heath, *J. Biol. Chem.*, 1979, **254**, 4101.
[112] D. R. Bundle, *Can. J. Biochem.*, 1979, **57**, 367.

mice, but the kinetics of the response differ from those of *Salmonella typhosa* antigen and pneumococcal type III polysaccharide.[113]

Resistance against ascites tumour development and interferon-inducing activity has been demonstrated in the lipopolysaccharide derived from the protein–lipopolysaccharide complex of *Pseudomonas aeruginosa*.[114] Both the lipid A portion and the covalently linked polysaccharide–protein complex appear to be necessary for inhibition of tumour development, whereas the lipid A with amide-linked fatty acid is suficient to induce the *in vitro* formation of interferon. The lipopolysaccharide of *P. aeruginosa* has a more pronounced effect as immunogen, mitogen, and interferon inducer than the lipopolysaccharide of *Brucella melitensis*, although they have similar toxicity levels.[115] Since lipid A is responsible for most of these activities, the variations may be partially explained by structural differences in the lipopolysaccharides.

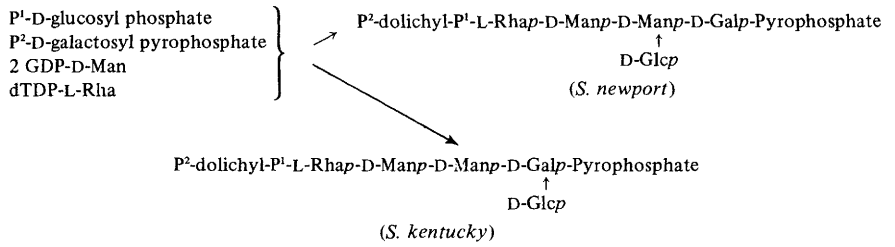
Further evidence has supported the theory that the interaction between lectins of host plants and *Rhizobium* is the key to the mechanism of host specificity in *Rhizobium*–legume symbiosis and that the lectin receptor of Rhizobial cells exists in the cell surface lipopolysaccharide.[116] No evidence was obtained to show interaction of extracellular polysaccharides with the lectins. It is possible that a specific recognition system may involve the specific binding of, for instance, pea (*Pisum sativum*) root hairs to an antigen that is situated on the lipopolysaccharide of infective strains of *R. leguminosarum*.[117]

Some aspects of the mechanism of O-specific polysaccharide biosynthesis in *Salmonella newport* and *S. kentucky* have been reported.[118] Cell envelope and soluble glycosyltransferase preparations from both organisms catalysed the formation of polyprenyl pyrophosphate oligosaccharides with carbohydrate units corresponding to the structures of the main-chain polysaccharides (Scheme 1). The D-glucosylation reaction occurs before the polymerization of the repeating units to the polysaccharide.

The binding sites for lipopolysaccharides on mouse lymphocytes have been identified as membrane proteins.[119] Active fractions isolated by affinity chromatography from B cell membranes were identified as immunoglobulins, possibly IgM and IgD, whilst the histocompatibility-2 complex proteins (H-2D, H-2K, and Ia antigens) were found to be binding sites for lipopolysaccharides on both B and T cells.

Some aspects of the chemistry and biology of bacterial lipopolysaccharides have been reviewed.[120]

[113] J. F. Breen and M. C. Apicella, *Infect. Immun.*, 1978, **22**, 195.
[114] K. I. Tanamoto, C. Abe, J. Y. Homma, and Y. Kojima, *Eur. J. Biochem.*, 1979, **97**, 623.
[115] I. Barasosin, M. L. Alonso, J. M. Rojo, E. Ronda, and A. Portoles, *Curr. Microbiol.*, 1978, **1**, 263.
[116] G. Kato, Y. Maruyama, and M. Nakamura, *Agric. Biol. Chem. (Jpn.).*, 1979, **43**, 1085.
[117] W. Kamberger, *FEMS Microbiol. Lett.*, 1979, **6**, 361.
[118] V. N. Shibaev, T. N. Druzhinina, A. N. Popova, S. S. Rozhnova, and V. A. Kilesso, *Eur. J. Biochem.*, 1979, **101**, 309.
[119] K. Yokoyama, J. Mashino, N. Kasai, T. Terao, and T. Osawa, *Z. Physiol. Chem.*, 1979, **360**, 587.
[120] C. Galanos, O. Lüderitz, E. T. Rietschel, and O. Westphal, in 'MTP International Review of Biochemistry', ed. T. W. Goodwin, 1977, Vol. 14, p. 239.



Scheme 1

4 Capsular Polysaccharides

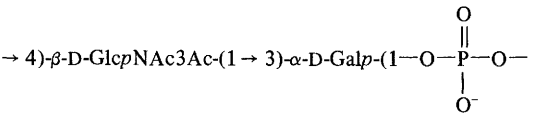
Colominic acid, a homopolymer of (2→8)-α-*N*-acetylneuraminic acid residues, is the *Escherichia coli* K1 capsular polysaccharide. Random *O*-acetylation at C-7 and C-9 of the polysaccharide, as determined by ^{13}C n.m.r. spectroscopy, has been shown to be responsible for the alternating antigenic change (form variation) associated with the organism.[121] A serological method has been established for the identification and isolation of the form variants.

X-Ray diffraction patterns for the capsular polysaccharides of *Escherichia coli* K29 and its M13 and M41 mutants have indicated that the molecular conformation and packing are the same for all the polysaccharides, suggesting that their primary structures are essentially unchanged.[122] Similar phage specificity and antibody reactions, together with the similarity of the diffraction patterns, indicated an overall chemical identity of the polysaccharides elaborated by the mutant strains with that of the parent strain. A model with anti-parallel two-fold helices at the corner and centre of the unit cell was proposed. The interaction of phage K29 with its host cell, *E. coli* K29, has been studied by use of combined virological and immunological methods.[123] The virion penetrates the capsule by a mechanism which involves receptor destruction and results in the release of DNA over a membrane-adhesion site.

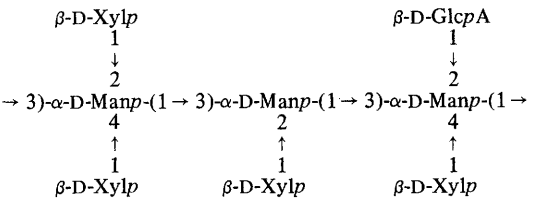
Fully methylated and fully acetylated disaccharides isolated from the capsular polysaccharide of *Escherichia coli* K100 and *Haemophilus influenzae* type b have been identified by m.s. as 2-*O*- and 1-*O*-D-ribofuranosyl ribitol, respectively.[124] The capsular antigen of *H. influenzae* type c has been purified by ion-exchange chromatography.[125] Structural investigations have identified the disaccharide (8) as the repeating unit of the polysaccharide.

The highly branched capsular polysaccharide from *Cryptococcus neoformans* serotype c consists of a backbone of (1→3)-α-D-mannopyranosyl residues with single chain non-reducing (1→2)- and (1→4)-β-D-xylopyranosyl- and (1→2)-β-

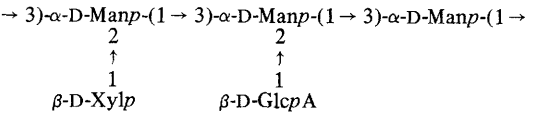
[121] F. Ørskov, I. Ørskov, A. Sutton, R. Schneerson, W. Lin, W. Egan, G. E. Hoff, and J. B. Robbins, *J. Exp. Med.*, 1979, **149**, 669.
[122] R. Moorhouse, W. T. Winter, S. Arnott, and M. E. Bayer, *J. Mol. Biol.*, 1977, **109**, 373.
[123] M. E. Bayer, H. Thurow, and M. H. Bayer, *Virology*, 1979, **94**, 95.
[124] B. A. Fraser, F. P. Tsui, and W. Egan, *Carbohydr. Res.*, 1979, **73**, 59.
[125] P. Branefors-Helander, B. Classon, L. Kenne, and B. Lindberg, *Carbohydr. Res.*, 1979, **76**, 197.



(8)



(9)



(10)

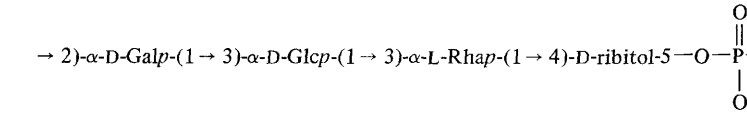
D-glucopyranosyl uronic acid residues.[126] Further examination of the polysaccharide (9) by alkaline degradation, followed by methylation, revealed that the D-glucuronic acid residues are located on D-mannosyl residues already substituted with a D-xylosyl residue.[127] The capsular polysaccharide of *C. neoformans* serotype D is less highly branched, but with many structural similarities to (10).[128]

When high molecular weight pneumococcal capsular polysaccharide is injected intraperitoneally into rats, the antigen is excreted in the urine.[129] Examination of the molecular weight profile of the excreted product indicates that significant degradation has taken place.

Pneumococcal type 19 polysaccharide is composed of residues of D-glucose, L-rhamnose, 2-acetamido-2-deoxy-D-mannose, and phosphate.[130] Strong acidic hydrolysis has been found to be accompanied by migration of the phosphate groups.

The structure of the capsular antigen of *Streptococcus pneumoniae* type 26 has been established (11).[131] The only difference between the type 26 and the type 6 antigen is that the α-L-rhamnopyranosyl residue is linked to O-4 of the

[126] A. K. Bhattacharjee, K. J. Kwon-Chung, and C. P. J. Glaudemans, *Immunochemistry*, 1978, **15**, 673.
[127] A. K. Bhattacharjee, K. J. Kwon-Chung, and C. P. J. Glaudemans, *Mol. Immunol.*, 1979, **16**, 531.
[128] A. K. Bhattacharjee, K. J. Kwon-Chung, and C. P. J. Glaudemans, *Carbohydr. Res.*, 1979, **73**, 183.
[129] J. D. Coonrod, *Proc. Soc. Exp. Biol. Med.*, 1979, **162**, 249.
[130] T. Yadomae, N. Ohno, and T. Miyazaki, *Carbohydr. Res.*, 1979, **75**, 191.
[131] L. Kenne, B. Lindberg, and J. K. Madden, *Carbohydr. Res.*, 1979, **73**, 175.



(11)

D-ribitol moiety in the former but to O-3 in the latter. The configuration of the 4,6-*O*-pyruvic acid acetal in *S. pneumoniae* type 27 has been established from ^{1}H and ^{13}C n.m.r. spectral data of the natural product and model compounds.[132] Inhibition of the homologous precipitin reaction by synthetic diastereoisomeric pyruvic acid acetal showed that the configuration of the pyruvic acid acetal contributes to the serological specificity of the type 27 polysaccharide.

As a result of the elucidation of the chemical structures of many *Pneumococci* and *Klebsiella* species, several previously uninterpretable serological cross-reactions have now been rationalized.[133]

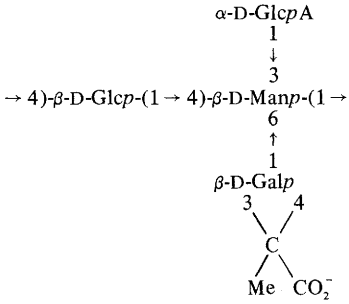
The acidic capsular polysaccharides of *Klebsiella* species have been visualized by application of polycationic derivatives of ferritin which bind electrostatically to anionic sites on the surface of other glutaraldehyde-fixed or unfixed cells.[134]

Pyruvic acid has been identified in approximately half of seventy-seven *Klebsiella* species investigated.[135] On the basis of o.r.d. measurements and their interpretation from Moffitt–Yang plots, a helical superstructure is proposed for *Klebsiella* capsular polysaccharides of serotypes K1, K5, K6, K8, K11, K56, and K57.[136] Measurements of the temperature dependence of the specific optical rotation reveal, in all cases, co-operative order–disorder transitions at temperatures between 25 and 40 °C.

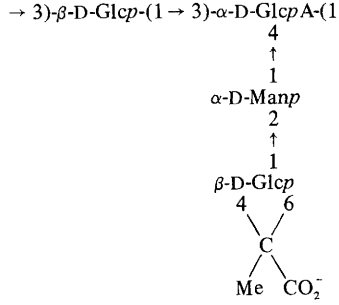
Structures have been established for the capsular polysaccharides of *Klebsiella* K30 (12),[137] K31 (13),[138] K33 (14),[139] K61 (15),[140] K63 (16),[141] and K64 (17).[142]

Limited *X*-ray studies on the *Klebsiella* K30 capsular polysaccharide suggest the existence of a trisaccharide-repeating structure with all the glycosidic linkages disposed 1,4-diequatorial and producing a two-fold helix with a ribbon-like appearance.[143] The antigenic determinant of hyporesponsiveness in BALB/c mice to *K. pneumoniae* type 47 has been reported to be L-rhamnose.[144]

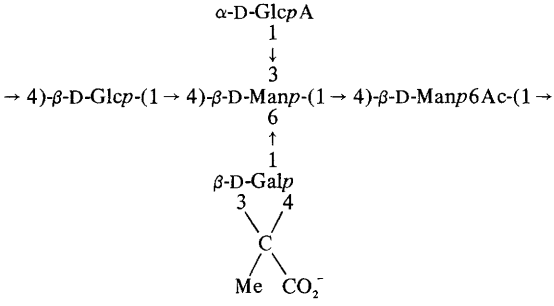
[132] L. G. Bennett and C. T. Bishop, *Immunochemistry*, 1977, **14**, 693.
[133] M. Heidelberger, W. Nimmich, K. Eriksen, and S. Stirm, *Acta Pathol. Microbiol. Scand.*, 1978, **86B**, 313.
[134] R. Weiss, H. G. Schiefer, and H. Kauss, *FEMS Microbiol. Lett.*, 1979, **6**, 435.
[135] W. Nimmich, *Z. Allg. Mikrobiol.*, 1979, **19**, 343.
[136] C. Wolf, U. Elsässer-Beile, S. Stirm, G. G. S. Dutton, and W. Burchard, *Biopolymers*, 1978, **17**, 731.
[137] B. Lindberg, F. Lindh, J. Lönngren, and I. W. Sutherland, *Carbohydr. Res.*, 1979, **76**, 281.
[138] C. C. Cheng, S. L. Wong, and Y. M. Choy, *Carbohydr. Res.*, 1979, **73**, 169.
[139] B. Lindberg, F. Lindh, J. Lönngren, and W. Nimmich, *Carbohydr. Res.*, 1979, **70**, 135.
[140] A. S. Rao and N. Roy, *Carbohydr. Res.*, 1979, **76**, 215.
[141] J. P. Joseleau and M. F. Marais, *Carbohydr. Res.*, 1979, **77**, 183.
[142] E. H. Merrifield and A. M. Stephen, *Carbohydr. Res.*, 1979, **74**, 241.
[143] H. F. Elloway, E. D. T. Atkins, and I. W. Sutherland, *Carbohydr. Res.*, 1979, **76**, 285.
[144] M. E. White-Scharf and L. T. Rosenberg, *Infect. Immun.*, 1978, **22**, 18.


α-D-Glc*p*A
1
↓
3
→ 4)-β-D-Glc*p*-(1 → 4)-β-D-Man*p*-(1 → 4)-β-D-Man*p*-(1 →
6
↑
1
β-D-Gal*p*
3 4
C
Me CO$_2^-$

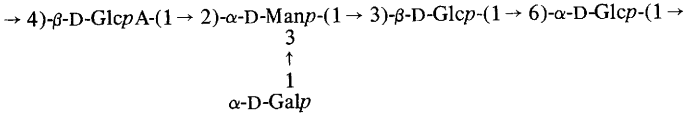
(12)


→ 3)-β-D-Glc*p*-(1 → 3)-α-D-Glc*p*A-(1 → 3)-β-D-Gal*p*-(1 →
4
↑
1
α-D-Man*p*
2
↑
1
β-D-Glc*p*
4 6
C
Me CO$_2^-$

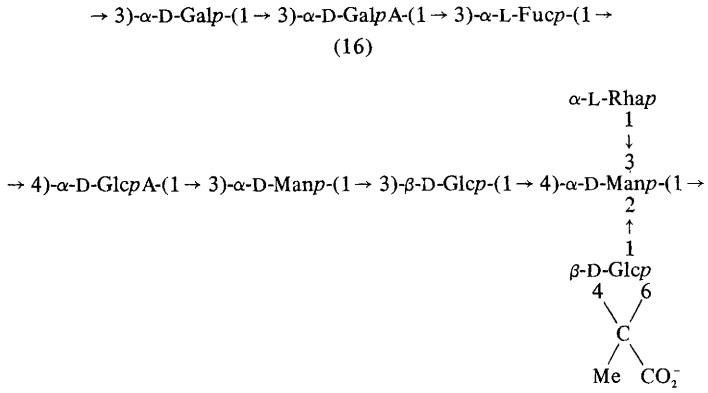
(13)


α-D-Glc*p*A
1
↓
3
→ 4)-β-D-Glc*p*-(1 → 4)-β-D-Man*p*-(1 → 4)-β-D-Man*p*6Ac-(1 →
6
↑
1
β-D-Gal*p*
3 4
C
Me CO$_2^-$

(14)


→ 4)-β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 →
3
↑
1
α-D-Gal*p*

(15)



(16)

(17)

Physical parameters, such as molecular weight, radius of gyration, and translational diffusional coefficient have been reported for group C meningococcal polysaccharide.[145] Viscosity, light scattering, and sedimentation results all showed that the polysaccharide forms aggregates in solution, the aggregates behaving like random-coiled polymers with no measurable shape factors.

5 Extracellular and Intracellular Polysaccharides

Several review articles on extracellular polysaccharides have dealt with such topics as the structures of acidic and neutral extracellular microbial polysaccharides,[146] the production of xanthan gums and other bacterial polysaccharides,[147] and the chemistry and biochemistry of microbial extracellular polysaccharides and polysaccharidases.[148]

Water-soluble and water-insoluble dextrans, synthesized by *Streptococcus mutans*, have been fractionated from solutions of alkali.[149] The insoluble fraction contained a greater proportion of (1 → 3)-linkages and was less susceptible to dextranase than was the soluble fraction.

The water-soluble dextrans produced by a strain of *Streptococcus mutans* have been fractionated by gel chromatography into three types having different molecular sizes, chemical structures, and serological properties.[150] One of the fractions and a water-insoluble dextran, also synthesized by the organism, appear to represent different physical states of an inherently identical D-glucan.

The molecular compression of dextrans has been examined using viscosity measurements.[151] Marked osmotic compression of the flexible molecular struc-

[145] Y. Tsunashima, K. Moro, B. Chu, and T. Y. Liu, *Biopolymers*, 1978, **17**, 251.
[146] P. A. Sandford, *Adv. Carbohydr. Chem. Biochem.*, 1979, **36**, 265.
[147] M. E. Slodki and M. C. Cadmus, *Adv. Appl. Microbiol.*, 1978, **23**, 19.
[148] 'Microbial Polysaccharides and Polysaccharases', ed. R. C. W. Berkeley, G. W. Gooday, and D. C. Ellwood, Academic Press, London, 1979.
[149] K. Trautner and B. Felgenhauer, *Caries Res.*, 1979, **13**, 1.
[150] M. Inoue and T. Koga, *Infect. Immun.*, 1979, **25**, 922.
[151] A. G. Ogston and B. N. Preston, *Biochem. J.*, 1979, **183**, 1.

tures occurs within the semi-dilute concentration range. Decreases in molecular volumes may have important effects on transport processes such as the multi-component systems found in living organisms.

Chemically defined, highly branched dextrans have been studied by a ^{13}C spin–lattice relaxation method.[152] Correlations were made to the positions of carbon atoms associated with branching residues, permitting the values of *O*-substitution to be established for D-glucopyranosyl residues. Fourier-transform-i.r. difference spectra of dextrans have been correlated to the type and degree of branching, which had been established previously by permethylation for these polysaccharides.[153] A combination of ^{1}H n.m.r.- and Fourier-transform-i.r.-spectroscopy has been used to give complementary information on the structure of dextrans.[154]

The results of the high-temperature enhancement of ^{13}C n.m.r. chemical shifts of dextrans have been correlated with those of methylation analysis.[155] The diagnostic nature of the 70–75 p.p.m. spectral region with regard to the type of dextran branching, and an increase in resolution of the polysaccharide spectra at higher temperatures, are reported. A combination of chemical-ionization m.s., ammonia m.s., and ^{13}C n.m.r. spectroscopy has been used for the unambiguous identification of peracetylated aldononitrile derivatives of methylated dextrans.[156] The method has been used to distinguish α-D-glucosyl residues substituted at O-3 in the linear chain and branch-point positions, or only in the branch-point positions. Several ^{13}C n.m.r. signals that are diagnostic for 4,6-di-*O*-substituted α-D-glucopyranosyl residues have been identified.[157] Analytical data for g.l.c.-m.s. methylation fragmentation and periodate oxidation for numerous dextrans have shown that results obtained using the two techniques are in general agreement for slightly branched dextrans, but are divergent for highly branched dextrans.[158] Hitherto unrecognized effects of (1→2)-, (1→3)-, (1→4)-linkages in the dextrans were observed.

A water-insoluble α-D-glucan has been isolated from growing cultures of *Streptococcus salivarius*.[159] The results of methylation analysis and periodate oxidation studies indicated the presence of about 80% (1→3)-α-D-glucosidic linkages, together with short side-chains attached to the backbone by (1→4)-, (1→3)-, and (1→2)-α-D-glucosidic linkages. A linear (1→3)-α-D-glucan was recovered after periodate oxidation of the parent polysaccharide.[160]

[152] F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydr. Res.*, 1979, **72**, 229.
[153] F. R. Seymour and R. L. Julian, *Carbohydr. Res.*, 1979, **74**, 63.
[154] F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydr. Res.*, 1979, **74**, 77.
[155] F. R. Seymour, E. C. M. Chen, S. H. Bishop, and A. Jeanes, *Carbohydr. Res.*, 1979, **68**, 123.
[156] F. R. Seymour, R. D. Knapp, E. C. M. Chen, S. H. Bishop, and A. Jeanes, *Carbohydr. Res.*, 1979, **74**, 41.
[157] F. R. Seymour, R. D. Knapp, E. C. M. Chen, A. Jeanes, and S. H. Bishop, *Carbohydr. Res.*, 1979, **75**, 275.
[158] A. Jeanes and F. R. Seymour, *Carbohydr. Res.*, 1979, **74**, 31.
[159] Y. Tsumuraya and A. Misaki, *Carbohydr. Res.*, 1979, **74**, 217.
[160] K. Ogawa, A. Misaki, S. Oka, and K. Okamura, *Carbohydr. Res.*, 1979, **75**, C13.

Further studies have confirmed that immunoglobulin A (W3129) binds only to the non-reducing end of dextrans and not to any intercatenary segments of five D-glucopyranosyl residues.[161]

Extended *X*-ray absorption fine structure analysis of ferritin and imferon (a synthetic complex of iron oxide coated with dextran) show that the near-neighbour environment around the iron atom in the two macromolecules is identical.[162]

The action patterns of an intracellular dextranase and three α-D-glucosidases (oligo-D-glucanases) from *Pseudomonas* UQM733 have been used to determine the structure of two branched isomalto-oligosaccharides obtained by enzymic degradation of dextran.[163] The adherence of heat-killed *Streptococcus mutans* cells to glass, induced by incubation with sucrose and cell-free dextransucrase (EC 2.4.1.5), is markedly inhibited by low concentrations of dextranases.[164] The dextranases repress the production of water-insoluble α-D-glucan and reduce adhesiveness and cell-agglutinating activity of the D-glycans, by lowering the content of (1 → 6)-linkages and molecular weights, thus inhibiting the formation of D-glucan film and adherence of *S. mutans* cells on glass.

Three extracellular proteins having affinities for binding to *Streptococcus mutans* serotype *c* cells have been isolated by affinity chromatography on immobilized α-D-glucans containing (1 → 3)- and (1 → 6)-linkages.[165] Dextransucrase activity was found to be associated with two of the proteins. Neither of the proteins had a unique binding specificity for (1 → 3)-α-D-glucosyl residues, and they were able to bind to (1 → 6)-α-D-glucans, other dextrans, and also to the (1 → 3)-α-D-glucosyl regions in pseudonigeran.

The role of antibodies against cell-wall antigens of *Streptococcus mutans* in the protection against dental caries in humans has been reviewed.[166] Soluble antigen preparations containing dextransucrase and from one serotype of *S. mutans* have been shown to elicit a protection immune response against infection with cariogenic *S. mutans* from many or possibly all serotypes.[167] Significant protection from experimental dental caries has been achieved by oral administration of soluble dextransucrase in animal feeding experiments.[168] A survey of anti-dextransucrase activities in rabbit antisera against whole cells of *S. mutans* in terms of inhibition of cell-free and cell-associated dextransucrases has been reported.[169] Inhibition of antiserum against serotype *c* was restricted to dextransucrases of serotypes *c*, *e*, and *f* but the dextransucrases of serotypes *a*, *d*, and *g* were not affected.

[161] L. G. Bennett and C. P. J. Glaudemans, *Carbohydr. Res.*, 1979, **72**, 315.
[162] E. C. Theil, D. E. Sayers, and M. A. Brown, *J. Biol. Chem.*, 1979, **254**, 8132.
[163] M. T. Covacevich and G. N. Richards, *Carbohydr. Res.*, 1979, **70**, 283.
[164] T. Koga and M. Inoue, *Arch. Oral Biol.*, 1979, **24**, 191.
[165] R. B. B. Russell, *J. Gen. Microbiol.*, 1979, **112**, 197.
[166] T. H. J. Huis in't Veld, W. H. van P. Helderman, and O. B. Dirks, *J. Microbiol Serol.*, 1979, **45**, 25.
[167] D. J. Smith, M. A. Taubman, and J. L. Ebersole, *Infect. Immun.*, 1978, **21**, 843.
[168] D. J. Smith, M. A. Taubman, and J. L. Ebersole, *Infect. Immun.*, 1979, **26**, 82.
[169] S. Hamada and J. Mizuno, *FEMS Microbiol. Lett.*, 1979, **6**, 1.

The production, purification, and properties of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F have been reported.[170]

The glycosyltransferase complexes [dextransucrase and levansucrase (EC 2.4.1.10)] from two strains of *Streptococcus mutans* have been resolved by polyacrylamide gel electrophoresis.[171] An improved method for the purification of dextransucrase involves the use of the non-ionic detergent Tween 80 to prevent aggregation of the enzyme, and affinity chromatographic absorption of the enzyme on insoluble dextran.[172] Ammonium sulphate precipitation of dextransucrase appears to favour aggregation of the enzyme to a form which catalyses the synthesis of insoluble α-D-glucan containing mainly (1→3)-linkages. Gel filtration (hence dilution of the enzyme) promotes disaggregation to a low molecular weight form that catalyses only the synthesis of water-soluble α-D-glucans, which contain predominantly (1→3)-linkages.[173]

An intracellular polysaccharide, mol. wt. 3×10^5, isolated from *Bacteroides fragilis*, has been characterized as being of the glycogen-type, as it stains with iodine and is degraded to D-glucose by glucoamylase.[174] The intracellular glycogen-like polysaccharide present in the cells of two strains of *Streptococcus mutans* at various stages of growth has been measured by quantitative electron microscopy.[175]

The nature of the binding sites for the substrates, activators, and inhibitors located on each of the four sub-units of adenosine diphosphate-D-glucose pyrophosphorylase (EC 2.7.7.27) from *Escherichia coli* have been investigated.[176] The binding of adenosine triphosphate and D-fructose 1,6-diphosphate to the enzyme is synergistic, showing heterotrophic co-operativity, and the two phosphorylated compounds must also be present together to inhibit effectively the binding of adenosine monophosphate. A mechanism has been proposed to explain some of the kinetic and binding properties in terms of asymmetry in the distribution of the conformational states of the four identical sub-units. Quantitative correlation of the changes in the rates of synthesis of glycogen and the utilization of D-glucose with simultaneous changes in the cellular levels of both D-glucose 6-phosphate and D-fructose 1,6-diphosphate have been reported for *E. coli*.[177] An equation has been developed which describes the *in vivo* velocity of ADP-D-glucose pyrophosphorylase, and the rate of glycogen synthesis.

Conditions have been described which cause significant changes in glycogen accumulation during periods of nitrogen starvation.[178,179] Differences have been

[170] J. F. Robyt and T. F. Walseth, *Carbohydr. Res.*, 1979, **68**, 95.
[171] W. R. Figures and J. R. Edwards, *Biochim. Biophys. Acta*, 1979, **577**, 142.
[172] W. R. Figures and J. R. Edwards, *Carbohydr. Res.*, 1979, **73**, 245.
[173] S. B. Mohan, B. M. Newman, and J. A. Cole, *FEMS Microbiol. Lett.*, 1979, **5**, 69.
[174] J. G. E. M. Linder, J. H. Marcelis, N. M. De Vos, and J. A. A. Hoogkamp-Korstanje, *J. Gen. Microbiol.*, 1979, **111**, 93.
[175] J. R. Dipersio, S. J. Mattingly, M. L. Higgins, and G. D. Shockman, *Microbios*, 1978, **21**, 109.
[176] T. H. Haugen and J. Preiss, *J. Biol. Chem.*, 1979, **254**, 127.
[177] D. N. Dietzler, M. P. Leckie, W. L. Sternheim, J. M. Ungar, D. L. Crimmins, and J. W. Lewis, *J. Biol. Chem.*, 1979, **254**, 8276.
[178] D. N. Dietzler, M. P. Leckie, C. J. Lias, D. A. Henry, J. H. Rothert, and R. M. Ferguson, *J. Biol. Chem.*, 1979, **254**, 8288.
[179] D. N. Dietzler, M. P. Leckie, J. W. Lewis, S. E. Porter, T. L. Taxman, and C. J. Lias, *J. Biol. Chem.*, 1979, **254**, 8295.

observed in the values of velocity of ADP-D-glucose synthetase in the intact *E. coli* cell, when the carbon source is varied from D-glucose to D-fructose, glycerol, or succinate.[180] These differences are a result of the different cellular levels of adenosine 3′,5′-phosphate in the cells using different carbon sources. A kinetic mechanism describing the action of the D-glucose 6-phosphate-dependent form of glycogen synthase (EC 2.4.1.11) of *Neurospora crassa* has been derived from initial velocity experiments and inhibition studies and has proved to be consistent with a rapid equilibrium random Bi–Bi mechanism.[181]

X-Ray diffraction studies on the (1→3)-β-D-glucan, curdlan, from *Alcaligenes faecalis* have shown the existence of microfibril formation, and a triple helical structure is proposed for the crystalline form of this polysaccharide.[182]

The maximum production of the D-fructan, levan, has been shown to occur during the mid-exponential to the late exponential phase of growth of *Actinomyces viscosus* and is followed by a rapid decline as a result of the production of levan hydrolase activity.[183] The levan produced by *Streptococcus mutans* OMZ 176 appears to have a structure similar to other known D-fructans and inulin.[184] On the basis of chemical shift displacements of the ^{13}C n.m.r. spectra, resulting from *O*-substitution at specific carbon atoms, resonances have been assigned to the carbon atoms of the β-D-fructofuranosyl residues in levans.[185] A variety of different levans show almost identical ^{13}C n.m.r. spectra in contrast to a wide divergence in the corresponding spectra of dextrans.[186]

The extracellular polysaccharide isolated from *Arthrobacter stabilis* NRRL B3225 contains residues of D-glucose, D-galactose, pyruvate, succinate, and acetate (in molar ratio 6:3:1:1:1.5).[187] The viscosity of aqueous salt-free solutions is relatively low, but atypical of anionic polysaccharides, increasing rapidly in the presence of salts, acids, and alkali.

The improvements of the rheological properties of microbial exopolysaccharides through mutagenesis and mutant selection has been demonstrated for *Escherichia coli* and *Enterobacter* (*Klebsiella aerogenes*).[188] The chemical composition of each group of polysaccharides produced by the mutants did not show any significant differences from those originating from the parent strain.

Antigenic polysaccharides from *Escherichia coli* 29M and *E. coli* 72M have been isolated from chicken intestine and the structures of their repeating

[180] D. N. Dietzler, M. P. Leckie, J. L. Magnani, M. J. Sughrue, P. E. Bergstein, and W. L. Sternheim, *J. Biol. Chem.*, 1979, **254**, 8308.
[181] H. Takahara and K. Matsuda, *J. Biochem. (Tokyo)*, 1978, **84**, 1381.
[182] R. H. Marchessault and Y. Deslandes, *Carbohydr. Res.*, 1979, **75**, 231.
[183] C. H. Miller and P. J. B. Somers, *Infect. Immun.*, 1978, **22**, 266.
[184] A. J. Corrigan and J. F. Robyt, *Infect. Immun.*, 1979, **26**, 387.
[185] F. R. Seymour, R. D. Knapp, J. E. Zweig, and S. H. Bishop, *Carbohydr. Res.*, 1979, **72**, 57.
[186] F. R. Seymour, R. D. Knapp, and A. Jeanes, *Carbohydr. Res.*, 1979, **72**, 222.
[187] C. A. Knutson, J. E. Pittsley, and A. Jeanes, *Carbohydr. Res.*, 1979, **73**, 159.
[188] I. W. Sutherland, *J. Appl. Biochem.*, 1979, **1**, 60.

units, (18) and (19) respectively, have been established using the standard methodology.[189,190]

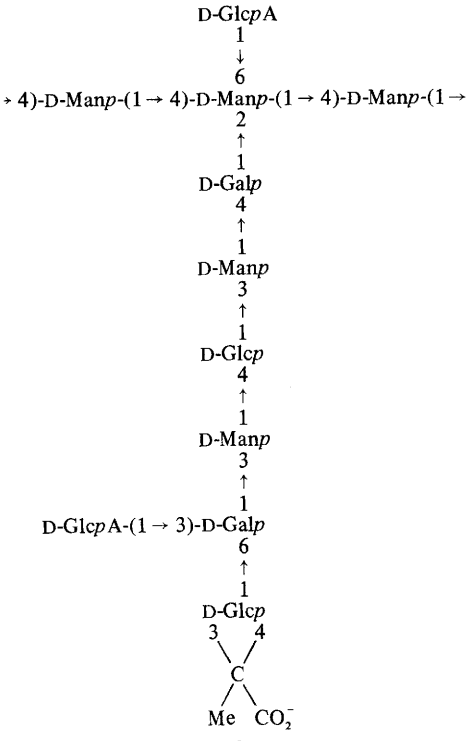
When grown on methanol an obligate methylotroph, *Methylocystis parvus*, originally isolated as a methane-utilizing bacterium, elaborates an extracellular viscous heteropolysaccharide composed of residues of D-glucose and L-rhamnose (6 : 1).[191] The extracellular polysaccharide of a methylotrophic culture contains residues of D-glucose, D-galactose, D-mannose, L-fucose, and L-rhamnose when grown on methane as the sole carbon source.[192] Transfer of the culture from methane to methanol results in the loss of its ability to utilise methane as the sole carbon source and is accompanied by a loss of its ability to synthesize 6-deoxyhexoses in the extracellular polysaccharide.

An acidic polysaccharide that exhibits unusual properties, such as formation of a brittle water-insoluble gel in the presence of Ca^{2+}, has been isolated from a *Pseudomonas* species when grown on methanol.[193] The polysaccharide is composed of a main chain of (1 → 3)-linked β-D-galactopyranosyl residues, with additional residues of D-glucose, D-mannose, D-glucuronic acid, and D-allose in undetermined positions. The slime polysaccharide synthesized by *Pseudomonas* PB1 contains D-glucose, D-galactose, acetate, and pyruvate and therefore differs from many exopolysaccharides formed by other pseudomonads in not containing residues of hexuronic acid.[194] Solutions of the polysaccharide were pseudoplastic, in that the measured viscosity was shear rate dependent, and formed gels in the presence of Fe^{3+} and Al^{3+}.

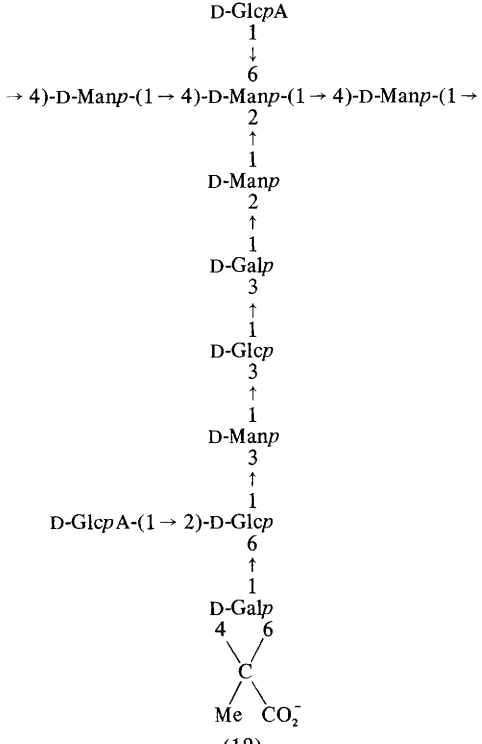
D-Fructose favours the production of exopolysaccharides by *Rhizobium meliloti*.[195] In contrast, D-glucose is metabolized to 2-ketogluconic acid, an inhibitor of the exopolysaccharide biosynthesis. Growth yields, polysaccharide production, and energy conservation in chemostat cultures of *R. trifolii* have been reported.[196] The extracellular polysaccharides of different strains of *R. meliloti* have been found to induce the production of polygalacturonase by roots of *Medicago sativa* seedlings.[197] A comparative study of polysaccharide production and monosaccharide composition of partially purified fractions capable of inducing the enzyme demonstrated differences according to their origins.

The structure (20) of the heptasaccharide repeating unit of an extracellular polysaccharide from *Rhizobium trifolii* has been established by a combination of methylation analysis, sequential oxidation, and elimination of oxidized

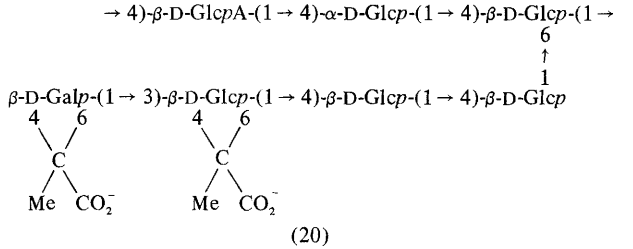
[189] A. Kamei, N. Takeuchi, S. Akashi, and K. Kagabe, *Chem. Pharm. Bull. (Jpn.)*, 1978, **26**, 3395.
[190] A. Kamei, N. Takeuchi, S. Akashi, and K. Kagabe, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 335.
[191] C. T. Hou, A. I. Laskin, and R. N. Patel, *Appl. Environ. Microbiol.*, 1979, **37**, 800.
[192] M. N. Huq, B. J. Ralph, and P. A. D. Rickard, *Aust. J. Biol. Sci.*, 1978, **31**, 311.
[193] A. Misaki, Y. Tsumuraya, M. Kakuta, H. Takemoto, and T. Igarashi, *Carbohydr. Res.*, 1979, **75**, C8.
[194] A. G. Williams, J. W. T. Wimpenny, and C. J. Lawson, *Biochim. Biophys. Acta*, 1979, **585**, 611.
[195] B. Courtois, J. P. Hornez, and J. C. Derieux, *Can. J. Microbiol.*, 1979, **25**, 1191.
[196] J. A. De Hullander, C. W. Bettenhaussen, and A. H. Stouthamer, *J. Microbiol. Serol.*, 1979, **45**, 401.
[197] A. Palomares, E. Montoya, and J. Olivares, *Microbios*, 1978, **22**, 7.


D-Glcp A
1
↓
6
→ 4)-D-Manp-(1 → 4)-D-Manp-(1 → 4)-D-Manp-(1 →
2
↑
1
D-Galp
4
↑
1
D-Manp
3
↑
1
D-Glcp
4
↑
1
D-Manp
3
↑
1
D-Glcp A-(1 → 3)-D-Galp
6
↑
1
D-Glcp
3 4
C
Me CO2-

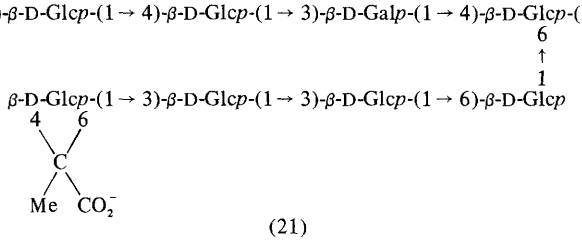
(18)


D-Glcp A
1
↓
6
→ 4)-D-Manp-(1 → 4)-D-Manp-(1 → 4)-D-Manp-(1 →
2
↑
1
D-Manp
2
↑
1
D-Galp
3
↑
1
D-Glcp
3
↑
1
D-Manp
3
↑
1
D-Glcp A-(1 → 2)-D-Glcp
6
↑
1
D-Galp
4 6
C
Me CO2-

(19)



(20)



(21)

residues.[198] This is not a repeating unit in the true sense as approximately one third of the units lack the terminal β-D-galactopyranosyl residues. Comparative studies on the polysaccharides elaborated by *R. meliloti*, *Alcaligenes faecalis*, and *Agrobacterium radiobacter* indicate that they have a common structure (21) except for the position of acylation.[199]

A D-gluco-D-mannan (mol.wt. 1.4×10^6) has been isolated from *Rhodococcus erythropolis*.[200]

The nutritional requirements for the production of xanthan by *Xanthomonas campestris* have indicated that D-glucose and sucrose are the best sources of carbon for the production of the polysaccharide.[201] Xanthan has been shown to possess a rigid rod-like conformation, 6000 Å in length and independent of ionic strength $>10^{-2}$ M.[202] The conformation has been studied as a function of temperature, ionic strength, and polymer concentration.[203] C.d. measurements indicate that the polysaccharide exists in a combination of random and ordered forms regardless of these conditions.

The virulence of the plant pathogen *Erwinia amylovora* has been correlated with the ability of the organism to produce extracellular polysaccharide.[204]

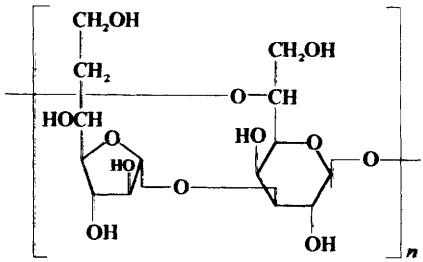
A soluble carrier protein (HPr), one of the common components of all phosphoenolpyruvate-dependent phosphotransferase systems, has been purified from the phosphoenolpyruvate-dependent phosphotransferase system of

[198] P. E. Jansson, B. Lindberg, and H. Ljunggren, *Carbohydr. Res.*, 1979, **75**, 207.
[199] T. Harada, A. Amemura, P. E. Jansson, and B. Lindberg, *Carbohydr. Res.*, 1979, **77**, 285.
[200] P. Rapp, C. H. Beck, and F. Wagner, *Eur. J. Appl. Microbiol. Biotechnol.*, 1979, **7**, 67.
[201] P. Souw and A. L. Demain, *Appl. Environ. Microbiol.*, 1979, **37**, 1186.
[202] M. Rinaudo and M. Milas, *Biopolymers*, 1978, **17**, 2663.
[203] M. Milas and M. Rinaudo, *Carbohydr. Res.*, 1979, **76**, 189.
[204] A. R. Ayers, S. B. Ayers, and R. N. Goodman, *Appl. Environ. Microbiol.*, 1979, **38**, 659.

Escherichia coli.[205] The protein has been co-purified with a (1→6)-α-D-glucan (mol.wt. 2.5×10^3). Results of steady-state kinetic measurements of the phosphotransferase activity demonstrate that the polysaccharide works as an activator of the enzymic system.

6 Miscellaneous Cell-wall Polysaccharides

The polysaccharide antigen from *Eubacterium saburreum*, which has been isolated by gel- and ion exchange-chromatographic separation of proteolytic digestion products, contains residues of D-*glycero*-D-*galacto*-heptose and a 6-deoxyheptose.[206] Cross-reactivity with a related polysaccharide from strain L49 indicates that deoxyheptose is 6-deoxy-D-*altro*-heptose. The structure (22) of the corresponding antigen from *E. saburreum* O2 contains (1→6)-β-D-*glycero*-D-*galacto*-heptopyranosyl residues, all of which are substituted with 6-deoxy-α-D-*altro*-heptofuranosyl residues at O-3.[207] This differs from the previously identified polysaccharide from the L49 strain, which contains chains of alternating (1→3)- and (1→6)-β-D-*glycero*-D-*galacto*-heptopyranosyl residues, the latter being substituted with 6-deoxy-α-D-*altro*-heptofuranosyl residues at O-3. In addition this polysaccharide contains *O*-acetyl groups at O-7 of some heptosyl residues and at O-2 of some 6-deoxy-α-D-heptosyl residues. The antigenic determinant on the L49 polysaccharide may be the 2-*O*-acetyl-6-deoxy-α-D-*altro*-heptofuranosyl residues.



(22)

One common characteristic of the cell walls of halococci so far investigated is the high content of glycine. A 2,4-dinitrophenyl derivative of 2-deoxy-2-glycylamido-D-glucose has been isolated after hydrolysis of 2,4-dinitrophenylated cell walls.[208] It is postulated that glycine may play a role in connecting glycan strands through peptide linkages between the amino-group of 2-amino-2-deoxy-D-glucose and the carboxy-group of a uronic acid residue.

When incubated under appropriate conditions with tritiated L-methionine, *Mycobacterium smegmatis* accumulates significant amounts of labelled oligo-

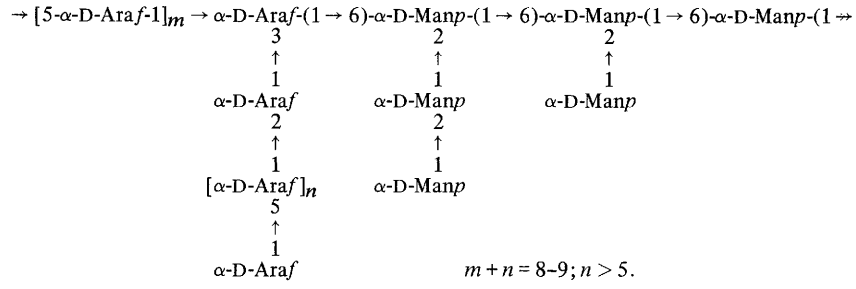
[205] G. Dooijewaard, F. F. Roossien, and C. T. Robillard, *Biochemistry*, 1979, **18**, 2990.
[206] N. Skaug and T. Hofstad, *Current Microbiol.*, 1979, **2**, 369.
[207] W. Kondo, N. Sato, and T. Ito, *Carbohydr. Res.*, 1979, **70**, 117.
[208] J. Steber and K. H. Schleifer, *Arch. Mikrobiol.*, 1979, **123**, 209.

saccharides that are related to known 3-*O*-methyl-D-mannose-containing polysaccharides.[209] A homologous series of oligosaccharides has been isolated, consisting predominantly of (1→4)-3-*O*-methyl-α-D-mannopyranosyl residues, terminated at the non-reducing end by D-mannopyranosyl residues or 3-*O*-methyl-α-D-mannopyranosyl residues and terminated at the reducing end of the chain by a methyl aglycone. These oligosaccharides are probably biosynthetic precursors of the larger 3-*O*-methyl-D-mannan homologues. ^{1}H N.m.r. spectroscopic studies suggest that the 3-*O*-methyl-D-mannan undergoes a major change in conformation on complexation with palmitic acid; the change is suggested to result from the tightening of a loosely coiled polymer.[210] The fatty acid is orientated, at least part of the time, with its carboxy-group near the *O*-methyl groups of the polysaccharide. The phylogenetically closely related bacterium, *Streptomyces griseus*, also produces an *O*-methyl-D-mannose-containing polysaccharide, which in addition contains approximately four esterified acetyl groups. These *O*-acetyl groups in the polysaccharide are located at position 6 of approximately half of the ten contiguous (1→4)-3-*O*-methyl-α-D-mannosyl residues.[211] The affinity of this polysaccharide for palmitic acid suggests that it plays a role similar to the 3-*O*-methyl-D-mannose-containing polysaccharide of *M. smegmatis* in its regulation of the bacterium's fatty acid synthase.

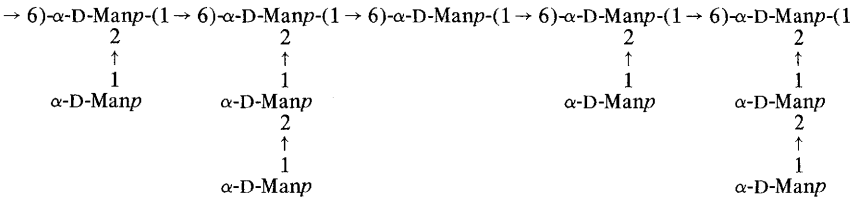
An acidic arabino-D-mannan isolated from *M. smegmatis* also contains two phosphate, six monoesterified succinate, and four ether-linked lactate groups per molecule.[212] Saponification of the polysaccharide releases a phosphorylated- and a non-phosphorylated polysaccharide, the main features of which are the presence of chains of contiguous (1→5)-arabinofuranosyl residues attached to O-4 of L-arabinopyranosyl residues. A serologically active D-arabino-D-mannan, isolated from the cells of *M. tuberculosis*, is composed of (1→5)-α-D-arabinofuranosyl residues and (1→6)- and (1→2)-D-mannopyranosyl residues.[213] Methylation analysis and enzymic degradation studies established the presence of a structure (23) containing short side-chains of (1→2)-α-D-mannosyl residues attached to a main backbone of (1→6)-α-D-mannopyranosyl residues. In addition, a serologically inactive α-D-mannan whose structure (24) resembles that of the core L-arabino-D-mannan has been isolated.

In addition to a high molecular weight lipopolysaccharide, the cell surface of *Rhizobium* contains a low molecular weight (1→2)-β-D-glucan.[214] Curie point m.s. of a polysaccharide isolated from the cell walls of the cyanobacterium *Spirulina platensis* resembles that of the (1→2)-β-D-glucan of *Agrobacterium tumefaciens*.[215] It is speculated that this polysaccharide may be the innermost of four layers of the cell wall.

[209] H. Yamada, R. E. Cohen, and C. E. Ballou, *J. Biol. Chem.*, 1979, **254**, 1972.
[210] K. K. Yabusaki, R. E. Cohen, and C. E. Ballou, *J. Biol. Chem.*, 1979, **254**, 7282.
[211] B. E. Kari and G. R. Gray, *J. Biol. Chem.*, 1979, **254**, 3354.
[212] P. L. Weber and G. R. Gray, *Carbohydr. Res.*, 1979, **74**, 259.
[213] A. Misaki, I. Azuma, and Y. Yamamura, *J. Biochem. (Jpn.)*, 1977, **82**, 1759.
[214] L. P. T. M. Zevenhuizen and H. J. Scholten-Koerselman, *J. Microbiol. Serol.*, 1979, **45**, 165.
[215] C. Vaneykelenburg, *J. Microbiol. Serol.*, 1978, **44**, 321.

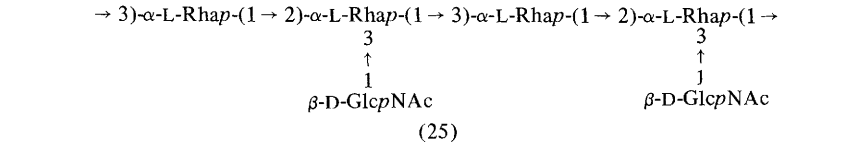


(23)

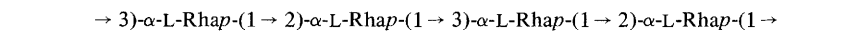


(24)

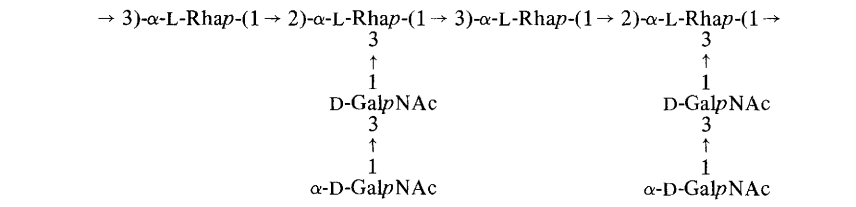
Chemical and immunochemical studies have been used in the assignment of the structures (25)–(27) of the group-specific carbohydrates of Groups A, A-variant, and C streptococci, respectively.[216] A close relationship among the L-rhamnan structures is revealed. Human antibodies to Group A and Group C carbohydrate have been measured in a radioimmunoassay system.[217] The Group-specific antibodies arise after infection or persistent colonization of the pharynx with either Group A or Group C streptococci.



(25)



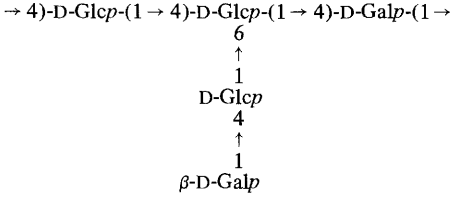
(26)



(27)

[216] J. E. Coligan, T. J. Kindt, and R. M. Krause, *Immunochemistry*, 1978, **15**, 755.
[217] B. Aasted, D. Bernstein, D. G. Klapper, A. El Kholy, and R. M. Krause, *Scand. J. Immunol.*, 1979, **9**, 61.

Several characteristic differences in the chemical composition of the cell walls among some serological types of *Streptococcus mutans* have been investigated through their susceptibility to *Flavobacterium* L-11 enzyme.[218] Generally, non-peptidoglycan components of the walls are responsible for serological differences in bacterial strains. The glycan isolated from *Streptococcus faecalis*, the structure of which (28) has been previously established, behaves abnormally on periodate oxidation.[219] Cyclic acetals are formed following periodate oxidation and this leads to the protection of hydroxy-groups during methylation. Inter-residue hemiacetals were identified between the aldehyde groups at C-2 or C-3 of oxidized residues and hydroxy-groups at C-3 or C-2 of an adjacent unoxidized residue.



(28)

The purification of *b*-antigen of *Streptococcus sanguis* has been achieved by separation from teichoic acid by affinity chromatography on immobilized anti-poly(glycerol phosphate) columns.[220] The antigen, which contains residues of D-glucose, L-rhamnose, and 2-acetamido-2-deoxy-D-glucose, appears to contain a single antigenic determinant related to isomaltose. A new Group-specific antigen, designated W-antigen, has been characterized for five strains of *Streptococcus sanguis*.[221] Two routes exist in *Streptococcus faecalis* for the synthesis of TDP-D-galactose, an inducible and a constitutive route.[222] Incorporation studies with TDP-D-[^{14}C]hexoses have shown that the sugar nucleotide serves as a donor for D-galactosyl moieties for the synthesis of immunogenic glycans in the cell wall of *Streptococcus faecalis*.

Antibodies to a heteroglycan of *Streptococcus bovis* have been purified by an affinity chromatographic method.[223]

7 Fungal Polysaccharides

Specific areas of the chemistry and biochemistry of fungi have been reviewed: the autolysis of fungi including the degradation of carbohydrates,[224] the bio-

[218] M. Inoue, S. Hamada, T. Ooshima, S. Kotani, and K. Kato, *Microbiol. Immunol.*, 1979, **23**, 319.
[219] L. S. Forsberg and J. H. Pazur, *Carbohydr. Res.*, 1979, **75**, 129.
[220] B. Appelbaum and B. Rosan, *Infect. Immun.*, 1978, **21**, 896.
[221] S. D. Henrisken and J. Eriksen, *Immunochemistry*, 1978, **15**, 761.
[222] J. H. Pazur, *J. Carbohydr., Nucleosides, Nucleotides*, 1978, **5**, 443.
[223] J. H. Pazur, Y. Tominaga, K. L. Dreher, L. S. Forsberg, and B. M. Romanic, *J. Carbohydr., Nucleosides, Nucleotides*, 1978, **5**, 1.
[224] K. H. Riemay and R. Tröger, *Z. Allg. Mikrobiol.*, 1978, **18**, 617.

synthesis of fungal cell walls,[225] the biochemistry of dimorphism in *Mucor*,[226] and cell wall and hyphal growth.[227]

Fractions from the cell wall and protoplasts of *Aspergillus fumigatus* have been shown to contain common antigenic components.[228] Differences in both immunological reactivity and chemical structure may be linked to their cellular location and may reflect incomplete structures in the process of biosynthesis and transfer to wall sites where they become immobilized.

Electron microscopic studies of the conidial wall surface of the deuteromycetes *Cladobotrytum varium*, *Cladosporium macrocarpum*, *Aspergillus niger*, and *Penicillium* species, and of the sporangiophore surface of the zygomycete *Mycotypha microspora* have been reported.[229] The wall layers, isolated from *A. niger*, exhibited distinct differences in total carbohydrate, monosaccharide, amino-sugar, peptide, and lipid composition. In *Phycomyces blakesleeanus*, during the formation of the germ-tube cell-wall, the composition shifts from that of a D-glucan, in the formant spore wall, to that of a chitin-like polymer in the mycelial wall.[230] Heat-induced germination of the sporangiophore results in an increase in the content of chitin and a decrease in the D-glucan contents.[231]

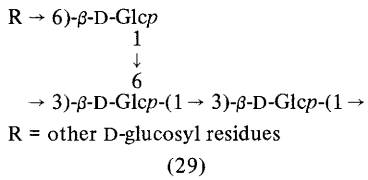
In the presence of 2-deoxy-D-arabinohexose, *Rhodosporidium toruloides* synthesizes higher proportions of D-glucan and chitin, while the relative contents of D-mannan and D-galactan decrease drastically.[232]

D-Glucans. – A study designed to ascertain whether nigeran might serve in determining phylogenetic relationships within the large and complex genera *Aspergillus* and *Penicillium* has shown that the nigeran content, used in conjunction with traditional morphological characteristics, can provide a useful and convenient tool for the determination of natural groupings of these two species.[233]

An alkali-soluble D-glucan which contains minor proportions of D-xylose, D-mannose, 2-amino-2-deoxy-D-glucose, and amino-acids has a large proportion of (1→4)-α-D-glucopyranosyl residues and a small proportion of (1→3)- and (1→6)-β-D-glucosyl residues.[234] On the basis of the identification of 2-*O*-α-D-glucopyranosylerythritol, it appears that segments of the (1→4)-α-D-glucopyranosyl residues are joined by single (1→3)-linkages.

The relationship between the chemical structure and anti-tumour activity of D-glucans prepared from *Grifora umbellata* has been investigated.[235] The anti-tumour D-glucans appear to contain the basic structural unit (29) although the length of the branches, branching frequency, molecular size, and molecular

[225] V. Farkaš, *Microbiol. Rev.*, 1979, **43**, 117.
[226] P. S. Sypherd, P. G. Borgia, and J. L. Paznokas, *Adv. Microb. Physiol.*, 1978, **18**, 67.
[227] A. P. J. Trinci, *Sci. Prog.*, 1978, **65**, 75.
[228] V. M. Hearn and D. W. R. Mackenzie, *J. Gen. Microbiol.*, 1979, **112**, 35.
[229] G. T. Cole, T. Sekiya, R. Kasai, T. Yokoyama, and Y. Nozawa, *Exp. Mycol.*, 1979, **3**, 132.
[230] A. J. Van Laere, A. R. Carlier, and A. J. Van Assche, *Arch. Mikrobiol.*, 1977, **112**, 303.
[231] B. Furch and L. Pambor, *Microbios Lett.*, 1978, **8**, 71.
[232] M. Sipiczki and V. Farkaš, *Folia Microbiol.*, 1979, **24**, 389.
[233] T. F. Bobbitt, and J. H. Nordin, *Mycologia*, 1978, **70**, 1201.
[234] C. B. Bottom and D. J. Siehr, *Carbohydr. Res.*, 1979, **77**, 169.
[235] T. Miyazaki, N. Oikawa, T. Yadomae, H. Yamada, Y. Yamada, H. Y. Hsu, and H. Ito, *Carbohydr. Res.*, 1979, **69**, 165.



(29)

conformation are also important. An extracellular (1 → 3)-β-D-glucan (mol. wt. 7×10^4) with single (1 → 6)-β-D-glucosyl side chains on every second D-glucosyl residue of the main chain has been isolated from *Monilinia fructigena*.[236]

Alkali-soluble and alkali-insoluble polysaccharides from the fruiting bodies of the basidiomycetes *Laetiporus sulphureus* and *Piptoporus betulinus* have been examined by *X*-ray diffraction, u.v. spectroscopy, and chemical methods.[237] The presence of (1 → 3)-α-D-glucan, (1 → 3)-β-D-glucan, and chitin was established. Variations in the levels of these polysaccharides were noted in different parts of the fungal structure.

Potato, red kidney bean, and soybean plants respond to highly purified β-D-glucan from *Phytophthora megasperma* by accumulating phytoalexins.[238] The plants appear to respond to the fungus following recognition of the same mycelial wall component and it is possible that plants may respond to a variety of fungi by recognizing the same or similar mycelial wall components.

Cell-wall formation in *Saccharomyces cerevisiae* appears to be the result of two main patterns of deposition of wall material, *viz.*, around the whole periphery of the non-budding cells and mainly at the tip of the daughter cell, or at the cross-wall that separates dividing cells.[239] Synthesis and secretion of β-D-glucan is unaffected by inhibition of RNA and protein synthesis. Growth of *S. cerevisiae* under conditions of phosphate limitations results in a decreased content of D-glucan and increased protein content as compared with control cells.[240]

Papulacandin B, an antibiotic produced by the deuteromycete *Papularia sphaerosperma*, inhibits the growth of yeasts, but has no effect on other fungi, bacteria, or protozoa.[241] The mode of action involves the inhibition of synthesis of alkali-insoluble β-D-glucan of *Saccharomyces cerevisiae* and *Candida albicans* in sphaeroplasts and causes lysis of cells by osmotic rupture.[241] Echinocandin B and a structurally related antibiotic aculeacin A also inhibit β-D-glucan synthesis in yeast, possibly in a similar manner.[242] Papulacandin B has been shown to inhibit the transfer of D-glucose from UDP-D-glucose, but not D-mannose from GDP-D-mannose, into dolichyl phosphate.[243]

[236] F. Santamaria, F. Reyes, and R. Lahoz, *J. Gen. Microbiol.*, 1978, **109**, 287.
[237] J. Jelsma and D. R. Kreger, *Arch. Mikrobiol.*, 1978, **119**, 249.
[238] K. Cline, M. Wade, and P. Albersheim, *Plant Physiol.*, 1978, **62**, 918
[239] L. Rodriguez, F. Laborda, and R. Sentandreu, *Curr. Microbiol.*, 1979, **2**, 293.
[240] A. M. Ramsay and L. J. Douglas, *J. Gen. Microbiol.*, 1979, **110**, 185.
[241] B. C. Baguley, G. Römmele, J. Gruner, and W. Wehrli, *Eur. J. Biochem.*, 1979, **97**, 345.
[242] J. Mizoguchi, T. Saito, K. Mizuno, and K. Hayano, *J. Antibiotics*, 1977, **30**, 308.
[243] B. C. Baguley, *Experientia*, 1978, **34**, 1667.

Some properties of the β-D-glucan synthetase from *Saccharomyces cerevisiae* have been described.[244] UDP-D-Glucose is a competitive inhibitor and D-glucono-1,4-lactone is a non-competitive inhibitor of this enzyme.

High yields of laminaribiose (3-*O*-β-D-glucopyranosyl-D-glucose) have been achieved by treatment of the (1→3)-β-D-glucan of *Saccharomyces cerevisiae* with an *endo*-(1→3)-β-D-glucanase from *Kluyveromyces phaseolosporus*.[245]

Glycogen metabolism in resting and growing cells of *Saccharomyces carlsbergensis* has been shown to be regulated by a variety of endogenous and environmental factors.[246]

A ^{13}C n.m.r. study of the gel networks of the branched (1→3)-β-D-glucans lentinan (from *Lentinus edodes*) and schizophyllan (from *Schizophyllum commune*) has been reported.[247] Conformational changes of the helical to random-coil forms were induced by treatment with aqueous sodium hydroxide.

The effect of low pH on the growth of *Aureobasidium pullulans* has been shown to prevent transformation of the cells into the yeast phase, with the elaboration of small amounts of pullulan.[248]

The main degradation product (75–90%) resulting from the action of selected α-amylases on the α-D-glucan of *Elsinoe leucospila* has been identified as the trisaccharide *O*-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→4)-D-glucose.[249] This confirms previous reports that the polysaccharide consists chiefly of maltotriose units and a small proportion of maltotetraose units joined by (1→3)-α-D-glucosyl linkages.

Cell extracts of *Cryptococcus laurentii*, a yeast which synthesizes amylose when grown at low pH, contain an α-D-glucan phosphorylase (EC 2.4.1.1) which has been characterized.[250] Evidence for a possible role of this enzyme in the biosynthesis of amylose was reported.

The ability of yeast cells to liberate sphaeroplasts following treatment with (1→3)-β-D-glucanases has been proposed as a taxonomic criterion for differentiating basidiomycetous from ascomycetous yeasts and for classifying the yeasts of the fungi imperfecti.[251]

Purified preparations of extracellular β-D-glucans from *Sphacelia sorghi* and from the cell walls of *Claviceps purpurea* have been shown to contain enzymically active protein.[252] The binding of the protein, which exhibited β-D-fructofuranosidase and acid phosphatase activities, to the D-glucan appeared to be non-covalent. When the enzymes were liberated from the D-glucan they showed enhanced activity, although the K_m values remained the same, suggesting that the matrix acts in the same manner as a non-competitive inhibitor. The solubility characteristics of β-D-glucans in the fungal cell wall have been shown to

[244] E. Lopez-Romero and J. Ruiz-Herrera, *J. Microbiol. Serol.*, 1978, **44**, 329.
[245] T. G. Villa, H. J. Phaff, and V. Notario, *Carbohydr. Res.*, 1979, **74**, 369.
[246] J. U. Becker, H. J. Vohmann, and C. Eilers-König, *Arch. Mikrobiol.*, 1979, **123**, 143.
[247] H. Saito, T. Ohki, and T. Sasaki, *Carbohydr. Res.*, 1979, **74**, 227.
[248] K. Ono, N. Yasuda, and S. Ueda, *Agric. Biol. Chem. (Jpn.)*, 1977, **41**, 2113.
[249] Y. Tsumuraya and A. Misaki, *J. Appl. Biochem.*, 1979, **1**, 235.
[250] M. S. Foda and H. J. Phaff, *Z. Allg. Mikrobiol.*, 1978, **18**, 95.
[251] J. M. Bastide, E. H. Hadibi, and M. Bastide, *J. Gen. Microbiol.*, 1979, **113**, 147.
[252] A. G. Dickerson and R. C. F. Baker, *J. Gen. Microbiol.*, 1979, **112**, 67.

undergo changes after specific removal of chitin.[253] Two types of β-D-glucans were isolated, one water-soluble and highly branched, and the other alkali-soluble and with single residue branches.

D-Mannans. – Six (1 → 6)-α-D-manno-oligosaccharides have been isolated after partial acid hydrolysis of proteolytic digests of an extracellular glycan of *Absidia cylindrospora*.[254] A pentasaccharide and higher molecular weight oligosaccharides inhibited significantly the precipitation reaction of intracellular glycans from *A. cylindrospora*, *Mucor hiemalis*, and *Rhizopus nigricans* with anti-*A. cylindrospora* serum.

On the basis of the ^{1}H n.m.r. spectra of the cell-wall D-mannans, and the base composition and sequence similarities of the deoxyribonucleic acids, no distinction could be made between strains of *Candida utilis* and *Hansenula jadinii* although differences could be detected between *H. jadinii* and *H. petersonii*.[255] The catabolic and anabolic transformation of D-glucose by a *Candida* species into cell wall D-glucan and D-mannan has been reported.[256] Approximately 20–40% of the hexose of these cell wall polysaccharides has been derived from the resynthesis of hexoses *via* the pentose phosphate pathway.

Methylation analysis and periodate oxidation studies have been used to characterize the extracellular β-D-mannan produced by *Rhodotorula rubra* as being composed of alternating (1 → 3)- and (1 → 4)-β-D-mannopyranosyl residues.[257] Of eight oligosaccharides isolated from the cell walls of *R. glutinis* after lysis with an enzyme from *Penicillium lilacinum*, two have been characterized as 3-*O*-β-D-mannopyranosyl-D-mannose and 4-*O*-β-D-mannopyranosyl-D-mannose.[258] The remaining oligosaccharides, which contained both D-glucose and D-mannose, were composed of (1 → 3)- and (1 → 4)-β-D-mannopyranosyl residues with (1 → 6)-β-D-glucopyranosyl side chains.

The transformation of D-glucose by a *Candida* species has been followed catabolically and anabolically.[259] Maximal incorporation into D-glucan and D-mannan is only achieved at a critical concentration of D-glucose. Dolichyl monophosphate-D-mannose is not an intermediate in the synthesis of the outer chains of the D-mannan-protein of *Saccharomyces cerevisiae* and dolichyl diphosphate oligosaccharides are synthesized by different D-mannosyltransferases.[260]

An osmotically-sensitive mutant of *Saccharomyces cerevisiae* S288 contains more β-D-glucan, less α-D-mannan, and less alkali-soluble glycogen than the parent strain.[261] The D-mannan has been characterized as containing more short

[253] J. H. Sietsma and J. G. H. Wessels, *J. Gen. Microbiol.*, 1979, **114**, 99.
[254] T. Miyazaki, O. Hayashi, Y. Ohshima, and T. Yadomae, *J. Gen. Microbiol.*, 1979, **111**, 417.
[255] P. L. Manachini, *J. Microbiol. Serol.*, 1979, **45**, 451.
[256] K. H. Rademacher and Y. Koch, *Z. Allg. Mikrobiol.*, 1979, **19**, 65.
[257] N. P. Elinov, G. A. Vitovskaya, V. A. Marikhin, Y. B. Marjukhta, and T. V. Kozlova, *Carbohydr. Res.*, 1979, **75**, 185.
[258] M. Arai and S. Murao, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 1651.
[259] B. Röber and G. Reuter, *Z. Allg. Mikrobiol.*, 1979, **19**, 187.
[260] A. J. Parodi, *J. Biol. Chem.*, 1979, **254**, 8343.
[261] G. Reuter, U. Märkisch, V. Hering, and P. V. Venkov, *Z. Allg. Mikrobiol.*, 1979, **19**, 411.

side-chains and fewer long side-chains than does the parent D-mannan and also contains a different proportion of (1 → 2)- and (1 → 3)-α-D-mannosyl residues.

Chitin. – The ability of lytic β-D-glucanases of *Arthrobacter* GJM-1 to dissolve the cell wall of *Saccharomyces cerevisiae* with the exception of the chitin component has been reported.[262] Electron microscopic examination of such wall residues isolated from various stages of the budding cycle shows a correspondence to the formation of an annular structure found as part of the bud scar after cell division. This annular chitin-rich structure could not be isolated at either cell-cycle stages preceding the bud emergence or at the earliest stages of bud development. Observations confirmed that the annular structure (chitin ring) formed during bud growth represents a major part of the total chitin present in the bud scar after septum closure.

Chitin synthetase (EC 7.4.1.16) has been located, mostly in an inactive form, associated with membranous fractions of *Neurospora crassa*, and could be activated either endogenously or by addition of exogenous proteases.[263]

Plasma membranes from *Schizophyllum commune* protoplasts have been stabilized against fragmentation by coating the protoplasts with concanavalin A.[264] Approximately half of the chitin synthase activity is associated with the plasma membranes in the form of a zymogen and could not be detached from the membranes. It is suggested that this inactive form represents the cytoplasmic transport form of the enzyme. The nature and location of proteases of *Aspergillus nidulans* and the interaction of one of these with the zymogen of chitin synthetase have been described.[265]

A potential industrial method for the production and isolation of chitosan from the hyphal walls of *Mucor rouxii* as an alternative to the product derived from shellfish chitin has been reported.[266]

Miscellaneous Polysaccharides. – An extracellular polysaccharide of *Aspergillus parasiticus* has been characterized as being a homopolymer of 2-amino-2-deoxy-D-galactosyl residues, of which approximately 50% are *N*-acetylated and the remainder unsubstituted.[267] The occurrence of the free amino-groups may result from enzymic *N*-deacetylation, since an enzyme isolated from the organism is capable of *N*-deacetylation of oligosaccharides containing fourteen or more 2-acetamido-2-deoxy-D-galactosyl residues, at the same rate as the polysaccharide is degraded. A D-galacto-D-mannan, isolated from the mycelia of *A. fumigatus*, reacts with antibodies specific for circulating antigen in invasive aspergillosis in humans and rabbits.[268] The polysaccharide is composed of a backbone of (1→6)-D-mannopyranosyl residues with trisaccharide side chains terminated with residues of D-galactofuranose. An enzyme-linked immunoadsorbent assay (ELISA)

[262] M. Vršanská, Z. Krátký, P. Biely, and S. Machala, *Z. Allg. Mikrobiol.*, 1979, **19**, 357.
[263] A. Arroyo-Begovich, and J. Ruiz-Herrera, *J. Gen. Microbiol.*, 1979, **113**, 339.
[264] C. A. Vermeulen, M. B. J. M. Raeven, and J. G. H. Wessels, *J. Gen. Microbiol.*, 1979, **114**, 87.
[265] J. M. Campbell and J. F. Peberdy, *FEMS Microbiol. Lett.*, 1979, **6**, 65.
[266] S. A. White, P. R. Farina, and I. Fulton, *Appl. Environ. Microbiol.*, 1979, **38**, 323.
[267] Y. Araki, H. Takada, N. Fujii, and E. Ito, *Eur. J. Biochem.*, 1979, **102**, 35.
[268] E. Reiss and P. F. Lehmann, *Infect. Immun.*, 1979, **25**, 357.

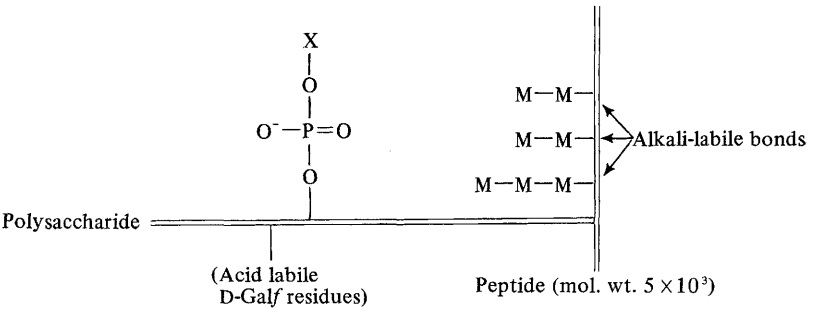
for immunoglobulin (IgG and IgE) antibodies to protein and antigens of *A. fumigatus* has been reported.[269]

D-Mannans, D-gluco-D-mannans, and D-galacto-D-mannans have been identified in phytopathogenic strains of *Ceratocystis paradoxa* and *C. fimbriata* isolated from different hosts, including tropical fruits and sugar cane.[270] Isolates of *Ceratocystis* within the same species were found to synthesize different polysaccharides, depending on their original hosts. Different polysaccharides could also be synthesized by the same strain at different temperatures. No correlation was made between host specificity and synthesis of a specific polysaccharide. It is possible that adherence of the fungus to the plant cells involves either specific or non-specific affinity reactions, which in turn do not correlate with the capacity of the micro-organism to cause disease.

In a study of the carbohydrate composition of *Geotrichum, Trichosporon*, and allied genera, the intact cells of *Geotrichum* were characterized as containing predominantly D-galacto-D-mannans.[271]

L-Iduronic acid and D-glucuronic acid have been identified by g.l.c.–m.s. of the *O*-TMS ethers of the corresponding hexuronolactones prepared from the glycuronan 'protuberic acid' from *Kobayasia nipponica*.[272]

A family of glycopeptides of disperse molecular weights, charge, and sugar composition have been isolated from the culture filtrates of *Fulvia fulvia*.[273] D-Galactose, D-glucose, and D-mannose were identified as the major sugar components, together with smaller amounts of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, D-glucuronic acid, and diester phosphate. The molecular organization of these polymers was investigated using mild alkali- and acid-hydrolysis, and acetolysis of the residual polysaccharide. The proposed structure (30) is similar to that suggested for the peptidophosphogalactomannan isolated from *Penicillium charlesii*.



X = residues of D-Glc, D-Man, and D-Gal

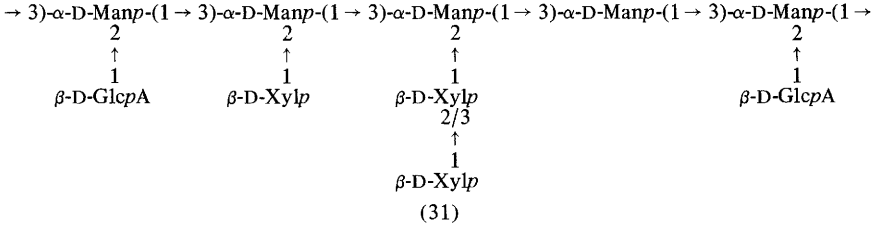
(30)

[269] R. Sepulveda, J. L. Longbottom, and J. Pepys, *Clin. Allergy.*, 1979, **9**, 359.
[270] C. S. Alviano, P. A. J. Gorin, and L. R. Travassos, *Exp. Mycol.*, 1979, **3**, 174.
[271] A. C. M. Weijman, *J. Microbiol Serol.*, 1979, **45**, 119.
[272] T. Miyazaki, H. Tsuchihashi, H. Yamada, and T. Yadomae, *Carbohydr. Res.*, 1979, **77**, 281.
[273] J. M. Dow and J. A. Callow, *J. Gen. Microbiol.*, 1979, **113**, 57.

The pathogenic fungus of rice blast disease, *Piricularia oryzae*, contains a cell-wall heteroglycan–protein complex containing residues of D-mannose, D-glucose, and D-galactose in the molar ratio 6 : 2 : 1.[274] Methylation analysis, partial acid hydrolysis, and digestion with an α-D-mannanase have shown the presence in the carbohydrate portion of a backbone of (1→6)-α-D-mannopyranosyl residues with side chains terminated by D-glucopyranosyl-, D-galactofuranosyl-, (1→2)- and (1→3)-α-D-mannopyranosyl residues. The heteroglycan is attached to the protein *via* O^3-D-mannopyranosyl-L-serine or O^3-D-mannopyranosyl-L-threonine linkages. Further studies have shown that the side chains comprise one to four D-mannosyl units, some of which are terminated by D-glucosyl- or D-galactofuranosyl-residues.[275]

Histochemical staining at the cell surface of yeast-like forms, hyphae, and conidia of *Sporothrix schenckii* cell-types with iron(III) hydroxide and ferritin has been shown to correspond to the locations of concanavalin A-reactive peptido-L-rhamno-D-mannan complexes in the cells.[276]

Several acidic oligosaccharides, isolated from the acidic heteroglycan of the edible mushroom *Tremella fuciformis*, have been characterized by methylation analysis and ^{1}H n.m.r. spectroscopy and the structure (31) has been proposed for the parent polysaccharide.[277] A similar structure has been proposed for a cell-wall polysaccharide isolated from growing cultures of haploid cells of two strains of *T. fuciformis*; two extracellular polysaccharides showed only minor variations from this structure.[278]



(31)

Water-soluble polysaccharides have been isolated from cultures of lichen mycobionts and phycobionts.[279] The polysaccharides of the mycobionts show close similarities with those of the parent lichens, whereas the polysaccharides of the phycobionts possess different features. The mycobiont of *Parmelia caperta* contained an α-D-glucan having equal proportions of (1→3)- and (1→4)-linkages, and identical to a D-glucan of the parent lichen. The phycobiont of *Ramalina crassa* contains a major water-soluble D-galactan, whose properties are different from those of the intact parent lichen and its mycobiont.

[274] T. Nakajima, K. Tamari, and K. Matsuda, *J. Biochem. (Jpn.)*, 1977, **82**, 1647.
[275] T. Nakajima, H. Sasaki, M. Sato, K. Tamari, and K. Matsuda, *J. Biochem. (Jpn.)*, 1977, **82**, 1657.
[276] M. Benchimol, W. De Souza, and L. R. Travassos, *Infect. Immun.*, 1979, **24**, 912.
[277] S. Ukai, T. Kiho, and C. Hara, *Chem. Pharm. Bull. (Jpn.)*, 1978, **26**, 3871.
[278] M. Kakuta, Y. Sone, T. Umeda, and A. Misaki, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 1659.
[279] K. Takahashi, T. Takeda, and S. Shibata, *Chem. Pharm. Bull. (Jpn.)*, 1979, **27**, 238.

5

Glycoproteins, Glycopeptides, and Animal Polysaccharides

BY R. J. STURGEON

A number of specialist books dealing with a variety of aspects of glycoproteins have been published. One volume of a two volume treatise on the glycoconjugates has dealt with general aspects of analysis and structural determination, and mucous and blood glycoproteins.[1] The second volume includes material on proteoglycans and structural glycoproteins, glycoprotein biosynthesis, glycoprotein catabolism, surface membranes, and immunological aspects and lectins.[2] A book entitled 'Complex Carbohydrates of Nervous Tissue' covers the area of nervous tissue complex carbohydrates from the standpoints of structure, biosynthesis, metabolism, and localization at the cellular and subcellular levels.[3] The series 'Methods in Enzymology' has continued with a volume devoted to complex carbohydrates, and includes methods for the isolation and identification of glycoproteins and antigens.[4]

The similarity of the structures of the terminal carbohydrate sequences of glycoproteins and glycolipids have been reviewed.[5]

1 Microbial Glycoproteins

Bacterial and Fungal Glycoproteins. – No evidence has been found to substantiate the claim for the presence of covalently-bound carbohydrate in energy-transducing adenosine triphosphatases of *Escherichia coli*.[6] Incomplete removal of sodium dodecyl sulphate (SDS) after SDS-electrophoresis of the protein results in a positive reaction with the periodate–Schiff staining method.

Tunicamycin and 2-deoxy-D-*arabino*-hexose have been shown to inhibit the *in vivo* glycosylation of polypeptides in polysomes from *Aspergillus niger*, but have no effect on the *in vitro* glycosylation.[7] Although no new oligosaccharide chains were synthesized, pre-existing chains were extended.

[1] 'The Glycoconjugates, Vol. I, Mammalian Glycoproteins and Glycolipids', ed. M. I. Horowitz and W. Pigman, Academic Press, New York, 1977.
[2] 'The Glycoconjugates, Vol. 2, Mammalian Glycoproteins, Glycolipids, and Proteoglycans', ed. M. I. Horowitz and W. Pigman, Academic Press, New York, 1978.
[3] 'Complex Carbohydrates of Nervous Tissue', ed. R. U. Margolis and R. K. Margolis, Plenum Press, New York, 1979.
[4] *Methods Enzymol.*, 1978, **50**.
[5] H. Rauvala and J. Finne, *FEBS Lett.*, 1979, **97**, 1.
[6] C. M. Nalin, R. L. Cross, J. J. Lucas, and W. E. Kohlbrenner, *FEBS Lett.*, 1979, **104**, 209.
[7] S. D. Long and M. J. Rudick, *Arch. Biochem. Biophys.*, 1979, **198**, 212.

The applicability of natural abundance ^{13}C n.m.r. spectroscopy to studies on the structure and mobility of the carbohydrate residues of glycoproteins has been evaluated by examining the spectra at 63.4 kG of native and denatured glucoamylase from *A. niger*.[8] Integrated intensities reveal an average of about one hundred and twenty carbohydrate residues per molecule, composed of about eight α-D-mannopyranosyl, ten α-D-glucopyranosyl, and a maximum of fifteen β-D-mannopyranosyl residues. All of the observed carbohydrate residues are involved in *O*-glycosidic linkages to L-serine, L-threonine, or other carbohydrate residues.

Although the neutral- and amino-sugar composition of the plasma membrane of *Dictyostelium discoideum* has been observed to remain unchanged during the early stages of differentiation, the amounts of glycoproteins increase and that of one particular component (mol. wt. 1.25×10^5) decreased dramatically.[9] More than half of the spherule-wall fragments of the slime mould *Physarum polycephalum* are composed of a glycoprotein fraction.[10] In addition, proteoglycans having a high carbohydrate to protein ratio were identified.

A family of glycopeptides having disperse molecular weights, charge, and sugar composition have been isolated from the culture filtrates of the tomato-leaf fungal pathogen *Fulvia fulvia*.[11] D-Galactose, D-glucose, and D-mannose are the principal sugars with smaller amounts of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and D-glucuronic acid. The proposed structure (see Chapter 4, p. 52, Fig. 30) is similar to that suggested for the peptidophospho-D-galacto-D-mannan secreted from *Penicillium charlesii*.

The extension of the oligosaccharide chains of the glycoprotein of *Neurospora crassa* does not require the presence of a lipid intermediate.[12] After the synthesis of the simple D-mannosyl-protein core, a second D-mannosyl unit is transferred directly from GDP-D-mannose. Different patterns of glycosylation have been identified for the *exo*-(1 → 3)-β-D-glucanases and *endo*-(1 → 3)-β-D-glucanases of *Pichia polymorpha*.[13] Contrary to other reports, the *endo*-(1 → 3)-β-D-glucanases like the *exo*-(1 → 3)-β-D-glucanases are glycoproteins, containing residues of D-glucose and D-mannose. Studies on the excretion of these enzymes by both protoplasts and whole cells appear to support the hypothesis that glycosylation takes place before or during secretion of the enzymes into the periplasmic space and no further glycosylation occurs during their secretion into the supernatant culture.

The properties of β-D-fructofuranosidase (invertase) of *Saccharomyces cerevisiae*, and of the modified enzyme after removal of about 90% of the carbohydrate residues, have been found to differ considerably, not only in stability but also in their capacity to be renatured following inactivation by guanidinium chloride.[14] The covalently bound carbohydrate of yeast β-D-fructo-

[8] K. Dill and A. Allerhand, *J. Biol. Chem.*, 1979, **254**, 4524.
[9] N. R. Gilkes, K. Laroy, and G. Weeks, *Biochim. Biophys. Acta*, 1979, **551**, 349.
[10] K. Zaar, P. Beyer, and H. Kleinig, *Biochim. Biophys. Acta*, 1979, **582**, 21.
[11] J. M. Dow and J. A. Callow, *J. Gen. Microbiol.*, 1979, **113**, 57.
[12] M. M. Gold and H. J. Hahn, *Phytochemistry*, 1979, **18**, 1269.
[13] T. G. Villa, V. Notario, and J. R. Villanueva, *J. Gen. Microbiol.*, 1978, **109**, 371.
[14] F. K. Chu, R. B. Trimble, and F. Maley, *J. Biol. Chem.*, 1978, **253**, 8691.

furanosidase has been shown to be involved in the promotion of folding of the protein to its most stable conformation.[15] After removal by *endo*-β-2-acetamido-2-deoxy-D-glucanase H of about 90% of the carbohydrate associated with this enzyme, no significant differences were detected in the catalytic and physical properties of the carbohydrate-depleted and the unmodified enzyme, although the former was much less stable to multiple freeze–thaw treatment, and to digestion by proteolytic enzymes. Many proteins and glycoproteins secreted by mutants of *S. cerevisiae* deficient in killer toxin expression show altered electrophoretic mobility and isoelectric points when compared with those of the parent strain.[16] The observed differences indicate that secretion in these mutants is abnormal, probably at the level of protein processing.

An unusual glycoprotein, isolated from the spores of *Ustilago tritia*, contains residues of L-fucose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, D-galactose, D-glucose, and D-mannose.[17]

2 Viral Glycoproteins

The following aspects of viral glycoproteins have been reviewed; glycoprotein and protein precursors to plasma membranes in vesicular stomatitis virus infected HeLa cells,[18] membrane glycoproteins of enveloped viruses,[19] membrane assembly, including the synthesis and intracellular processing of vesicular stomatitis virus,[20] and the structure and replication of α-viruses.[21]

The adherence of streptococci to canine kidney cells has been shown to involve the recognition of viral haemagglutinin, a glycoprotein whose synthesis is blocked by tunicamycin and streptovirudin.[22] Antibody in the sera of chickens has been measured for reactivity to determinants of the envelope glycoprotein of endogenous and exogenous avian tumour viruses.[23] The pattern of reactivity of animals infected with exogenous sarcoma virus served to define a class of determinants present on the envelope glycoprotein of exogenous viruses and absent from that of the endogenous virus Rous-associated virus-O.

Significant changes in the transport of D-glucose and in glycolysis have been observed after treatment of normal chicken fibroblasts with the major glycoprotein from avian myoblastosis virus.[24] An avian sarcoma virus envelope glycoprotein (mol. wt. 8.5×10^4), which specifically binds to chick embryo

[15] J. A. Brown, H. I. Segal, F. Maley, R. B. Trimble, and F. Chu, *J. Biol. Chem.*, 1979, **254**, 3689.
[16] D. T. Rogers, D. Saville, and H. Bussey, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 187.
[17] Nasir-Ud-Din, J. K. Wold, I. Matsumoto, M. I. D. Chughtai, and E. Walker-Nasir, *Phytochemistry*, 1978, **17**, 2059.
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[20] F. N. Katz, J. E. Rothman, D. M. Knipe, and H. F. Lodish, *J. Supramol. Struc.*, 1977, **7**, 353.
[21] L. Kääriäinen and H. Söderlund, *Curr. Top. Microbiol. Immunol.*, 1978, **82**, 15.
[22] Y. T. Pan, J. W. Schmitt, B. A. Sanford, and A. D. Elbein, *J. Bacteriol.*, 1979, **139**, 507.
[23] S. C. Marini, R. R. Friis, and M. S. Halpern, *J. Virol.*, 1979, **29**, 805.
[24] J. D. Papamatheakis and D. J. Marciani, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 2784.

fibroblasts, has been purified with retention of its biological binding activity.[25] A glycoprotein of this type should be useful for the analysis of early events of avian RNA tumour virus infection as well as for the purification and analysis of cellular receptor sites for avian type C viral envelope glycoproteins. Glycopeptides isolated from avian RNA tumour viral glycoproteins have been characterized by their broad molecular weight distribution.[26] The oligosaccharide chains of these glycopeptides are made up of both D-mannose-rich neutral chains and complex chains bearing terminal *N*-acetylneuraminic acid residues. A photoreactive glycolipid probe, 2-[12-(4-azido-2-nitrophenoxy)stearamido]-2-deoxy-D-[1-^{14}C]glucose has been used to map the envelope proteins and glycoproteins of Newcastle disease virus.[27]

The Bunyaviridae is a family of arthropod-borne viruses characterized by possession of genomes consisting of three distinct size classes of RNA, designated L (large), M (medium), and S (small). M (medium) RNA has been shown to code for two viral glycoproteins (G1 and G2) of snowshoe hare (SSH) virus, LaCrosse (LAC) virus, and an LAC/SSH recombinant virus.[28]

A correlation between Epstein–Barr virus membrane antigen and three surface glycoproteins (mol. wts. 2.36, 2.12, and 1.41×10^5) has been established on the basis of radioimmunoprecipitation and immunoadsorption experiments.[29]

The physical location in viral DNA of the templates specifying the major viral glycoproteins of Herpes simplex virus and of mutations causing alterations in the behaviour of infected cells has been reported.[30] In Herpes simplex virus type 1 a glycoprotein (mol. wt. 5.2×10^4) containing an oligo-D-mannosyl core (mol. wt. 1.8×10^3) is processed by further glycosylation and sialylation to produce a more acidic and heterogeneous molecule, which was identified as the type-common envelope glycoprotein D (CP-1 antigen).[31] Three glycoprotein antigens of Herpes simplex virus type 1 react as targets for immunocytolysis reactions in the presence of either complement or mononuclear leukocytes from human peripheral blood.[32]

A glycoprotein with affinity for the Fc region of immunoglobulin G has been isolated from extracts of cultured cells infected with Herpes simplex virus type 1.[33] Evidence is provided that it could account for at least some, and possibly all, of the Fc-binding activity detected on the surfaces of infected cells. The glycoprotein accumulates in two electrophoretically distinct forms (mol. wts. 8.0 and 6.5×10^4) and is distinct from glycoproteins characterized previously from this virus.

[25] C. F. Moldow, F. H. Reynolds, J. Lake, K. Lundberg, and J. R. Stephenson, *Virology*, 1979, **97**, 448.
[26] L. A. Hunt, S. E. Wright, J. R. Etchison, and D. F. Summers, *J. Virol.*, 1979, **29**, 336.
[27] J. S. Bramhall, M. A. Shiflett, and B. J. Wisnieski, *Biochem. J.*, 1979, **117**, 765.
[28] J. R. Gentsch and D. H. L. Bishop, *J. Virol.*, 1979, **30**, 767.
[29] B. C. Strnad, R. H. Neubauer, H. Rabin, and R. A. Mazur, *J. Virol.*, 1979, **32**, 885.
[30] W. T. Ruyechan, L. S. Morse, D. M. Knipe, and B. Roizman, *J. Virol.*, 1979, **29**, 677.
[31] R. J. Eisenberg, C. Hydrean-Stearn, and G. H. Cohen, *J. Virol.*, 1979, **31**, 608.
[32] B. Norrild, S. L. Shore, and A. J. Nahmias, *J. Virol.*, 1979, **32**, 741.
[33] R. B. Baucke and P. G. Spear, *J. Virol.*, 1979, **32**, 779.

Herpes simplex virus cells labelled with D-galactose and 2-amino-2-deoxy-D-glucose have been subjected to proteolytic digestion.[34] High molecular weight fractions containing the amino-sugar, which were present in the digests of uninfected cells, were absent. Changes were also observed in the relative binding of the glycopeptides from the two types of cells to concanavalin A.

Two glycoproteins (GP1 and GP2, mol. wts. 1.05×10^5 and 8.2×10^4, respectively) isolated from influenza C virus have been found to share common amino-acid sequences.[35] The larger molecular weight glycoprotein is considered to be the primary viral gene product, which may be converted into the lower molecular weight glycoprotein by proteolytic cleavage, a mechanism that appears to be essential for maximal viral infectivity. Four different glycopeptides, each one only containing *N*-glycosidic linkages between 2-acetamido-2-deoxy-D-glucose and L-asparagine, have been isolated after proteolytic digestion of influenza A viral glycoproteins.[36] Two of the glycopeptides contained residues of 2-acetamido-2-deoxy-D-glucose, D-mannose, D-galactose, and L-fucose, whilst the other two contained residues of D-mannose and 2-acetamido-2-deoxy-D-glucose. The types of carbohydrate side chains associated with haemagglutinin, one of the two distinct glycoproteins on the surface of influenza A virions, has been found to vary depending on host cell type as well as virus strain.[37] Further, the distribution of oligosaccharide chains associated with the haemagglutinin varied among influenza virus strains of the same as well as different antigenic subtypes. Two possible mechanisms by which changes in amino-acid sequence can cause variation in the relative amounts of the two different types of glycopeptides have been considered. The variable genes of influenza A viruses coding for haemagglutinin and neuraminidase have been found to consist of a relatively small highly conserved region, which is presumably involved in the functional integrity of the gene products and a relatively large highly variable region, presumably involved in the immunological properties.[38] Evidence for the extrusion of haemagglutinin into membrane vesicles and its glycosylation during synthesis has been presented.[39]

Glycosylation of influenza viral glycoproteins has been investigated by pulse-labelling of infected baby hamster kidney cells with radioactive sugar precursors.[40] On the basis of this work and earlier work, steps involved in the biosynthesis of these glycoproteins have been postulated. The polypeptide portion of the viral glycoprotein is synthesized in association with rough membranes. During or immediately after its synthesis, oligosaccharide cores that contain D-mannose and 2-amino-2-deoxy-D-glucose are transferred from lipid-linked intermediates to glycosylation sites on the polypeptides. The glycoproteins then migrate from the rough to smooth membranes, where some

[34] S. Olofsson and J. Blomberg, *Arch. Virol.*, 1977, **55**, 293.
[35] G. Herrler, R. W. Compans, and H. Meier-Ewart, *Virology*, 1979, **99**, 49.
[36] W. Keil, H. D. Klenk, and R. T. Schwarz, *J. Virol.*, 1979, **31**, 253.
[37] K. Nakamura and R. W. Compans, *Virology*, 1979, **95**, 8.
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[39] K. T. Elder, J. M. Bye, J. J. Skehel, M. D. Waterfield, and A. E. Smith, *Virology*, 1979, **95**, 343.
[40] K. Nakamura and R. W. Compans, *Virology*, 1979, **93**, 31.

D-mannosyl residues are removed from a population of oligosaccharide cores and subsequently branching of the chains occurs by addition of D-galactosyl, L-fucosyl-, and 2-acetamido-2-deoxy-D-glucosyl residues in a stepwise manner. The remaining oligosaccharide cores do not undergo either this trimming process or the addition of sugars at branch points. After further migration from smooth membranes to plasma membranes, the two types of glycoproteins are incorporated into mature virions.

A particular monoclonal antibody specific for the influenza virus haemagglutinin molecule is capable of blocking virus-specific cytotoxic T cells in an *in vitro* assay.[41] The binding appears to be in the context of the histocompatibility antigen H-2D^d but not of H-2K^d.[42] Another monoclonal antibody that has been isolated is capable of blocking both sets of HA-specific effector T cells.

Particles of hepatitis B surface antigen have been labelled after treatment with neuraminidase and D-galactose oxidase, followed by reduction with sodium borotritide.[43] The incorporated label was detected chiefly in a glycoprotein (mol. wt. 2.8×10^4) that retained its serological activity after treatment with neuraminidase.

A radioimmunoassay procedure has demonstrated that a glycoprotein (mol. wt. 3.6×10^4) from a murine mammary tumour virus has both type-specific and group-specific antigenic determinants.[44,45] A glycoprotein antigen (mol. wt. 7.5×10^4), which competes with murine C-type viral glycoprotein in an interspecies radioimmunoassay, has been purified from the surface of EL-4 tumour cells.[46] Serological and structural studies show this glycoprotein to be distinct from any of the viral glycoproteins of similar molecular weight so far examined.

The glycosylated *env* gene precursor of Moloney murine leukaemia virus has been isolated by selective immunoprecipitation.[47] By use of tunicamycin to inhibit nascent glycosylation, it was demonstrated that the precursor contained an apoprotein (mol. wt. 6×10^4) as compared with the complete virion glycoprotein (mol. wt. 7×10^4). The precursor appears to contain two distinct major sites of glycosylation which are within a 3×10^4 dalton region of the apoprotein sequence. Other workers have observed that the molecular weight of the precursor of the polypeptide encoded by the *env* gene of Rauscher murine leukaemia virus is reduced from 8.5×10^4 to 6.8×10^4 when synthesized in the presence of tunicamycin.[48] Most, if not all, of the carbohydrate content of the larger molecular weight glycoprotein is linked to L-asparaginyl residues, and glycosylation appears to be necessary for normal processing of precursor proteins into viral particles.

[41] R. B. Effros, M. E. Frankel, W. Gerhard, and P. C. Doherty, *J. Immunol.*, 1979, **123**, 1343.
[42] M. E. Frankel, R. B. Effros, P. C. Doherty, and W. U. Gerhard, *J. Immunol.*, 1979, **123**, 2438.
[43] J. Skelly, C. R. Howard, and A. J. Zuckerman, *J. Gen. Virol.*, 1978, **41**, 447.
[44] Y. A. Teramoto and J. Schlom, *J. Virol.*, 1979, **31**, 334.
[45] S. L. Marcus, R. Kopelman, and N. H. Sarkar, *J. Virol.*, 1979, **31**, 341.
[46] W. L. NcLennan and J. N. Ihle, *Virology*, 1978, **89**, 547.
[47] O. N. White and D. F. Wirth, *J. Virol.*, 1979, **29**, 735.
[48] A. M. Schultz and S. Oroszlan, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 1206.

Immunoprecipitation has been used in the isolation of a major *gag*-related internal structural glycoprotein (mol. wt. 8×10^4) from Moloney murine leukaemia virus.[49] In cells, this glycoprotein appears to be further glycosylated to a glycoprotein (mol. wt. 9.5×10^4), which is rapidly cleaved and released from the cell as two soluble *gag*-related glycoproteins (mol. wts. 5.5×10^4 and 4.5×10^4, respectively). Analysis of the carbohydrate components of two ecotropic murine leukaemia viruses have indicated that these viruses possess complex glycopeptides, containing residues of D-mannose, D-galactose, L-fucose, and 2-amino-2-deoxy-D-glucose, as well as glycopeptides with high levels of D-mannosyl residues, but lacking D-galactosyl or L-fucosyl residues.[50] Glycosylation of the viral glycoproteins appears to be controlled by features of the viral polypeptide sequence as well as the host cell of origin.

The products of the major histocompatibility locus have been shown to interact with viral antigens on the cell surface of thymocytes.[51] A number of polypeptides bind to the major envelope glycoprotein of Rauscher murine leukaemia virus. The receptors for this glycoprotein, which probably involve lipoproteins, have been prepared from plasma membrane preparations from mouse cells.[52]

Evidence for a cell-associated precursor of two virion glycopeptides (GP1 and GP2) of lymphocytic choriomeningitis virus has been reported.[53] Proteolytic cleavage of the precursor molecule occurs at a single site or at spatially-close multiple sites releasing GP1, a glycopeptide containing residues of L-fucose, D-galactose, and 2-amino-2-deoxy-D-glucose, and GP2, a glycopeptide containing only residues of L-fucose of 2-amino-2-deoxy-D-glucose.

The Friend spleen focus-forming virus encodes a glycoprotein (mol. wt. 5.5×10^4).[54] Although it appears to be structurally related to the lymphatic leukaemia viral glycoprotein (mol. wt. 7.5×10^4), the former glycoprotein has distinctive structural features in the polypeptide portion of the molecule.

Cells infected with Rous sarcoma virus, and then treated with tunicamycin, synthesize a polypeptide (mol. wt. 6.2×10^4) containing antigenic determinants of both the envelope glycoproteins (mol. wts. 8.5×10^4 and 3.5×10^4) of the virus.[55] In addition, the polypeptide contained all the tryptic peptides of the precursor glycoprotein (mol. wt. 9.2×10^4) of the two envelope glycoproteins.

The binding of Semliki Forest virus and its spike glycoproteins to baby hamster kidney cells has been followed by electron microscopy and quantitative binding assays.[56] Binding of the virus to the cell involves two different modes; near neutral pH the virus binds to specific glycoproteins and at low pH it binds non-specifically to the lipids of the plasma membrane. Separation of the

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[50] M. C. Kemp, S. Basak, and R. W. Compans, *J. Virol.*, 1979, **31**, 1.
[51] D. R. Twardzik, A. K. Fowler, O. S. Weislow, G. A. Hegamyer, and A. Hellman, *Proc. Soc. Exp. Biol. Med.*, 1979, **162**, 304.
[52] V. S. Kalyanaraman, M. G. Sarngadharan, and R. C. Gallo, *J. Virol.*, 1978, **28**, 686.
[53] M. J. Buchmeier and M. B. A. Oldstone, *Virology*, 1979, **99**, 111.
[54] S. Dresler, M. Ruta, M. J. Murray, and D. Kabat, *J. Virol.*, 1969, **30**, 564.
[55] H. Diggelmann, *J. Virol.*, 1979, **30**, 799.
[56] E. Fries and A. Helenius, *Eur. J. Biochem.*, 1979, **97**, 213.

membrane glycoproteins E1 and E2 of this virus has been achieved by affinity chromatography using immobilized concanavalin A.[57] Glycopeptide E1, which binds to the lectin, contains two peripheral sugar branches on the polypeptide chain containing D-mannosyl-, D-galactosyl-, 2-acetamido-2-deoxy-D-glucosyl- and neuraminosyl- residues, whereas the glycopeptide E2 contains single chains containing D-mannosyl- and 2-acetamido-2-deoxy-D-glucosyl residues. Metabolic labelling of the precursor of the Semliki Forest viral envelope glycoproteins E2 and E3 has revealed that the precursor glycoprotein contains mainly glycan chains with a high level of D-mannosyl residues, whereas its products, the viral glycoproteins E2 and E3, contain an additional lactosamine-type glycan.[58] Semliki Forest virus membrane glycoproteins, after removal of their L-fucosyl, D-galactosyl, neuraminosyl, and distal 2-acetamido-2-deoxy-D-glucosyl residues, have been digested with an *endo*-β-D-2-acetamido-2-deoxy-glucanase (EC 3.2.1.96) to release a tetrasaccharide.[59] The absolute structure of this tetrasaccharide is not yet known, although it contains two α-D-mannosyl, one β-D-mannosyl, and one 2-acetamido-2-deoxy-D-glucosyl residues.

Semliki Forest virus glycoproteins have been desialylized and then subjected to hydrazinolysis to release glycans whose molecular sizes range from 1.85–2.15×10^3.[60]

Sendai virus, one of the paramyxovirus group, has two glycoproteins that constitute the spikes on the virus envelope.[61] The HN glycoprotein is responsible for the attachment of the virus to the cell membrane, resulting in the agglutination of cells, and is associated with neuraminidase activity. The F glycoprotein is required for envelope fusion and is also required for biological activities such as infectivity, haemolysis, and cell fusion. Disulphide bonds in the HN glycoprotein may be split using mild conditions with a concomitant loss of biological activity of the virus. More drastic conditions are necessary for the cleavage of interpeptide disulphide bonds between sub-units on the F glycoprotein, when both fragments remain on the virion. This is in contrast to the release of sub-units of influenza virus, and a model for the arrangement of these sub-units on viral membranes is proposed. A method has been developed for the reconstitution of biologically active membranes with the individual HN and F glycoproteins of Sendai virus.[62]

Two mouse L-cell variant lines (CL3 and CL6), selected for their resistance to ricin, are restricted in their ability to replicate Sindbis and Semliki Forest viruses.[63] The CL3 cells exhibit an increase in CMP-neuraminic acid:glycoprotein neuraminosyltransferase and G_{M_3}-synthase activities, whereas the CL6 cells contain decreased UDP-D-galactose:glycoprotein galactosyltransferase and UDP-2-acetamido-2-deoxy-D-glucose:glycoprotein 2-acetamido-2-deoxy-D-glucosyltransferase activities. The adsorption of Sindbis virus to CL6 cells was con-

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[58] H. Stenvall and D. Renkonen, *Biochim. Biophys. Acta*, 1979, **586**, 146.
[59] M. Pesonen, K. Haahtela, and O. Renkonen, *Biochim. Biophys. Acta*, 1979, **588**, 102.
[60] M. L. Rasilo and O. Renkonen, *Biochim. Biophys. Acta*, 1979, **582**, 307.
[61] M. Ozawa, A. Asano, and Y. Okada, *J. Biochem. (Jpn.)*, 1979, **86**, 1361.
[62] M. C. Hsu, A. Scheid, and P. W. Choppin, *Virology*, 1979, **95**, 476.
[63] C. Gottlieb, S. Kornfeld, and S. Schlesinger, *J. Virol.*, 1979, **20**, 340.

siderably reduced, suggesting a loss or inaccessibility of the receptors for the virus accounted for a major defect in the viral protein. In contrast, CL3 cell line synthesized Sindbis viral RNA and proteins, but was unable to convert the precursor glycoprotein, PE2, into the structural protein E2. The cleavage of PE2 to E2 was blocked in both CL3 and CL6 cells infected with Semliki virus.

A study of the tryptic peptides released from glycoproteins (mol. wt. 7×10^4) of ecotropic and amphotropic murine leukaemia viruses suggests that the viruses are structurally divergent.[64]

The glycoproteins isolated from complete and defective rabies viruses contain the same monosaccharides, *viz.* neuraminic acid, D-galactose, D-mannose, L-fucose, and 2-acetamido-2-deoxy-D-glucose.[65] The isoelectric points, molecular sizes, and glycosylation patterns of the two groups of glycoproteins were found to differ.

The carbohydrate moieties of the glycoproteins from the paramyxovirus SV5 contain residues of L-fucose, D-mannose, D-galactose, and 2-amino-2-deoxy-D-glucose (in the molar ratio 1:3:4:2).[66] Glycopeptides isolated from the HN_1, F_1, and F_2 glycoproteins contain three, three, and one carbohydrate chains, respectively. The structure (1) is unusual for one isolated from a eukaryotic source because of the presence of acetaldehyde in an acetal linkage. Ten percent of the carbohydrate chains are esterified with sulphate, located at O-6 of the amino-sugar residues.

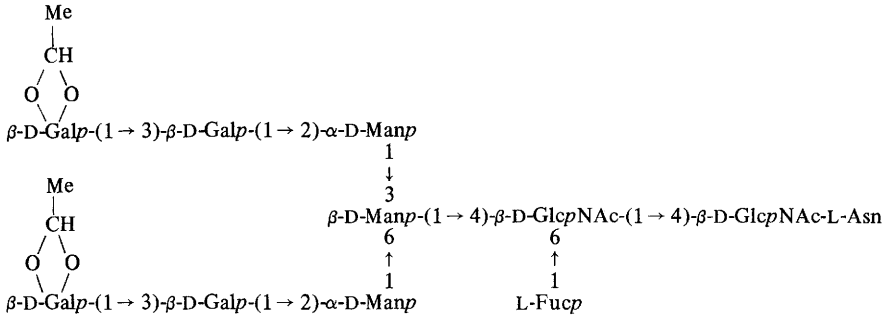
One or two moles of covalently bound fatty acid per mole of Sindbis virus glycoprotein E1 have been reported.[67] The covalent attachment of the fatty acids to the polypeptide is alkali-labile and is thought to occur during maturation of the viral glycoprotein.

The structure (2) of the oligosaccharide chains of the glycoprotein E2, isolated from growth of this virus in hamster and chicken cells, is based on sugar composition, linkage analysis, and sequential degradation with enzymes.[68] Glycoproteins isolated from the virus grown separately in the two hosts show no variation in the structure of the oligosaccharide chains. It is noteworthy that the neuraminic acid residues in the oligosaccharide chains of the Sindbis viral glycoprotein are linked exclusively to O-3 of the D-galactosyl residues, whereas in mammalian glycoproteins it is to O-6 or to a mixture of O-3 and O-6 D-galactosyl residues.

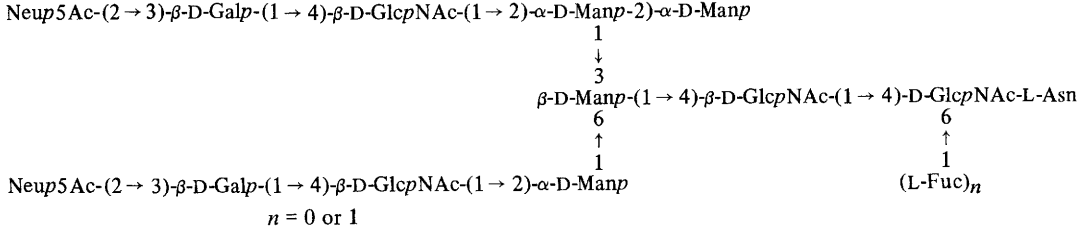
Glycoprotein–glycoprotein interactions have been proposed to account for the observations of hexagonal arrays of glycoproteins on the surface of Sindbis virus.[69]

A variant of Sindbis virus that contains a smaller molecular weight form of the viral glycoprotein E2 has been isolated.[70] The molecular weight of the

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[65] P. Atanisiu, P. Perrin, S. Favre, and J. Sisman, *Ann. Microbiol.*, 1979, **130A**, 85.
[66] P. Prehm, A. Scheid, and P. W. Choppin, *J. Biol. Chem.*, 1979, **254**, 9669.
[67] M. F. G. Schmidt, M. Bracha, and M. J. Schlesinger, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 1687.
[68] D. Burke and K. Keegstra, *J. Virol.*, 1979, **29**, 546.
[69] C. H. Von Bonsdorff and S. C. Harrison, *J. Virol.*, 1978, **28**, 578.
[70] M. Bracha and M. J. Schlesinger, *J. Virol.*, 1978, **26**, 126.


Me
CH
O O
β-D-Galp-(1→3)-β-D-Galp-(1→2)-α-D-Manp
1
↓
3
β-D-Manp-(1→4)-β-D-GlcpNAc-(1→4)-β-D-GlcpNAc-L-Asn
6 6
↑ ↑
1 1
L-Fucp

(1)


Neup5Ac-(2→3)-β-D-Galp-(1→4)-β-D-GlcpNAc-(1→2)-α-D-Manp-2)-α-D-Manp
1
↓
3
β-D-Manp-(1→4)-β-D-GlcpNAc-(1→4)-D-GlcpNAc-L-Asn
6 6
↑ ↑
1 1
Neup5Ac-(2→3)-β-D-Galp-(1→4)-β-D-GlcpNAc-(1→2)-α-D-Manp
(L-Fuc)n
n = 0 or 1

(2)

precursor glycoprotein and the glycosylation pattern of the smaller E2 glycoprotein are normal, thus indicating that the latter is formed by an aberrant proteolytic cleavage.

The envelope glycoprotein of Venezuelan equine encephalomyelitis virus is derived by proteolytic cleavage of a precursor glycoprotein, as has been shown with many other viruses.[71] A comparison of the envelope glycoproteins of representative strains of this virus, using polyacrylamide gel electrophoresis and isoelectric focusing has been presented, together with a description of a method for serological sub-typing of the virus strains using antisera to these glycoproteins.

A procedure has been described for the selective isolation of mutants of vesicular stomatitis virus, defective in the production of the viral glycoprotein.[72]

The glycoprotein of vesicular stomatitis virus has been selectively liberated from the virion membrane by the dialysable non-ionic detergent octyl β-D-glucopyranoside.[73] When mixtures of the viral glycoprotein and egg lecithin were dialysed until free from the detergent, glycoprotein vesicles formed spontaneously with spikes protruding in the same external orientation as the original virion membrane. Pulse-labelling of HeLa cells infected with vesicular stomatitis virus has resulted in the isolation from the bound ribosomes of a peptidyl-transfer RNA species specifically labelled in the carbohydrate moiety.[74] The size distribution of the nascent polypeptide chains that serve as a substrate for the cellular glycosyltransferases has been determined by polyacrylamide gel electrophoresis. Attachment of the carbohydrate chains starts when approximately half of the amino-acid sequence of the viral glycoprotein has been synthesized. The non-glycosylated proteins of the virus, which are produced by growing the virus in the presence of tunicamycin, are temperature-sensitive and undergo aggregation at elevated temperatures.[75] Proteins with different sequences of amino-acids appear to have differing requirements for glycosylation, and the glycosylation probably influences the folding and ultimate conformation of the glycoprotein.

The general effects of glycosylation and restriction on different bacteriophage T4 gene expression and replication have been examined.[76,77]

2 Plant Glycoproteins

Review articles dealing with chemical and biochemical aspects of glycoproteins,[78] and the involvement of the Golgi apparatus in the biosynthesis and secretion of plant glycoproteins and polysaccharides,[79] have been published.

[71] J. K. France, B. C. Wyrick, and D. W. Trent, *J. Gen. Virol.*, 1979, **44**, 725.
[72] H. F. Lodish and R. A. Weiss, *J. Virol.*, 1979, **30**, 177.
[73] W. A. Petri and R. R. Wagner, *J. Biol. Chem.*, 1979, **254**, 4313.
[74] J. Kruppa, *Biochem. J.*, 1979, **181**, 295.
[75] R. Gibson, S. Schlesinger, and S. Kornfeld, *J. Biol. Chem.*, 1979, **254**, 3600.
[76] K. Dharmalingam and E. B. Goldberg, *Virology*, 1979, **96**, 393.
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[78] R. G. Brown and W. C. Kimmins in 'MTP International Review of Biochemistry', ed. D. H. Northcote, 1977, Vol. 13, p. 183.
[79] D. H. Northcote, in 'Biomembranes', ed. L. A. Manson, Plenum Press, New York, Vol. 10, p. 51.

The inhibition of the *in vitro* transfer of 2-acetamido-2-deoxy-D-glucose and D-mannose from UDP-2-acetamido-2-deoxy-D-glucose and GDP-D-mannose, respectively, into lipid-linked intermediates has been demonstrated in mung beans and carrots in the presence of amphomycin.[80] The antibiotic prevents the D-mannosylation of protein, but does not block the *in vitro* transfer of D-glucose from UDP-D-glucose to β-D-glucans.

Infection of muskmelon (*Cucumis melo*) seedlings by the fungus *Colletotrichum lagenarium* causes a ten-fold increase in the level of glycoproteins containing L-hydroxyproline.[81] Observed increases in diseased plants accounted for a cell-wall enrichment of glycosylated L-hydroxyprolyl and L-seryl residues. The extent of accumulation of these glycoproteins was inhibited by treating the plants with ethylene, or growing them in the presence of L-*trans*-hydroxyproline.[82] The increase in cell wall L-hydroxyproline-rich glycoproteins mediated by treatment with ethylene was paralleled by an increasing resistance of the host to the pathogen. Inversely, inhibition of the synthesis of these glycoproteins in diseased plants was correlated to an accelerated and more intense colonization of the host by the pathogen.

An L-arabino-D-galactan–protein complex has been isolated from the style canal of *Gladiolus* by affinity chromatography on the D-galactose-binding lectin from the giant clam *Tridacna maxima*.[83] The procedure should prove useful in the isolation of polysaccharides and glycoproteins containing β-D-galactopyranosyl residues.

Three globulins isolated from the seeds of *Lupinus augustifolius* have been shown to contain six, four, and two major sub-units, which are all glycosylated to varying degrees with residues of D-mannose, D-galactose, and 2-amino-2-deoxy-D-glucose.[84] Proteolytic digestion of two of the glycoproteins has yielded glycopeptides (mol. wt. 5×10^3).

In addition to hemicellulose, pectic substances, and cellulose, high levels of L-hydroxyproline have been detected in the cellulose fraction of the endosperm wall of rice (*Oryza sativa*).[85] Glycoproteins containing this amino-acid may be bound to the cellulose microfibrils.

A series of oligosaccharides, containing up to four L-arabinosyl residues linked to L-hydroxyproline, has been isolated from the cell walls of *Phaseolus vulgaris*.[86] In cell suspensions of cultures of *P. vulgaris*, variations in the patterns of L-arabinosylation were observed only during the lag phase and early log phase of the cultures. A glycoprotein, containing only small amounts of L-hydroxyproline and consisting of a single polypeptide chain, has been isolated from *P. vulgaris*.[87] Treatment with alkali released two polypeptide chains, with most

[80] M. C. Ericson, J. T. Gafford, and A. D. Elbein, *Arch. Biochem. Biophys.*, 1978, **191**, 698.
[81] M. T. Esquerré-Tugayé and D. T. A. Lamport, *Planet Physiol.*, 1979, **64**, 314.
[82] M. T. Esquerré-Tugayé, C. Lafitte, D. Mazau, A. Toppan, and A. Touzé, *Plant Physiol.*, 1979, **64**, 320.
[83] P. A. Gleeson, M. A. Jermyn, and A. E. Clarke, *Anal. Biochem.*, 1979, **92**, 41.
[84] C. A. Eaton-Mordas and K. G. Moore, *Phytochemistry*, 1979, **18**, 1775.
[85] N. Shibuya and T. Iwasaki, *Agric. Biol. Chem. (Jpn.)*, 1979, **42**, 2259.
[86] F. M. Klis and E. Eeltink, *Planta*, 1979, **144**, 479.
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of the carbohydrate residues linked to L-serine on the polypeptide of lower molecular weight. Methylation analysis indicated the presence of 1,2-, 1,3-, or 1,5-substituted L-arabinofuranosyl residues, non-reducing terminal D-galactosyl residues, and a chain of (1 → 4)-β-D-glucosyl residues.

Soybean 7S protein contains residues of D-mannose and 2-acetamido-2-deoxy-D-glucose.[88] After proteolytic digestion, three related glycopeptides were isolated and structural analysis has allowed a possible structure (3) to be deduced.

The stem and root-infecting pathogen of soybean, *Phytophthora megasperma*, excretes glycoproteins.[89] They have proved to be poor, non-specific elicitors of phytoalexin accumulation.

Free- and membrane-bound ribosomes prepared from wheat germ have binding sites for concanavalin A.[90] Four glycoproteins (mol. wts. $1.4–2.4 \times 10^4$) were identified by sodium dodecyl sulphate–polyacrylamide gel electrophoresis in both classes of ribosomes.

Membrane preparations from the alga *Prototheca zopfii* incorporate D-mannose and 2-acetamido-2-deoxy-D-glucose from their respective sugar nucleotides into acid labile glycolipids whose properties are consistent with those of dolichyl D-mannopyranosyl phosphate.[91] The intermediate appears to be involved in the formation of glycoproteins containing high proportions of D-mannosyl residues of the type present in more-evolved eukaryotes.

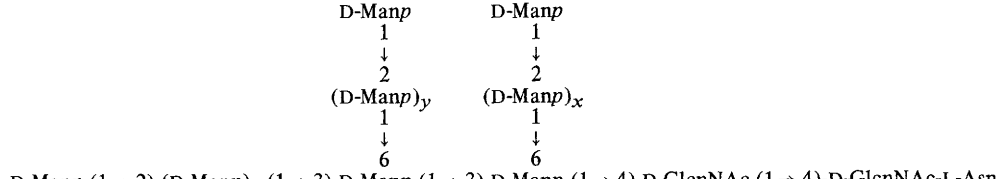
Fluorescein-conjugated lectins and lactoperoxidase-catalysed iodination reactions have been used to locate the cell surface glycoproteins of the multicellular alga *Volvox carteri*.[92]

3 Lectins

In a review, a comparison has been made of the specific combining sites of a number of lectins and those of antibodies to blood-group substances.[93] A comprehensive review of both plant and animal lectins has been published.[94] The application of lectins for the purification and characterization of enzymes and other proteins has been reviewed in order to define some of the variables that affect the binding of glycoproteins to lectins.[95] The role of the oligosaccharides of cell-wall surface glycoproteins in variants of plasma membranes of mammalian cells that are resistant to cytotoxic lectins has been assessed.[96]

A model has been proposed for the function of lectins as membrane components.[97] The basic feature lies in the emphasis on the interaction of lectins

[88] F. Yamauchi and T. Yamagishi, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 505.
[89] M. Wade and P. Albersheim, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4433.
[90] K. Yoshida, *J. Biochem. (Jpn.)*, 1978, **83**, 1609.
[91] P. A. Romero, H. E. Mopp, and R. Pont-Lezica, *Biochim. Biophys. Acta*, 1979, **586**, 545.
[92] N. Kurn and B. A. Sela, *FEBS Lett.*, 1979, **104**, 249.
[93] E. A. Kabat, *J. Supramol. Struct.*, 1978, **8**, 79.
[94] I. J. Goldstein and C. E. Hayes, *Adv. Carbohydr. Chem. Biochem.*, 1978, **35**, 127.
[95] J. T. Dulaney, *Mol. Cell. Biochem.*, 1978, **21**, 43.
[96] J. A. Wright, *Int. J. Biochem.*, 1979, **10**, 951.
[97] D. J. Bowles, *FEBS Lett.*, 1979, **102**, 1.



a, $x = 1, y = 2, z = 1$; b, $x = 2, y = 1, z = 1$.

(3)

with their endogenous glycosylated receptor molecules. Since this interaction is reversible, the consequent formation and destruction of pair complexes provides the cell with a rapid and efficient means of regulation.

Further evidence has substantiated the theory that the interaction between lectins of host plants and *Rhizobium* species is the key to the mechanism of host-specificity in *Rhizobium*–legume symbiosis and that the lectin receptors of the bacterial cells exist in the cell-surface lipopolysaccharides.[98]

The association constants of a number of lectin–carbohydrate complexes have been determined by affinity electrophoresis and are pH-dependent.[99]

In an investigation of the secondary structures of eight lectins by c.d., the β-pleated sheet was found to be the predominant structural form.[100] Only insignificant changes in the secondary structures of the lectins on binding to their respective monosaccharides were observed. A study of the cross-reactions of antisera raised to eight homogeneous plant lectins has indicated a high level of conservation of antigenic determinants, and indicated that the lectins may be structurally-related proteins.[101]

The adsorption behaviour of concanavalin A to a series of alkyl-agaroses at various temperatures and salt concentrations has been reported.[102] The affinity shown by concanavalin A for alkyl-agaroses under physiological conditions suggests that some of the biological properties attributed to the lectin might stem both from its hydrophobic character and its sugar-binding capacity. Various individual metal ions (Mn^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+}, and Pb^{2+}) or mixtures of these ions, when added to demetallized concanavalin A, convert the protein into a conformational form that has a high affinity for saccharides.[103] The rates of conversion of the demetallized lectin into this active form depend on the identity of the ions added and the sample pH.

Equilibrium dialysis techniques have been used to examine the affinity and stoicheiometry of various transition metal ions for apo-concanavalin A at pH 6.5.[104] The saccharide-binding activities of various transition metal ion–concanavalin A complexes were analysed using 4-methylumbelliferyl α-D-mannopyranoside. The presence of only one metal ion per sub-unit appears to be sufficient for saccharide binding, therefore the binding of a second Mn^{2+} or a second Ca^{2+} ion per sub-unit is completely incidental to saccharide-binding activity. This interpretation is contrary to an earlier proposal in which the induction of saccharide-binding activity was thought to involve two bound metal ions per sub-unit.[105]

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Spectroscopic effects observed in the near-u.v. of c.d. spectra of concanavalin A are associated with the binding of metals at sites S1 and S2.[106] Time-dependent changes, as measured by the intrinsic Cotton effects, for Ca^{2+} and the binding of methyl α-D-mannopyranoside were modulated by a symmetrical broad transition. A tentative structural hypothesis, which designates L-tyrosine-112 as an intrinsic reporter, is advanced to account for the spectroscopic observations.

The crystal structure of demetallized concanavalin A has been determined, at a resolution of 3.2 Å.[107] Observed differences between the native and demetallized proteins have formed the basis for a mechanistic model of the interactions in solution between the two metal-binding sites. Intact sub-units of concanavalin A have been isolated after ion-exchange chromatography of the lectin in the presence of H_4-edta.[108]

When concanavalin A is immobilized on Sephadex® and treated with glutaraldehyde, the L-lysine residues readily available for cross-linking occur only on the surface of the C-terminal half of the lectin protomer.[109] The ε-amino groups of the L-lysine residues are oriented in a direction roughly antipodeal to corresponding groups on neighbouring protomers, such that intramolecular cross-links are not possible. These exteriorly located L-lysine residues of each concanavalin A protomer are distributed in at least two 'clusters', since it is sterically possible for a single protomer to be linked to at least two other protomers arising from a different concanavalin A oligomer or oligomers.

Antibodies specific for the 'active site' of concanavalin A have been isolated from rabbit sera.[110] Two antibody fractions, which were isolated after affinity chromatographic separation on immobilized concanavalin A, showed distinct differences in biological activity. One population of antibodies was directed to the 'active site' of the lectin, and binding of the antibody in this case is inhibited by methyl α-D-mannopyranoside, whereas the other antibodies were directed to the antigenic determinants on the concanavalin A molecule.

Aggregation of concanavalin A by low concentrations of sodium dodecyl sulphate requires the presence of an intact saccharide–ligand binding site.[111] A rapid reversal of the detergent effect is achieved by addition of methyl α-D-mannopyranoside or methyl α-D-glucopyranoside. No evidence was obtained to suggest that the detergent binds to the hydrophobic pocket in the surface of the protein. A newly observed conformational change in concanavalin A, which is dependent on temperature and perhaps also on salt concentration and which perturbs the binding of methyl α-D-mannopyranoside, has been detected by measurements of the heat of binding of the sugar.[112]

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[108] F. Obata, R. Sakai and H. Shiokawa, *J. Biochem. (Jpn.)*, 1978, **84**, 103.
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[111] R. H. Glew and R. J. Doyle, *Carbohydr. Res.*, 1979, **73**, 219.
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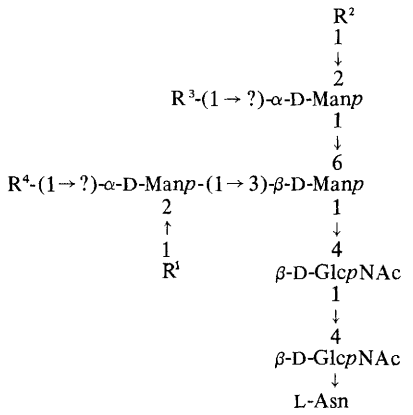
The effects of binding of a series of mono- and oligo-saccharides to Ca^{2+}–Mn^{2+}–concanavalin A on the solvent-water proton-relaxation rate over a range of magnetic fields have been measured.[113] The observed changes in relaxation rates were shown to be sensitive to the affinity constants of the saccharides, in that the effect was proportional to the amount of saccharide bound to the protein. Data provided suggest that all the mono- and oligo-saccharides that bind to concanavalin A induce the same protein conformational transition as monitored by the dispersion measurements. Although the possibility of an extended binding site could not be excluded, evidence from this and other work is of a single saccharide-residue-binding site.

Concanavalin A, purified by conventional methods, has been shown to behave heterogeneously in its interactions with 4′-nitrophenyl 2-*O*-α-D-mannopyranosyl-α-D-mannopyranoside and Sephadex® G-75.[114] Three protein fractions have been identified, one composed predominantly of fragmented polypeptide chains, one composed entirely of intact polypeptide chains, and one which appears to be a metal-deficient form of the lectin. Comparison of the association constants for binding of mono- and di-saccharides demonstrated that the intact concanavalin A has a nineteen-fold greater affinity for the chromogenic disaccharide than the monosaccharide glycoside 4-nitrophenyl α-D-mannopyranoside, thus strongly suggesting that the lectin interacts simultaneously with groups on both D-mannopyranosyl residues. The binding of the chromogenic disaccharide to the intact concanavalin A proceeds *via* a biphasic process as opposed to the monophasic second-order process observed for the monosaccharide.[115] The rate of formation of the first detectable lectin–disaccharide complex remained several orders of magnitude below that expected for a diffusion-controlled encounter, and appears to be different from that proposed to occur immediately after diffusion-controlled encounters between concanavalin A and its ligands.

Iodinated glycopeptides have been used to determine the association constants and binding of these molecules to macroporous agarose-immobilized concanavalin A.[116] The glycopeptides examined had a core structure (4). The presence of the two α-D-mannopyranosyl residues is essential for interactions with the saccharide binding site resulting in retention on immobilized concanavalin A. The presence of substituents at C-2 of the α-D-mannopyranosyl residues ($R^1 = R^2 =$ mono- or oligo-saccharides) did not reduce the association constants sufficiently to prevent binding; however, substantial removal of peripheral monosaccharides on chain branches arising from position O-2 results in a progressive increase in the association constants.

Concanavalin A dissociates to protomer and protomer fragments at very low concentrations.[117] The associating behaviour of the lectin occurring at increasing concentrations contributes to the concentration-dependent

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(4) $R^1 = R^2 = R^3 = R^4 =$ H or oligosaccharides

association of concanavalin A sub-units, which contribute to the observed positive co-operativity on the binding of concanavalin A to erythrocytes. By use of an aggregation assay, which provides a continuous measure of both the rate and degree of intercellular adhesion in the presence of controlled shear forces, the agglutination of human erythrocytes by concanavalin A has been resolved into three stages, two of which are observed only if the system is exposed to shear.[118] Concanavalin A has been used as a coupling agent to promote the binding and endocytosis of the lysosomal hydrolases, β-D-galactosidase and β-D-2-acetamido-2-deoxyhexosidase by genetically deficient fibroblasts.[119] The initial binding of the enzyme to the multivalent lectin molecules occurred at the cell surface, and was followed by an endocytotic process resulting in the internalization of both lectin and lectin-bound enzyme.

The lectin from *Bandeiraea simplicifolia* has been conjugated with fluorescein isothiocyanate and used as a histochemical probe for the detection of α-D-galactopyranosyl residues in glycoproteins of basement membranes, and on the surface of certain epithelial cells, and on the surface of TA3 murine mammary carcinoma.[120] The lectins from this source form a family of tetrameric glycoproteins composed of various combinations of two kinds of sub-units A and B.[121] Although the sub-units A and B have similar amino-acid compositions, the B unit contains one L-methionine residue and the A unit has none. The sub-units each contain residues of D-mannose, L-fucose, and D-xylose (8, 2, and 2 moles, respectively, per mole of sub-unit). Examination of the ability of biopolymers and synthetic glycoproteins to precipitate those lectins containing only A or B units led to the suggestion that hydrogen bonding occurs between the A and B sub-units and the hydrogen atoms of the C-3 and C-4 hydroxy-groups of

[118] R. G. Greig and D. E. Brooks, *Nature (London)*, 1979, **282**, 738.
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[120] B. P. Peters and I. J. Goldstein, *Exp. Cell Res.*, 1979, **120**, 321.
[121] L. A. Murphy and I. J. Goldstein, *Biochemistry*, 1979, **18**, 4999.

2-acetamido-2-deoxy-α-D-galactopyranosyl and α-D-galactopyranosyl residues, respectively. The binding site of the lectin containing four A subunits (A_4) is probably no longer than a disaccharide, with the oligosaccharides having the structures (5) and (6) being equally active.[122] In contrast, the binding site of the lectin containing four B sub-units (B_4) involves at least the sub-terminal 1,3-substituted D-galactosyl residue since the oligosaccharide (7) is most active, with the oligosaccharides (5) and (6) being less active.

α-D-Gal*p*NAc-(1 → 3)-D-Gal*p*

(5)

α-D-Gal*p*NAc-(1 → 3)-β-D-Gal*p*-(1 → 3)-D-Glc*p*NAc

(6)

α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 6)-R
2
↑
1
α-L-Fuc*p*

(7) R = further monosacharide residues

The inhibition of lysozyme conjugated with 4-aminophenyl β-D-galactopyranoside by the lectins of *Ricinus communis* specific for D-galactopyranosyl residues in glycoconjugates has been utilized for the assay of these lectins in the nanogram range.[123] The lectin-specific sugar derivative is conjugated to a residue of L-lysine near the active site of the lysozyme molecule. When this conjugated lysozyme is incubated with lectin, the enzyme is inhibited, owing to the binding of lectin to sugar. The lectins of *R. communis*, which are specific for D-galactopyranosyl residues, differ considerably in their affinity toward immobilized L-rhamnopyranosyl residues on affinity electrophoresis, thus enabling separation of the agglutinin and the toxin.[124] Variation of temperature, ionic strength, and capacity affects the interaction between ceruloplasmin, asialoceruloplasmin, and thyroglobulin on immobilized concanavalin A and immobilized *R. communis* lectin.[125] The capacity of the lectins varied on a weight basis in a narrow range (0.8–3.0 mg glycoprotein mg^{-1} lectin). The dissociation of the lectin–glycoprotein complex by simple glycosides depends on time, temperature, and glycoside concentration.

Changes in the capacity of hepatoma cells to the binding of *Ricinus communis* lectin have been noted after treatment with chondroitin lyase AC.[126] The binding sites for this lectin, but not those for concanavalin A, are masked with chondroitin 4-sulphate. By use of a direct method for studying the binding

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[125] J. T. Dulaney, *Anal. Biochem.*, 1979, **99**, 254.
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of lectins to glycoproteins and their sub-units using fluorescent microscopy and sodium dodecyl sulphate electrophoresis together, concanavalin A has been shown to bind to both the *R. communis* agglutinins (RCA I and RCA II) and not only to RCA I as has previously been reported.[127]

The binding of *Ricinus communis* agglutinin to the mitochondrial inner membrane now appears to be an artefact of the preparation of the *R. communis* mitochondria.[128] When endosperms from *R. communis* are homogenized in the presence of ^{3}H-labelled *R. communis* agglutinin, with and without the addition of lactose, the labelled lectin is only found associated with the mitochondrial membrane fraction in the absence of lactose.

A detailed examination of the specificity of the *Ricinus communis* lectins RCA I (an agglutinin) and RCA III (a toxin) for complex oligosaccharides containing residues of either 2-acetamido-2-deoxy-D-glucose or 2-acetamido-2-deoxy-D-galactose has been reported.[129] A number of iodinated glycopeptides containing these amino-sugars have been used in precipitation assays, enabling Scatchard plot analyses of the saturation curves to be obtained. RCA II lectin showed enhanced association constants for binding to glycopeptides containing 2-acetamido-2-deoxy-3-*O*-D-galactosyl-β-D-galactosyl disaccharide units when compared to the RCA I lectin. The interaction of the *R. communis* haemagglutinin (RCA I) with D-galactose and lactose has been studied by microcalorimetry, equilibrium dialysis, and analytical ultracentrifugation, to reveal two classes of binding sites on the molecule.[130]

Ricin, the toxin of *Ricinus communis*, has been adsorbed on guar gum, a polysaccharide containing D-galactosyl and D-mannosyl residues.[131] The non-binding toxic A chain is then isolated by reduction of the immobilized toxin with 2-mercaptoethanol, and the B chain is eluted with lactose. A nucleotide-binding domain in the A-chain was demonstrated from its interaction with Cibacron Blue F3GA.

Neither ricin nor the toxic lectin modeccin are capable of inhibiting the elongation factor (EF1)-dependent binding of L-[^{14}C]phenylalanyl-tRNA although they are specific inhibitors of translocation.[132] Inconsistencies in earlier reports may have arisen from an overestimation of the number of control ribosomes engaged in the binding reaction if trace amounts of EF2 contaminate the ribosomal preparations.

Ricin has been shown to exert its toxic effect on human neuroblastoma cells by inhibiting DNA polymerase α-activity, whereas DNA polymerase β-activity is inhibited by red kidney bean lectin.[133]

A hybrid protein molecule, which may be regarded as a structural analogue of ricin, has been synthesized from the toxic A sub-unit of the lectin and the cell-specific β-sub-unit of human chorionic gonadotrophin.[134] The biological

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[134] T. N. Oeltmann and E. C. Heath, *J. Biol. Chem.*, 1979, **254**, 1022.

activity of the hybrid has been studied by assaying the inhibition of protein synthesis in rat R2C Leydig cells and mouse L-cells.[135] For the A sub-unit to exhibit its toxic effect it must first bind to cells through receptors that are specific for the β-sub-unit of the hybrid. Protein synthesis in the rat cells but not the mouse cells is inhibited, indicating that only cells with β-type receptors are sensitive to the toxic effects, which human chorionic gonadotrophin is capable of blocking, while cells which are not target cells for the hormone are not subject to the toxic effects.

The binding, entry, and action of ricin, abrin, and modeccin on eukaryotic cellular systems have been reviewed.[136] Modeccin binds to surface receptors containing terminal D-galactosyl residues, and like abrin and ricin it inhibits protein synthesis by inactivating the 60 S ribosomal sub-units.[137] Competition experiments with various glycoproteins have indicated that the modeccin receptors are different from abrin receptors. Mutant cell lines resistant to abrin and ricin were not resistant to modeccin and *vice-versa*.

Two anti-tumour proteins, abrin A and abrin B, have been isolated from *Abrus precatorius* by ion-exchange and affinity chromatographic methods.[138] Both proteins have similar molecular weights (6.5×10^4), exist as dimers, and have similar, but not identical, amino-acid compositions. ^{131}I-Labelled abrin A and abrin B bind to Sarcoma 180 cells, but the patterns of inhibition of binding by different sugars are different.

The physicochemical and carbohydrate-binding properties of succinylated wheat-germ agglutin have been compared with those of the unmodified lectin.[139] The dramatic decrease in the apparent number of cell-surface receptors detected using the modified lectin is discussed on the basis of a decrease in the isoelectric point of the lectin and in terms of the acidic properties of the cell surface.

Concanavalin A and wheat-germ agglutinin inhibit endocytosis of lysosomal enzymes, in a mechanism by which the cell surface receptors for the enzymes interact either directly with the lectins or are closely linked to lectin receptors.[140]

Synthetic glycoconjugates, prepared by reductive amidation of *NN'*-diacetylchitobiose and *NN'N''*-triacetylchitotriose to poly-L-lysine, have been used to examine binding specificities of wheat-germ agglutinin and *Bandeiraea simplicifolia* II lectin.[141] These conjugates are effective precipitating antigens for the lectins, and the results are in agreement with conclusions reached using other methods.

The carbohydrate binding site of wheat-germ agglutinin is well characterized as being complementary to 2-acetamido-2-deoxy-D-glucose and its (1 → 4)-β-

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[139] M. Monsigny, C. Sene, A. Obrenovitch, A. C. Roche, F. Delmotte, and E. Boschetti, *Eur. J. Biochem.*, 1979, **98**, 39.
[140] H. Beeck, K. Ullrich, and K. von Figura, *Biochim. Biophys. Acta*, 1979, **583**, 179.
[141] B. M. Cederberg and G. R. Gray, *Anal. Biochem.*, 1979, **99**, 221.

linked oligosaccharides. The binding of this lectin to *N*-acetylneuraminic acid and to several of its derivatives and to 2-acetamido-2-deoxy-D-galactose has also been reported.[142] Affinity chromatography of glycoconjugates on macroporous agarose-immobilized wheat-germ agglutinin columns has confirmed that *N*-acetylneuraminyl residues play an important role in the binding of sialoglycoproteins to this lectin.[143] The binding of a glycoprotein to the immobilized wheat-germ agglutinin appears to be influenced by the density of the binding carbohydrate residues (2-acetamido-2-deoxy-D-glucose and *N*-acetylneuraminic acid) on the glycoprotein and by the density of the lectin molecules on the gel beads. Both the acetamido-group and the hydroxy-group at C-4, each in an equatorial position in 2-acetamido-2-deoxy-D-glucose and *N*-acetylneuraminic acid, have been shown to be the main determinants in the binding of these sugars to the lectin.[144] The interaction between wheat-germ agglutinin and glycoconjugates containing *N*-acetylneuraminic acid is shown also to be dependent on a charge effect due to the high isoelectric point of the lectin and on an avidity effect.

The composition and properties of a number of lectins are listed in Table 1. Examination of the amino-acid sequence of the α-sub-unit of pea (*Pisum sativum*) lectin shows considerable homology to concanavalin A and near identity to the α-sub-unit of the lentil (*Lens culinaris*) lectin.[158] As in the case of the α-sub-unit of the lentil lectin, the binding site(s) for methyl α-D-glucopyranoside are not present in this region nor are the S1 and S2 metal-binding sites. The seeds of *Vicia cracca* contain two lectins, a D-glucose-specific lectin, which agglutinates erythrocytes non-specifically, and a blood group A specific lectin.[159] A comparison of the amino-acid sequences has revealed extensive homologies between the *N*-terminal regions of the α- and β-chains of the D-glucose-specific lectin and the corresponding chains of the lentil and pea lectins.

[142] B. P. Peters, S. Ebisu, I. J. Goldstein, and M. Flashner, *Biochemistry*, 1979, **18**, 5505.
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[146] M. Tsuda, *J. Biochem. (Jpn.)*, 1979, **86**, 1451.
[147] M. Sakakibara, H. Noguchi, and S. Makino, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 1647.
[148] K. D. Hapner and J. E. Robbins, *Biochim. Biophys. Acta*, 1979, **580**, 186.
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[151] A. Falasca, C. Franceschi, C. A. Rossi, and F. Stirpe, *Biochim. Biophys. Acta*, 1979, **577**, 71.
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[156] M. Tsuda, *J. Biochem. (Jpn.)*, 1979, **86**, 1463.
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[159] C. Baumann, H. Rudiger, and A. D. Strosberg, *FEBS Lett.*, 1979, **102**, 216.

Table 1 *Composition and properties of some purified lectins*

Source	Mol. wt.	Sub-units	Carbohydrate %		Human blood type	Sugar specificity	Ref.
			Neutral	Amino-sugar			
Plants							
Cucurbita pepo	2.0×10^4					Chito-oligosaccharides	145
Rice bran (*Oryza sativa*)	3.7×10^4	2				GlcNAc	146
Lathyrus odoratus I	5.2×10^4	4				Methyl α-D-Man	147
II		4				Methyl α-D-Man	147
III	5.5×10^4	4				Methyl α-D-Man	147
Onobrychis viciifolia	5.2×10^4	2	2.6			α-D-Man, α-D-Glc	148, 149
Vicia faba	2.5×10^4	2	3.0			α-D-Man, α-D-Glc	150
Vicia sativa	7.0×10^4		9.4	1.0	O	α-D-Man, α-D-Glc	151
Wisteria floribunda	2.2×10^5	8	4.6	4.6	O	D-Gal, D-GalNAc	152
	6.6×10^4	2	10.3	2.9		D-Man	153
Animals							
Oncorhynchus tshawytscha	1.22×10^5				B	D-Gal, L-Rha	154
Micro-organisms							
Streptomyces sp.	7.0×10^4				O	L-Fuc, D-Man	155
Flammulina veltipes	2.0×10^4	2				Complex carbohydrate	156
Neurospora crassa	3.9×10^4				ABO	GlcNAc, chito-oligosaccharides	157

However, no homology was detected between the α- and β-chains of the blood group A specific *V. cracca* lectin. The two *V. cracca* lectins appear to be products of homologous genes that diverged during evolution and the data confirm the existence of at least two genes coding for the lectins. Several well characterized lectins from leguminous plants and specific for D-galactose, L-fucose, and 2-acetamido-2-deoxy-D-galactose have been examined for immunological compatibility.[160] The cross-reactions observed indicated that most of the lectins studied are evolutionarily closely related proteins.

Affinity chromatography has been used to purify *Ulex europaeus* lectin II.[161] The lectin precipitates to different extents with blood group A_1, A_2, B, H, Le^a, Le^b, and I precursor substances as well as with milk oligosaccharides. The maximum inhibition of the precipitin reaction occurs with H-specific oligosaccharide and to a lesser extent with the related oligosaccharide (8), 2′-*O*-α-L-fucosyl-lactose, and oligomers of 2-acetamido-2-deoxy-D-glucose.

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 6)-R

(8) R = further monosaccharide units

Two isolectins from red kidney beans (*Phaseolus vulgaris*) have been isolated by affinity chromatography on immobilized thyroglobulin.[162] One isolectin was unable to agglutinate erythrocytes but had a high activity for stimulating and agglutinating lymphocytes, whereas the other isolectin showed a high activity for the agglutination of erythrocytes but no mitogenic activity. A glycopeptide, isolated from the leukoagglutinating phytohaemagglutinin of *P. vulgaris*, contains a branched oligosaccharide moiety, linked glycosidically to L-asparagine and composed of five D-mannosyl and two 2-acetamido-2-deoxy-D-glucosyl residues, similar to that found in many mammalian glycoproteins.[163]

Human Tamm–Horsfall glycoprotein inhibits lymphocyte transformation induced by the leukoagglutinin and haemagglutinin of *P. vulgaris* owing to competition between the glycoprotein and carbohydrate receptors on the lymphocytes for the two lectins.[164] Interactions between the Tamm–Horsfall glycoprotein and the *P. vulgaris* lectins have been used in the development of a procedure for the separation of the two lectins by affinity chromatography on immobilized Tamm–Horsfall glycoprotein.

The lectin from the thorn apple, *Datura stramonium*, purified by affinity chromatography on immobilized fetuin, agglutinates lymphocytes as well as erythrocytes.[165] The agglutination of both cell types was specifically inhibited by oligomers of 2-acetamido-2-deoxy-D-glucose and appears to be caused by a single lectin. However, these activities are distinct from an associated mitogenic

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[161] M. A. E. Pereira, F. Gruezo, and E. A. Kabat, *Arch. Biochem. Biophys.*, 1979, **194**, 511.
[162] M. Monsigny, K. H. Jeune-Chung, and Y. Perrodon, *Biochimie*, 1978, **60**, 1315.
[163] R. Hsu, J. B. Miller, S. Yachnin, and G. Dawson, *Biochim. Biophys. Acta*, 1979, **579**, 386.
[164] F. Serafini-Cessi, C. Franceschi, and S. Sperti, *Biochem. J.*, 1979, **183**, 381.
[165] D. C. Kilpatrick, *Plant Sci. Lett.*, 1979, **15**, 279.

activity of similar saccharide specificity. Immunofluorescent staining of cotyledon sections of *D. stramonium* fixed with formaldehyde show that the lectin is localized in the cytoplasm with little association with the cell walls.[166] The location and developmental regulation of the lectin does not rule out the possibility of a recognition function for the lectin.

The lectin from potato, *Solanum tuberosum*, has been purified by affinity chromatography on an immobilized $NN'N''$-triacetyl-chitotriose matrix.[167]

Comparative studies of the lectins from the Indian jack fruit (*Artocarpus integrifolia*), betelnut (*Areca catechu*), and sponge (*Geodia* species) with the alkali-labile and alkali-stable carbohydrate chains of glycoproteins have been reported.[168]

L-Arabino-D-galactan, cross-linked with epichlorohydrin to produce an insoluble gel, has been used to purify the β-D-galactosyl-specific lectins from *Ricinus communis*, *Glycine max*, *Arachis hypogaea*, wild cucumber (*Echinocystis lobata*), sun hemp (*Crotalaria juncea*), and bitter gourd (*Momordica charantia*) to homogeneity.[169] The lectin of *Agaricus bisporus* recognizes the *O*-α-glycosyl linkage between 2-acetamido-2-deoxy-D-galactose and L-serine, in addition to the 3-*O*-β-D-galactosyl-2-acetamido-2-deoxy-D-galactosyl sequence for which *Arachis hypogaea* (peanut) and *Bauhinia purpurea* lectins are specific.[170]

Relatively stable proteins isolated from human heart and from human and rhesus monkey skeletal muscles, are capable of binding to β-D-galactosides.[171] Gel precipitation and radioimmunoassay studies with rabbit antiserum to calf heart lectin revealed antigenic cross-reactions between the primate and bovine muscle lectins. The lectin from calf heart shows a greater specificity towards the terminal disaccharide (lactosamine) units of blood-group antigen precursor chains than does the *Ricinus communis* lectin.[172]

Extracts from human glomerular basement membranes, solubilized with pepsin or bacterial collagenase, agglutinate normal or transformed human cells.[173] Cytoagglutination is inhibited by 2-acetamido-2-deoxy-hexoses. The maintenance of tissue differentiation is governed by the interaction of cells with particular sites of the basement membrane consisting of an association between collagen and matrix glycoprotein.[174]

A hepatic lectin responsible for the clearance of desialylated serum glycoproteins has been immobilized on Sepharose.[175] The binding site was examined

[166] D. C. Kilpatrick, M. M. Yeoman, and A. R. Gould, *Biochem. J.*, 1979, **184**, 215.
[167] N. N. Desai and A. K. Allen, *Anal. Biochem.*, 1979, **93**, 88.
[168] B. Chatterjee, P. Vaith, S. Chatterjee, D. Karduck, and G. Uhlenbruck, *Int. J. Biochem.*, 1979, **10**, 321.
[169] T. Majumdar and A. Surolia, *Indian J. Biochem. Biophys.*, 1979, **16**, 200.
[170] R. Kaifu and T. Osawa, *Carbohydr. Res.*, 1979, **69**, 79.
[171] R. A. Childs and T. Feizi, *Biochem. J.*, 1979, **183**, 755.
[172] R. A. Childs and T. Feizi, *FEBS Lett.*, 1979, **99**, 175.
[173] J. Gerfaux, F. Chany-Fournier, P. Bardos, J. P. Muh, and C. Chany, *C.R. Hebd. Seances Acad. Sci., Ser. D*, 1978, **288**, 915.
[174] J. Gerfaux, F. Chany-Fournier, P. Bardos, J. P. Muh, and C. Chany, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 5129.
[175] M. Sarkar, J. Liao, E. A. Kabat, T. Tanabe, and G. Ashwell, *J. Biol. Chem.*, 1979, **254**, 3170.

using a competitive binding assay with a ^{3}H-labelled precursor blood group Ii glycoprotein and various glycoproteins, polysaccharides, monosaccharides, and glycosides as inhibitors. A lectin from embryonic chick muscle has been isolated in a high molecular weight form, presumably as an aggregate.[176] Haemagglutination activity was inhibited by heparin, dermatan sulphate, and heparan sulphate.

A lectin (mol. wt. 4.3×10^4) has been isolated from the mucous of the snail *Arion empiriconum*.[177] Unlike the *Helix* lectins, this one has no blood-group specificity and no well-defined carbohydrate specificity. It precipitates some serum proteins and possesses proteinase-inhibition properties. The isolectins of *H. pomatia* have been separated by isoelectric focusing into proteins having identical molecular weights (8×10^4), but different amino-acid compositions.[178] Certain similarities between invertebrate and vertebrate 'serum-lectins' with respect to their specificity for binding D-galactans have been observed.[179]

The marine sponge *Geodia cydonium* contains a D-galactose-specific lectin which does not promote aggregation of single cells but prevents the anti-aggregation receptor from disaggregating cell clumps.[180] Competition experiments have shown that the lectin inactivates the anti-aggregation receptor by binding to it, most probably *via* its terminal D-galactosyl group.

Four lectins, two of which precipitate human erythrocytes, have been isolated from the sponge *Axinella polypoides*.[181] Inhibition of agglutination was obtained with 6-*O*-β-D-galactosyl-D-galactose. Another sponge, *Aaptos papillata*, contains three lectins, whose binding is inhibited by *NN'N''*-triacetyl-chitotriose. The lectins are stored in vesicles of the 'spherulous cells' of *Axinella polypoides*.[182]

A strain of *Conidiobolus*, a parasite of various insects and mammals, contains a chitin-binding haemagglutinin.[183] Cholera toxin (choleragen), through its binding to the oligosaccharide portion of a glycolipid, exhibits lectin-like activity, which results in the agglutination of liposomes and erythrocytes, containing the toxin receptor (ganglioside G_{M_1}).[184] The enzyme D-galactose oxidase demonstrates lectin-like properties by interacting with immobilized D-galactosyl residues and related immobilized- and free-sugars under conditions of affinity electrophoresis, and by agglutination of neuraminidase-treated human erythrocytes.[185] The sugar-binding and haemagglutinin activities were preserved even after removal of Cu^{2+}, which is essential for enzymic activity.

[176] D. Kobiler and S. H. Barondes, *FEBS Lett.*, 1979, **101**, 257.
[177] L. Habets, U. C. Vieth, and C. Hermann, *Biochim. Biophys. Acta*, 1979, **582**, 154.
[178] P. Vretblad, R. Hjorth, and T. Låås, *Biochim. Biophys. Acta*, 1979, **579**, 52.
[179] G. Uhlenbruck and D. Karduck, *Naturwissenschaften*, 1979, **66**, 524.
[180] W. E. G. Müller, B. Kurelec, R. K. Zahn, I. Müller, P. Vaith, and G. Uhlenbruck, *J. Biol. Chem.*, 1979, **254**, 7479.
[181] H. Bretting, H. Kalthoff, and S. Fehr, *J. Invert. Pathol.*, 1978, **32**, 151.
[182] H. Bretting and K. Köningsmann, *Cell Tissue Res.*, 1979, **201**, 487.
[183] F. Ishikawa, K. Oishi, and K. Aida, *Appl. Environ. Microbiol.*, 1979, **37**, 1110.
[184] R. L. Richards, J. Moss, C. R. Alving, P. H. Fishman, and R. O. Brady, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 1673.
[185] V. Hořejší, *Biochim. Biophys. Acta*, 1979, **577**, 383.

Pallidin, a lectin from the cellular slime mould *Polysphondylium pallidum*, has been separated into three isolectin forms, each consisting of two or three sub-units.[186]

4 Blood-group Substances

Biochemical studies on ABO and Lewis antigens and the role of the secretor gene have been reviewed.[187] The Haworth Memorial Lecture given by R. U. Lemieux and entitled 'Human Blood Groups and Carbohydrate Chemistry' has been published.[188]

$$\beta\text{-D-Gal}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{NAc-}1 \rightarrow$$
(9)

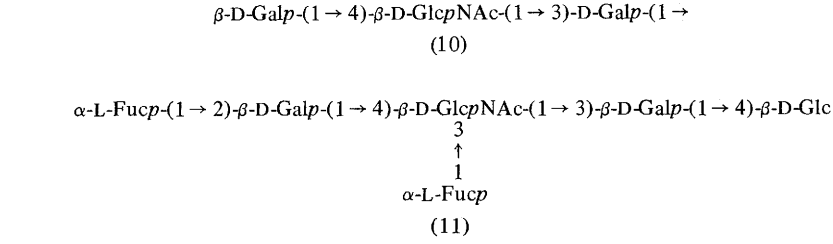
Oligosaccharide inhibition studies using anti-I Ma (group 1) have been used to define the structure (9) of the anti-I Ma (group 1) combining site.[189] The amino-sugar in turn is linked to a D-galactosyl residue, but the data require that the intact D-galactosyl residue does not necessarily contribute to binding beyond the C-6 methylene group. Identical results have been obtained for anti-I Ma by use of oligosaccharides in a radioimmunoassay technique.[190] A glycoprotein that cross-reacts with anti-I Ma serum is formed when β-D-galactosyl residues are transferrred to a precursor glycoprotein prepared from blood-group H substance from which terminal L-fucosyl and D-galactosyl groups had been sequentially removed.[191] A double-antibody radioimmunoassay has been developed for blood group Ii antigens.[192] The method can be applied to the measurement of blood group Ii antigens on intact human erythrocytes and on erythrocytes after treatment with pepsin and neuraminidase.

Blood-group antigens M, N, and T (Thomsen–Friedenrich) have been isolated from red-cell stroma.[193] In a radioimmunoassay system, rabbit anti-(blood group M) displays a higher affinity for the M glycoprotein than for the M peptide. No specific assay could be obtained for the N antigen. These methods should facilitate studies on the release of the antigens from normal and pathological membranes. Two glycopeptides and one peptide fragment have been obtained by treatment of glycophorins, glycoproteins having MM, NN, and MN antigenic activity, from human erythrocyte membranes by treatment with cyanogen bromide.[194] The fragments include an eight-residue glycopeptide,

[186] S. D. Rosen, J. Kaur, D. L. Clark, B. T. Pardos, and W. A. Frazier, *J. Biol. Chem.*, 1979, **254**, 9408.
[187] B. Boettcher in 'The Biochemical Genetics of Man', ed. D. J. H. Brock and O. Mayo, Academic Press, New York, 1978, p. 325.
[188] R. U. Lemieux, *Chem. Soc. Rev.*, 1978, **7**, 423.
[189] E. A. Kabat, J. Liao, and R. U. Lemieux, *Immunochemistry*, 1978, **15**, 727.
[190] T. Feizi, E. Wood, A. Augé, S. David, and A. Veyrières, *Immunochemistry*, 1978, **15**, 801.
[191] H. Schenkel-Brunner, E. A. Kabat, and J. Liao, *Eur. J. Biochem.*, 1979, **98**, 573.
[192] E. Wood, J. Lecomte, R. A. Childs, and T. Feizi, *Mol. Immunol.*, 1979, **16**, 813.
[193] M. J. King and A. Ebringer, *Immunology*, 1979, **38**, 129.
[194] O. O. Blumenfeld and K. V. Puglia, *Biochim. Biophys. Acta*, 1979, **579**, 95.

originating from the *N*-terminus of the glycoprotein, a large molecular weight glycopeptide from the middle of the molecule bearing L-asparagine-linked oligosaccharides, and a residual peptide fragment of about fifty amino-acid residues from the *C*-terminus of the molecule. The amino-acid and carbohydrate composition and size of the glycoprotein are in agreement with earlier sequence data.[195] The levels of A, H, I, and i blood-group antigens have been measured in normal plasma by an agglutination-inhibition method.[196] The level of H antigen in the plasma directly correlated with the amount in the red cells and was affected by the donor's secretor status.

No correlation was observed between the I and i antigen in normal plasma, favouring the theory that there is no biochemical relationship between these two specificities. The blood-group I- and i-antigen activities of a number of synthetic oligosaccharides, as determined by radioimmunoassay, have shown that oligosaccharides containing structure (10) have inhibitory activity toward the majority of anti-I and anti-i antibodies.[197] The levels of Le^b-active oligosaccharides, chiefly lacto-*N*-difucohexaose I (11) in urine and plasma of pregnant and lactating women belonging to different ABO and Lewis blood groups have been measured by radioimmunoassay.[198] An ultracentrifugal-difference method has been used to determine the extent of complex formation between lysozyme and mucous glycoproteins.[199] The interaction is strongest with those glycoproteins of blood group Le^a specificity.



(10)

(11)

Equilibrium density-gradient centrifugation has been used for the isolation and characterization of glycoproteins with blood group P_1 activity from sheep hydatid-cyst fluid.[200] Variations in the proportions of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, D-mannose, and D-glucose were noted in fractions of differing specific activity. Ovomucoid from turtle dove exhibits blood-group P_1 antigen activity, but differs in both the protein and carbohydrate moieties from hen ovomucoid.[201]

[195] M. Tomita, H. Furthmayr, and V. T. Marchesi, *Biochemistry*, 1978, **17**, 4756.
[196] P. Rouger, D. Riveau, and C. Salmon, *Vox Sanguinis*, 1979, **37**, 78.
[197] E. Wood and T. Feizi, *FEBS Lett.*, 1979, **104**, 135.
[198] D. A. Zopf, V. Ginsberg, P. Hallgren, A. C. Jonsson, B. S. Lindberg, and A. Lundblad, *Eur. J. Biochem.*, 1979, **93**, 431.
[199] J. M. Creeth, J. L. Bridge, and J. R. Horton, *Biochem. J.*, 1979, **181**, 717.
[200] A. Cossey, A. DiMichiel, and J. Dunstone, *Eur. J. Biochem.*, 1979, **98**, 53.
[201] C. Francois-Gerard, G. Gerday, and J. G. Beeley, *Biochem. J.*, 1979, **177**, 679.

The structures [(12) and (13)] of two new oligosaccharides of A^+ hog submaxillary glycoproteins have been identified after their elimination from the glycoprotein by alkaline borohydride elimination.[202] Oligosaccharides from H^+ hog sub-maxillary glycoprotein have been isolated and characterized, and compared with those found in A^+ and A^-H^- glycoproteins.[203] The distinction between H^+ and A^-H^- glycoproteins appears to be dependent on the amounts and ratios of the various oligosaccharides in two glycoproteins.

Neu*p*5Ac-(2→6)-D-Gal*p*-(1→3)-D-GalNAc-ol

(12)

D-Gal*p*-(1→3)-D-GalNAc-ol

(13)

5 Collagens

A book dealing with collagen in the context of the physiology and pathology of connective tissues,[204] and a practical handbook for the preparation and analysis of basement membranes,[205] have been published.

Collagens and procollagens have been visualized directly in polyacrylamide gels since the absorbance maxima for complexes of these glycoproteins with Coomassie Blue R250 occurs at 520–535 nm compared with the maxima for other proteins of 550–560 nm.[206] The technique has been used to detect procollagens in human diploid fibroblast cultures. Human procollagen has been isolated in quantities suitable for detailed chemical analysis following adsorption chromatography on controlled pore glass beads.[207] A high molecular weight type 1 human procollagen, isolated using this procedure from culture medium of human foreskin fibroblasts, contains two α-1 and one α-2 procollagen chains, linked by disulphide bonds *via* carboxyterminal propeptides. Amino-acid sequencing and other studies have established the occurrence of an *O*-β-D-galactopyranosyl-L-hydroxylysine residue at position 564 and a 2-*O*-α-D-glucopyranosyl-*O*-β-D-galactopyranosyl-L-hydroxylysine residue at position 603 in the αI(II) chains of bovine cartilage collagen.[208] Two further amino-acid residues at positions 648 and 657 were tentatively identified as glycosylated L-hydroxylysine.

The pattern of excretion of the collagen metabolites L-hydroxyproline, L-hydroxylysine, and L-hydroxylysine glycosides in urine by patients with Paget's disease of the bone, and carcinoma with bone metastases, is reported to be a

[202] D. Aminoff, M. M. Baig, and W. D. Gathmann, *J. Biol. Chem.*, 1979, **254**, 1788.
[203] D. Aminoff, W. D. Gathmann, and M. M. Baig, *J. Biol. Chem.*, 1979, **254**, 8909.
[204] S. Gay and E. J. Miller in 'Collagen in the Physiology and Pathology of Connective Tissue', Gustav Fischer Verlag, Stuttgurt, 1978.
[205] 'Biology and Chemistry of Basement Membranes', ed. N. A. Kefalides, Academic Press, New York, 1978.
[206] P. J. McCormick, S. Chandrasekhar, and A. J. T. Mills, *Anal. Biochem.*, 1979, **97**, 359.
[207] S. Gerard and W. M. Mitchell, *Anal. Biochem.*, 1979, **96**, 433.
[208] G. Francis, W. T. Butler, and J. E. Finch, *Biochem. J.*, 1978, **175**, 921.

more specific indicator of the presence of bone disease than is the measurement of any individual collagen metabolite.[209] The effect of adjuvant-induced arthritis on the urinary excretion of L-hydroxyproline and L-hydroxylysylglycosides in rats has been reported.[210]

E.s.r. studies of spin-labelled rat-skin collagen has permitted a more detailed picture of the thermal denaturation of native salt-soluble collagen to be obtained.[211] Based on an examination of the spectral changes, it is apparent that the microenvironment around the spin-label undergoes a series of hysterectic changes. The activation energies calculated from the Arrhenius plots provide an indication of the energetics of the expansion of the microenvironment about the labelled site. Kangaroo tail tendon has been studied by *X*-ray diffraction.[212] A stereochemically acceptable solution was found for one of two possible interchain hydrogen-bonding schemes. Anterior lens collagen has been cleaved by treatment with cyanogen bromide to release three polypeptides, each with varying quantities of L-hydroxylysl glycosides.[213] Structural differences have been reported among the procollagens associated with the rough and smooth microsomes from chick embryo cartilage.[214] Glycosylation of both collagenous and non-collagenous regions is indicated early in the processing of the polypeptides in the rough endoplasmic reticulum, and after formation of disulphide bonds, the glycosylation of the non-collagenous region is continued in the same location as well as in the Golgi apparatus, where the appropriate glycosyl transferases have been shown to occur. Residues of D-mannose can be identified in both the *N*- and *C*-terminal properties of type II procollagen of chick embryo cartilage.[215] The addition of D-mannose to the propeptides occurs under conditions in which folding of the collagen domain into a triple helical conformation is prevented.

The synthesis of collagen *in vitro* and *in vivo* in rats is inhibited by D-penicillamine, probably due to its ability to chelate Fe^{2+}, a cofactor for proline hydroxylase (EC 1.14.11.2).[216]

The effects of dexamethasone on transformed human cells result in the accumulation of both collagen and fibronectin.[217]

[209] P. C. Kelleher, *Clin. Chim. Acta*, 1979, **92**, 373.
[210] S. Orloff, V. H. Rao, and L. Verbruggen, *Ital. J. Biochem.*, 1979, **28**, 173.
[211] M. T. W. Hearn, M. L. Jenkinson, D. B. Myers, and B. M. Peake, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1542.
[212] R. D. B. Fraser, T. P. MacRae, and E. Suzuki, *J. Mol. Biol.*, 1979, **129**, 463.
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[216] H. Nakagawa, K. Ohkoshi, and S. Tsurufuji, *Biochem. Pharmacol.* 1979, **28**, 1771.
[217] L. T. Furcht, D. F. Mosher, G. Wendleschafer-Crabb, D. A. Woodbridge, and J. M. Foidart, *Nature (London)*, 1979, **277**, 393.

6 Glycogen

The chemistry and biochemistry of glycogen-storage diseases have been reviewed.[218]

An unusual type of glycogen, whose synthesis is stimulated by thyroid hormones and other serum factors, has been isolated from chick embryo sternum.[219] The polysaccharide has been characterized as a (1 → 4)-α-D-glucan with a low degree of branching. The fate of glycogen in rats, which have been fasted and then re-fed, has been investigated.[220] Results from pulse-labelling reveal that D-glucosyl residues in newly-synthesized glycogen are preferentially located on the outer chains. An almost equal distribution of residues between the inner and outer chains was attained after a time and increased with increasing amounts of pre-existing glycogen. In the degradation of the glycogen, the labelled D-glucosyl residues that had been incorporated into the glycogen at various times of synthesis were removed during glycogenolysis in a defined order; those residues that had been incorporated first were removed last and *vice versa*. Since the labelling of the outer chains was not markedly different, this order cannot be explained by the initial removal of outer chains.

Rat-liver glycogen synthetase has been isolated in an active form free from contaminating synthetase kinases and phosphoprotein phosphatase.[221] This 3′,5′-cyclic AMP-independent synthetase is more specific than the 3′,5′-cyclic AMP-dependent protein kinase in catalysing the phosphorylation and conversion of the synthetase into an inactive form. The level of glycogen synthetase *b* phosphatase is low in the liver of alloxan diabetic rats.[222] Pre-treatment of the diabetic animals with insulin results in a return to normal levels for the conversion of glycogen synthetase *b* phosphatase into glycogen synthetase *a* phosphatase. Glycogen mediated by glycogen synthetase has been identified as the major acceptor of D-xylose in polysomal preparations of chick embryo cartilage cultures.[223] Electrophoretic heterogeneity of glycogen synthetase I from rabbit skeletal muscles has been demonstrated.[224] The existence of the enzyme in the form of an equilibrium of oligomer forms capable of reversible association–dissociation was suggested. The phosphorylation of skeletal muscle glycogen synthetase is catalysed by a protein kinase that is stimulated by a Ca^{2+}-dependent regulator protein.[225]

The synthesis of glycogen in rat adipose tissue is catalysed by multiple D-glucose 6-phosphatase-dependent forms of glycogen synthetase.[226] Hormones

[218] S. Palmer in 'Pediatric Nutrition in Developmental Disorders', ed. S. Palmer and S. Ekvall, C. C. Thomas, Springfield, 1978, p. 261.
[219] B. J. Segen, J. R. Dunstone, and K. D. Gibson, *Arch. Biochem. Biophys.*, 1979, **193**, 267.
[220] P. Devos and H. G. Hers, *Eur. J. Biochem.*, 1979, **99**, 161.
[221] M. F. Jett and T. R. Soderling, *J. Biol. Chem.*, 1979, **254**, 6739.
[222] A. H. Gold, D. Dickemper, and D. M. Haverstick, *Mol. Cell, Biochem.*, 1979, **25**, 47.
[223] J. H. Kimura, and A. I. Caplan, *Arch. Biochem. Biophys.*, 1978, **191**, 687.
[224] G. A. Solov'eva, O. L. Evastaf'eva, and I. S. Sakharova, *Biochemistry (USSR)*, 1977, **42**, 1751.
[225] A. K. Srivastava, D. M. Waisman, C. O. Brostrom, and T. R. Soderling, *J. Biol. Chem.*, 1979, **254**, 583.
[226] H. R. Kaslow, R. D. Eichner, and S. E. Mayer, *J. Biol. Chem.*, 1979, **254**, 4674.

regulate the metabolism of the polysaccharide by promoting the reversible interconversions between these forms. No evidence was obtained to show that the D-glucose 6-phosphate independent form of the glycogen synthetase exists in intact adipose tissue.

The incorporation of D-glucose from D-[^{14}C]glucose into glycogen in rat brain slices is stimulated by Li^+, in a manner similar to that of skeletal muscle.[227] Enzyme patterns occurring during the synthesis of glycogen in hepatocytes from starved and fed rats have been described.[228] In addition to the classical *a* and *b* forms of glycogen phosphorylase, the existence of other forms that vary in their activity is apparent.

A non-phosphorylated form of glycogen phosphorylase B (EC 2.4.1.1) has been isolated from rabbit liver.[229] Co-operative interactions between the substrate-binding sites become more pronounced in the presence of inhibitors, ATP, and D-glucose, and less pronounced in the presence of the activator AMP. Isoenzymes of glycogen phosphorylase and glycogen synthetase are preserved in chick embryo liver and in the liver of adult chicken.[230] The changes which were observed in the ratio between phosphorylases *a* and *b*, on the one hand, and glycogen synthetases I and D on the other, do not fit into the Hers scheme for the liver of adult mammals.

The properties of phosphorylase *b* from the deep sea fish *Indocibium guttattam* and rabbit muscle phosphorylase *b* have been compared.[231] Specific antibodies against rabbit-muscle phosphorylase are capable of binding to the liver isoenzyme of phosphorylase.[232] Under these conditions, the apparent affinity (K_m) for D-glucose 1-phosphate was improved four-fold at saturating concentrations of AMP, while at concentrations of D-glucose 1-phosphate lower than the K_m, the antibodies increased enzyme activity more than ten-fold. The structural character of the *N*-terminal regions of the liver and muscle phosphorylase *b* isoenzymes may be, at least partially, responsible for their differing affinities for D-glucose 1-phosphate.

7 Glycosaminoglycuronans, Glycosaminoglycans, and their Protein and Peptide Derivatives

A comprehensive book dealing with biological, chemical, and medical aspects of proteoglycans has been published.[233] Detailed coverage is given to the nomenclature of the macromolecules, their occurrence, function, isolation, and component analysis. Further chapters give detailed information on their

[227] R. A. Mickel, L. Hallidy, N. Haugaard, and E. S. Haugaard, *Biochem. Pharmacol.*, 1978, **27**, 799.
[228] J. Katz, S. Golden, and P. A. Wals, *Biochem. J.*, 1979, **180**, 389.
[229] T. B. Eronina, G. V. Silonova, and N. B. Livanova, *Biochemistry (USSR)*, 1977, **42**, 197.
[230] G. Yurovitskii, I. P. Krivopishin, and L. S. Mil'man, *Biochemistry (USSR)*, 1978, **43**, 1260.
[231] G. Soman and G. Philip, *Biochim. Biophys. Acta*, 1977, **482**, 35.
[232] A. M. Janski and D. J. Graves, *J. Biol. Chem.*, 1979, **254**, 1644.
[233] J. F. Kennedy in 'Proteoglycans – Biological and Chemical aspects in Human Life', Elsevier, Amsterdam, 1979.

primary, secondary, and tertiary structures, enzymic susceptibilities, chemical modification, qualitative and quantitative determination, chemical synthesis and biosynthesis. Chapters dealing with the more medical aspects detail the effects of drugs *etc.* upon biosynthesis, and processes of health and of disease in relation to proteoglycans and glycosaminoglycans.

In a review on proteoglycans and atherosclerosis, the characterization of arterial proteoglycans and their probable role in atherosclerosis has been discussed.[234] A review on rheumatoid disease in cultured human synovial cells has dealt with a biochemical study of glycosaminoglycans, proteins, and plasma membranes of synovial fibroblasts in culture.[235] Aspects of glycosaminoglycans and their binding to biological macromolecules have been reviewed.[236]

An alternative, and hopefully simplified, rational nomenclature for proteoglycans has been proposed.[237]

Analysis. – Glycosaminoglycuronans in urine and blood have been measured following formation of soluble complexes with Alcian Blue.[238] The method is based on differences in absorption spectra of the dye and dye–glycosaminoglycuronan complexes. The extent of complex formation between Alcian Blue and glycosaminoglycuronans in solutions of dimethylsulphoxide has been shown to depend both on the concentration of polysaccharide and the composition of the dimethylsulphoxide solution.[239]

A simple purpose-built fluorimeter has been constructed for the titrimetric assay of glycosaminoglycans with Acridine Orange.[240]

All known glycosaminoglycans have been separated from each other on cellulose acetate using a discontinuous electrophoretic method.[241] The separation of glycosaminoglycans by electrophoresis on wedge-shaped cellulose acetate strips has been developed to deal with low levels of polysaccharides.[242] Short migration times, high sensitivity, and improved resolution for multisample separation of glycosaminoglycans have been reported.[243] An electrophoretic method employing agarose gel has been used for the separation of glycosaminoglycans on both an analytical and a preparative scale.[244] The method avoids the limitations of sample size imposed by cellulose acetate electrophoresis.

An 'improved' method for the microdetermination of glycosaminoglycuronans after digestion with chondroitin sulphate lyase ABC and chondroitin sulphate lyase AC has been reported.[245] However, it is based on the already reported

[234] P. L. Muthiah, *Artery*, 1978, **4**, 183.
[235] E. Vuorio, *Scand. J. Clin. Lab. Invest., Suppl. 149*, 1977, **37**, 3.
[236] U. Lindahl and M. Höök, *Annu. Rev. Biochem.*, 1978, **47**, 385.
[237] L. B. Jaques, *Artery*, 1978, **4**, 144.
[238] E. W. Gold, *Anal. Biochem.*, 1979, **99**, 183.
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[240] G. P. Diakun, H. E. Edwards, J. C. Allen, G. O. Phillips, and R. B. Cundall, *Anal. Biochem.*, 1979, **94**, 378.
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[242] S. Rufini, M. Ghebregzabher, G. Castellucci, and M. Lato, *Clin. Chim. Acta*, 1979, **95**, 443.
[243] R. Cappelletti, M. Del Roso, and V. P. Chiarugi, *Anal. Biochem.*, 1979, **93**, 37.
[244] J. L. Funderburgh and J. W. Chandler, *Anal. Biochem.*, 1978, **91**, 464.
[245] M. Koseki, A. Kimura, and K. Tsurumi, *J. Biochem. (Jpn.)*, 1978, **83**, 553.

colour produced by reaction of thiobarbituric acid with the β-formyl pyruvic acid liberated from periodate oxidized disaccharides containing D-gluco-4-enepyranosyluronic acid, which are released by the *endo*-eliminases. The unsaturated disaccharides liberated from chondroitin sulphate and dermatan sulphate after treatment with chondroitin sulphate lyase ABC have been estimated with a higher sensitivity than conventional methods by h.p.l.c.[246]

Occurrence, Isolation, and Structure. – In an ultrastructural cytochemical investigation into complex carbohydrates in leucocyte granules, an overlapping distribution of glycosaminoglycans and acid phosphatase has been observed.[247] Biochemical data suggest that the glycosaminoglycans inhibit the acid hydrolases and may prevent premature granule destruction. A complex population of glycosaminoglycans has been identified in macrophage cells, some being present both as cell surface and cytoplasmic components while others, such as chondroitin 4-sulphate and heparan sulphate, seem to be surface molecules.[248] Glycosaminoglycans, mainly hyaluronic acid, have been identified in embryonic chick cardiac jelly and other embryonic matrices.[249]

Cultures of chondrocytes from rabbit ear cartilage produce chondroitin 4- and 6-sulphate and keratan sulphate but no dermatan sulphate.[250] Hence, rabbit ear chondrocytes produce peptidoglycans comparable to those isolated from hyaline cartilage. The secretion of proteoglycans by chondrocytes can be influenced by colchicine, cytochalasin B, and 4-nitrophenyl β-D-xylopyranoside.[251] Data suggest that the microtubules might have a facilitory rather than an obligatory role in the intracellular translocation and secretion of proteoglycans in these cells. The enhancement of aggregation of chicken retina cells by physiological concentrations of glycosaminoglycans appears to be due to the steric exclusion of cells by the polysaccharides.[252] The glycosaminoglycans, by virtue of their excluded volumes, may also have important non-specific influences on cell migration and cell recognition during morphogenesis.

Freeze-etching and small angle *X*-ray data indicate that there are at least four, and probably as many as seven, bands of glycosaminoglycan along the axis of rat tail collagen, with four of the most prominent bands circumscribing the collagen fibrils.[253]

The ^1H n.m.r. spectra of monomers and dimers of glycosaminoglycans have been recorded in solutions of [$^2H_6$]dimethyl sulphoxide; the NH- and OH-resonances were partially assigned.[254] The temperature-dependence of the spectra suggests hydrogen bonding to the solvent with the exception of the C-4

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[250] K. Madsen and S. Lohmander, *Arch. Biochem. Biophys.*, 1979, **196**, 192.
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[252] J. E. Morris, *Exp. Cell Res.*, 1979, **120**, 141.
[253] R. Lam, W. J. Claffey, and P. H. Geil, *Biophys. J.*, 1978, **24**, 613.
[254] F. Heatley, J. E. Scott, and B. Casu, *Carbohydr. Res.*, 1979, **72**, 13.

hydroxy-group of sodium D-glucuronate, which was consistently downfield and relatively temperature insensitive. Spectroscopic results also indicate that the acetamido-groups occur in well-defined and discretely different environments in different glycosaminoglycans, and they suggest that some polymers have preferred conformations in solution.[255] In general the n.m.r. results support the conclusions drawn from periodate oxidation studies and extend the findings of *X*-ray crystallographic studies. Several glycosaminoglycans (heparin, hyaluronic acid, chondroitin sulphate, and dermatan sulphate) interact with neutral D-glucans, such as glycogen and dextran, to form precipitates in 50% aqueous ethanol.[256] It is likely that the formation of D-glucan–polysaccharide–polyelectrolyte complexes in ethanol is a general phenomenon.

A procedure has been described for the isolation of proteoglycans from cultures of human embryonic skin fibroblasts.[257] The proteoglycans are separated into sub-fractions with different proportions of heparan sulphate and dermatan sulphate with distinctly different structures for the galactosaminoglycan component. Sulphated peptidoglycans secreted from human skin fibroblasts bind in molar sodium chloride with macroporous agarose-immobilized concanavalin A, although the glycosaminoglycans derived from the proteoglycans do not bind to the immobilized lectin.[258] The binding appears to be between the protein core of the peptidoglycan and the sugar-binding sites of the concanavalin A. Invertebral disc proteoglycans are intrinsically of smaller hydrodynamic size than cartilage proteoglycans. The difference in size does not arise from degradation during extraction.[259] The glycosaminoglycans from human greater omentum have been separated by cellulose acetate electrophoresis into three fractions with mobilities of chondroitin sulphate, heparan sulphate, and hyaluronic acid.[260] Proteoglycans extracted from human gingival epithelium appear to contain a proportion of molecules that will interact with hyaluronic acid to form macromolecular aggregates.[261] In contrast, proteoglycans from underlying tissue behaved differently. The aggregation properties of proteoglycans from human adult costal cartilage have been reported.[262]

Digestion of equine gastric mucosa with proteolytic enzymes releases hyaluronic acid, chondroitin 4- and 6-sulphate, and dermatan sulphate.[263] This pattern of glycosaminoglycans differs from that found in pig mucosa, which contains heparin, dermatan sulphate, and chondroitin 4-sulphate, and in dog mucosa which contains hyaluronic acid and dermatan sulphate.

Two proteoglycan–hyaluronate complexes have been isolated from bovine aorta under conditions of minimal proteolytic degradation.[264] Both complexes

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[256] R. J. Doyle and V. F. Holmes, *Carbohydr. Res.*, 1979, **73**, 342.
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[258] J. Schwermann, R. Prinz, and K. von Figura, *Biochem. Biophys. Res. Commun.*, 1978, **83**, 241.
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[263] H. Masuda, S. Shichijo, and M. Takeuchi, *Int. J. Biochem.*, 1979, **10**, 337.
[264] J. McMurtrey, B. Radhakrishnamurthy, E. R. Dalferes, G. S. Berenson, and J. D. Gregory, *J. Biol. Chem.*, 1979, **254**, 1621.

contained large amounts of hyaluronic acid, and chondroitin sulphates and dermatan sulphate constituted the remainder of the glycosaminoglycans. Chondroitin sulphates have been identified as the predominant glycosaminoglycan constituents in the intima of bovine aorta, whereas hyaluronic acid, dermatan sulphate, and heparan sulphates and a related glycosaminoglycan were the predominant constituents of the adventitia.[265] The molecular weight distribution of the glycosaminoglycans was reported.

Electron microscopic studies in native and fragmented proteoglycans from bovine nasal cartilage indicate that the aggregated molecules contain variably long central filaments of hyaluronic acid with an average length of 1057 nm.[266] The peptidoglycans attached to hyaluronic acid appeared as side-chain filaments of variable length (249 nm), and were distributed along the central filament at average distances of about 36 nm. Purified proteoglycans from bovine nasal cartilage have been separated into fractions enriched in aggregating and non-aggregating proteoglycans.[267] The non-aggregating proteoglycans may have structural features different from aggregating proteoglycans and are unlikely to be derived from the aggregating proteoglycans by a simple degradation process.

A theoretical model for bovine nasal cartilage proteoglycan with uniform side chains of chondroitin sulphate (40 disaccharide residues) and keratan sulphate (15 disaccharide residues) and a random-coil polypeptide backbone has been used to estimate the unperturbed radius and it was found to be about 19 nm for the proteoglycan of molecular weight 1.5×10^6.[268]

A proteoglycan sub-unit isolated from bovine nasal cartilage has been degraded by several different proteinases to produce glycosaminoglycan–peptides of varying structures.[269] The results obtained from gel permeation chromatography, viscometry, electrophoresis, and amino-sugar and amino-acid analyses have been interpreted: it is suggested that the proteoglycan core protein may have short constant sequences at the points of attachment of the glycosaminoglycan chain and variable sequences between the chains. The length and composition of the variable sequences determine the susceptibility of each interchain peptide to cleavage by a particular proteinase and hence account for the multiplicity of degradation products. The proteoglycan sub-units from articular cartilage have a smaller size, owing to degradation of a common core protein or to shorter chondroitin sulphate chains, rather than to the synthesis of a different core protein.

Carbodi-imide-induced modification of about 20% of the total carboxy-groups of bovine nasal cartilage proteoglycan result in a change in molecular weight from $0.8–2.2 \times 10^6$ to $6–9 \times 10^5$.[270] Neither the protein nor the carbohydrate moiety is considered to be cleaved in this reaction.

The major high-density proteoglycan isolated from bovine articular cartilage is polydisperse, and contains relatively constant amounts of keratan sulphate.[271]

[265] K. Murata, *Connect. Tissue Res.*, 1978, **6**, 131.
[266] D. Heinegard, S. Lohmander, and J. Thyberg, *Biochem. J.*, 1978, **175**, 913.
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[271] D. A. Swann, S. Powell, and S. Sotman, *J. Biol. Chem.*, 1979, **254**, 945.

A hyaluronic acid-binding region appears to be absent from this fraction. An oligosaccharide component of bovine articular proteoglycan monomer has been shown to contain residues of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, D-galactose, and neuraminic acid (in the molar ratio 1:1:1:2).[272] The presence of this oligosaccharide in the proteoglycan monomer was demonstrated at all stages of foetal and adult life.

Porcine ovarian follicular fluid proteoglycans consist of about 20% protein, 50% dermatan sulphate, and 20% oligosaccharides rich in neuraminic acid, D-galactose, D-mannose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose.[273] The amino-acid composition of this proteoglycan is significantly different from that of cartilage proteoglycans, with a higher proportion of L-aspartic acid, L-threonine, and L-lysine, and lower amounts of L-proline and glycine. Dermatan sulphate (mol. wt. 5.6×10^4) is released on treatment with alkali. In contrast, cartilage proteoglycans under similar treatment release chondroitin sulphate (mol. wt. 2.5×10^4). A pentasaccharide containing residues of neuraminic acid, D-galactose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose (in the molar ratio 1:2:1:1) and a hexasaccharide containing residues of neuraminic acid, D-galactose, D-mannose, and 2-amino-2-deoxy-D-glucose (in the molar ratio 1:2:1:2) are also released on treatment with alkali. The follicular fluid proteoglycans, unlike those from cartilage, do not interact with hyaluronic acid, and are susceptible to digestion with plasmin.

A previously unrecognized cartilage-matrix protein associated with proteoglycans was identified when the proteoglycans of bovine cartilage were isolated in the absence of dissociative solvents.[274] The protein (mol. wt. 2×10^5) which co-fractionated with proteoglycan in all systems contains sub-units (mol. wt. 6×10^4).

More than 90% of the proteoglycans of sheep lung parenchyma have been solubilized in a non-enzymic sequential procedure.[275]

Proteoglycans have been isolated from porcine lungs and characterized after associative density-gradient centrifugation into fractions of different buoyant density and different proteoglycan composition.[276]

The properties of proteoglycans from normal porcine nucleus pulposus, normal human annulus fibrosus, and normal human nucleus pulposus have been compared with the current structural model for proteoglycans of hyaline cartilage.[277] Like the cartilage proteoglycans, those of invertebral disc contain both keratan sulphate and chondroitin sulphate attached to a protein core and able to aggregate with hyaluronic acid. The protein core likewise has three regions, one lacking glycosaminoglycans, another rich in keratan sulphate, and a third rich in chondroitin sulphate. However, the disc proteoglycans contain more keratan sulphate and protein and less chondroitin sulphate and have lower

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molecular weights than cartilage proteoglycans. Electric birefringence studies have confirmed the existence of dimers of pig laryngeal proteoglycans under conditions of low concentration and ionic strength.[278] Chondroitin 4-sulphate is the only sulphated glycosaminoglycan identified in the proteoglycans released from the surface of rabbit platelets during ADP-induced aggregation, and in the proteoglycan released from the platelet granules after exposure to thrombin.[279]

Glycosaminoglycans have been isolated from guinea-pig basophilic leukocytes after treatment with proteolytic enzymes.[280] Further treatment with chondroitin lyase AC, chondroitin lyase ABC, and heparan sulphate lyase was used to identify chondroitin sulphate (55%), dermatan sulphate (30%), and heparan sulphate (15%).

After partial hepatectomy, the nuclear content of glycosaminoglycans of rat liver tends to decrease and is replaced by a sulphated glycopeptide, and another polyanionic component containing hexose, D-ribose, and phosphate.[281] Glycosaminoglycans isolated from the rat-liver mitochondria have been fractionated into those insoluble in trichloracetic acid, namely dermatan sulphate and chondroitin 4-sulphate (in the molar ratio 2:1), and those soluble in trichloracetic acid, namely chondroitin 4- and 6-sulphate, dermatan sulphate, and heparan sulphate (in the molar ratio 1:3:12).[282] Vasopressin stimulates the incorporation of sulphate into proteoglycans by foetal rat chondrocytes in monolayer culture.[283] The chemical and physical characteristics of proteoglycans and glycosaminoglycans that are synthesized by chondrocytes of embryonic chicks grown in high-density cultures are similar to those observed in proteoglycans from other hyaline cartilages.[284] The proteoglycans of two limb regions of chicken that remain cartilaginous the longest (articular and epiphyseal) have been examined for heterogeneity.[285] These functionally and anatomically distinct cartilages synthesize proteoglycans that are similar in size and in their ability to react with hyaluronic acid, but show microheterogeneities in their chemical composition and their interaction with collagen.

The use of antibodies to fragments of proteoglycans has been advocated in identifying variations in proteoglycan structure in normal and degenerated tissues.[286] Antibodies have been raised to proteoglycan monomers, the isolated hyaluronic acid-binding region, and peptides from the proteoglycan link-protein. The effect of reduction and alkylation on the antigenicity of proteoglycans of chick epiphyseal cartilage has been reported.[287] Immunodiffusion and radio-

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[287] K. Sugahara and A. Dorfman, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 1193.

immunoprecipitation assays indicate that the proteoglycan sub-units have both species-specific and species-common antigenic determinants. Reduction and alkylation abolishes the antigenicity of the species-specific determinants of both chick and rat chondrosarcoma.

A radioimmunoassay procedure has been used to estimate the small amounts of link-protein that remain associated with the proteoglycan after purification.[288] Partially degraded hyaluronic acid, immobilized on a support of macroporous agarose, has been used to isolate two link-proteins from crude cartilage extracts.[289]

The complex of hyaluronic acid binding region, link-protein and hyaluronic acid has been isolated from trypsin digests of bovine nasal cartilage proteoglycan aggregates and the hyaluronic acid-binding region was then purified from the complex.[290] The effects of dansylation and acetylation on the interaction between hyaluronic acid and the hyaluronic acid-binding region were determined. Separate sites exist on the hyaluronic acid-binding region molecules, which are protected from dansylation when both hyaluronic acid and the link-protein are present or when only hyaluronic acid is present, suggesting that the hyaluronic acid-binding region of the proteoglycan molecule interacts with the link-protein as well as with hyaluronic acid in forming proteoglycan aggregates. Structural similarities in two major link-proteins from bovine nasal cartilage have been identified after proteolysis and examination of their peptide patterns.[291] The major difference between them is in their carbohydrate content. Extraction of Swarm rat chondrosarcoma with guanidinium chloride under 'associative' conditions solubilizes approximately half of the extractable proteoglycan, most of which is in the form of aggregate.[292] The link-protein is a major component of the aggregate and only trace amounts of other proteins are detected. Link-protein strengthens the binding properties of proteoglycan to hyaluronic acid, as shown by viscosity and analytical ultracentrifugation studies.[293] Under physiological conditions it probably 'locks' the proteoglycan to the hyaluronate chain.

For maximal binding to proteoglycan, oligosaccharides derived from hyaluronic acid must be at least ten sugar residues long and be terminated at the non-reducing and reducing ends with a D-glucuronosyl and a 2-acetamido-2-deoxy-D-glucosyl residue, respectively.[294] Sugar residues extended beyond this basic decasaccharide do not interact with the hyaluronate binding site on the proteoglycan.

Solvolytic desulphation of chondroitin sulphate has been achieved by treatment of pyridine salts of chondroitin 4- and 6-sulphates in dimethyl sulphoxide containing 10% water or methanol, with the production of high yields and low

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[293] T. E. Hardingham, *Biochem. J.*, 1979, **177**, 237.
[294] J. E. Christner, M. L. Brown, and D. D. Dziewiatkowski, *J. Biol. Chem.*, 1979, **254**, 4624.

sulphate contents.[295] The method will probably be adaptable for the desulphation of other sulphated polysaccharides.

Chondroitin sulphate proteoglycan, devoid of any affinity to hyaluronic acid, and dermatan sulphate proteoglycan have been isolated after extraction of proteoglycans from pig skin with guanidinium chloride at low temperatures and in the presence of protease inhibitors.[296] Some chemical and physical properties of the products were reported. However, a chondroitin sulphate proteoglycan from cultured human glial cells has been shown to bind to hyaluronic acid.[297]

The proteoglycans, isolated from the cartilage of homozygotes in a colony of rats having a deficiency in D-glucuronosyltransferase activity, are indistinguishable from the proteoglycans of the heterozygotes of the same age.[298]

The proportions of chondroitin 4- and 6-sulphate of the intima and media layers of normal human aortae vary with age, with the two isomers in approximately equal amounts in those of young individuals, whereas chondroitin 6-sulphate is more abundant in those of adult individuals.[299] This increase is even more pronounced for the intima and media from atherosclerotic aortae. With increasing age, the ratio of 4-sulphated disaccharide units to 6-sulphated disaccharide units from chondroitin sulphate of bovine articular cartilage decreases significantly.[300] Although a decrease in the level of sulphation of chondroitin sulphate in rat costal cartilage is observed with ageing, no significant difference in the molecular size of the glycosaminoglycan was observed between young and old animals.[301]

An enzyme preparation from cultured chick embryo vertebral chondrocytes cleaves oligosaccharides from the non-reducing terminus of chondroitin sulphate in a recycling pathway involving β-D-glucuronidase, a 4- or 6-sulphatase, and a β-D-2-acetamido-2-deoxygalactosidase.[302] In the presence of lactone-type inhibitors, chondroitin sulphate is degraded by an *endo*-glycanase system to oligosaccharides containing ten to fifteen monosaccharide units. The trisaccharide (14) is sequentially degraded from the non-reducing end to monosaccharides and sulphate by a lysosomal enzyme preparation from chick epiphyseal cartilage, thus confirming the hypothesis that oligosaccharides derived from glycosaminoglycans may be degraded by the concerted action of *exo*-enzymes.[303] The sequence is initiated by a sulphatase, which preferentially hydrolyses sulphate groups at O-6, followed by attack by the β-D-2-acetamido-2-deoxy-galactosidase and the β-D-glucuronidase.

A dodecasaccharide derived from chondroitin 4-sulphate is the lowest molecular weight substrate for hyaluronidase and a decasaccharide is the largest

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β-D-Gal*p*NAc4/6SO_3^--(1 → 4)-β-D-Glc*p*A-(1 → 3)-D-Gal*p*NAc4/6SO_3^-

(14)

size substrate for β-D-glucuronidase in the digestion of chondroitin 4-sulphate by the lysozomal hydrolases of canine liver.[304]

After digestion of chondroitin 4- and 6-sulphates with chondroitin lyase ABC, the liberated disaccharides have been separated by h.p.l.c. on a weak anion-exchange resin.[305] The method may be used in a study of the ratio of chondroitin 4- and 6-sulphates in cartilage.

Chondroitin 4- and 6-sulphates have been recognized as the major inhibitors of calcium oxalate crystal growth in normal urine.[306]

The enthalpies of dilution of aqueous solutions of sodium chondroitin 4- and 6-sulphates have been reported.[307] Chondroitin sulphate in aqueous solution may behave in such a way that the polyanion has a stretched conformation with disaccharide lengths of approximately 12.6 Å at infinite dilution and a conformation similar to that of the solid state at moderate concentration. The decrease in viscosity of chondroitin 4-sulphate and hyaluronic acid in acid and neutral solutions resembles that of cellulose derivatives in both magnitude and sign.[308] A new polymorph of sodium chondroitin 4-sulphate has been crystallized from an ethanolic aqueous solution at low pH.[309] The unit cell of the new polymorph is rectangular with dimensions in Å of $a = 16.0$, $b = 24.1$, and $c = 26.0$.

The thermodynamics of the partition of complexes of chondroitin sulphate and hexadecylpyridinium complexes in butan-1-ol–aqueous salt biphasic solutions have been reported.[310]

Amongst the proteoglycans isolated from chick embryonic cartilage, a hitherto unidentified proteoglycan has been characterized as being a 'copolymer' containing dermatan sulphate and chondroitin sulphate chains.[311] The complex has a low buoyant density, is susceptible to reduction with 2-mercaptoethanol, and forms aggregates in guanidinium chloride. Dermatan sulphate, isolated from pig skin, has been immobilized on macroporous agarose and used in the affinity chromatographic isolation of oligosaccharides with affinity for the glycosaminoglycan.[312] Oligosaccharides of decasaccharide size or larger with alternating sequences, arising from the degradation of aggregating dermatan sulphate with hyaluronidase, became partially bound to the ligand. The contact regions of the dermatan sulphate chains contain the sequences of D-glucopyranosyluronic acid

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and 2-acetamido-2-deoxy-D-galactopyranosyl 4-sulphate and of L-idopyranosyluronic acid and 2-acetamido-2-deoxy-D-galactopyranosyl 4-sulphate.

The aggregation of dermatan sulphate chains under various conditions has been investigated by light-scattering and low-shear viscometry.[313] Self-association of the glycosaminoglycan chains occurs in the presence of Na^+ and the dissociation is accomplished by increasing the ionic strength of sodium chloride or increasing the shear rate. The conformation of the L-idopyranosyluronate residues in dermatan sulphate has been shown by resolution-enhanced ^1H n.m.r. spectroscopy to be of the $^1C_4$ type.[314] This conclusion is at variance with results from *X*-ray crystallographic and periodate oxidation studies. The discrepancy relative to the *X*-ray data could be explained if the conformation of dermatan sulphate in solution is different from that in the solid state or if the *X*-ray pattern could arise from the D-glucopyranosyluronic acid-containing segments rather than from the major constituents of this heterogeneous polymer.

Dermatan [^{35}S]sulphate has been isolated after injection of [^{35}S]sulphate into rats, and treatment of the isolated glycosaminoglycans with protease, heparin hydrolase, and hyaluronidase.[315] Differences in the patterns of [^{35}S]sulphate-labelled glycosaminoglycans synthesized by cultures of normal and transformed hamster fibroblasts have been revealed.[316] The normal cells synthesize more dermatan sulphate than the transformed cells and produce a class of sulphated proteoglycans absent from the transformed cells. The transformed cells release a lower molecular weight class of proteoglycan, containing chondroitin 4- and 6-sulphate. Age-related changes in the composition and structure of human articular cartilage proteoglycan include increases in the protein and keratan sulphate content and a decrease in the hydrodynamic size of the dissociated protein with advancing age.[317] Proteoglycans from bovine articular cartilage also showed maturation related changes.[318] The chondroitin sulphate components contained shorter and fewer chains, and an increased number of keratan sulphate chains was also detected.[319]

Keratan sulphates I and II have been isolated and purified from bovine cornea and nasal cartilage, respectively.[320] Desulphation of keratan sulphate I yielded both water-soluble and water-insoluble products, but keratan sulphate II yielded only water-soluble products. The water-soluble form contains glycopeptides from the protein–polysaccharide linkage region, and oligosaccharides derived from the main chain of the glycosaminoglycan associate forming water-insoluble species.

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[320] M. M. U. McCarthy and J. R. Baker, *Carbohydr. Res.*, 1979, **69**, 151.

A comparison of the proteoglycan fractions isolated from non-calcified and calcified portions of bovine nasal cartilage indicates that the relative amount of keratan sulphate increases on calcification with a concomitant decrease in chondroitin sulphate.[321] An elevation of the ratio of chondroitin 4-sulphate to chondroitin 6-sulphate also appears to occur on calcification.

A 2-acetamido-2-deoxy-D-galactose 6-sulphatase capable of hydrolysis of sulphate groups on the non-reducing end of oligosaccharides derived from keratan sulphate has been isolated from an *Actinobacillus* species.[322] When this enzyme is used in conjunction with a 2-acetamido-2-deoxy-D-glucose 6-sulphate sulphatase, (*exo*-)β-D-2-acetamido-2-deoxyglucosidase and (*exo*-)β-D-galactosidase, the monosaccharide sequence of oligosaccharides of keratan sulphate may be identified.

Heparin with high anticoagulant activity has been recovered from crude commercial preparations by affinity chromatography on columns of immobilized human α-thrombin.[323]

Preparations of heparin and heparan sulphate have been degraded with nitrous acid, with the production of a mixture of disaccharides terminated with 2,5-anhydro-D-mannose units, which in turn are reduced to the corresponding 2,5-anhydromannitol derivatives.[324] Two non-sulphated disaccharides were identified as 2,5-anhydro-4-*O*-(β-D-glucopyranosyluronic acid)-D-mannitol and 2,5-anhydro-4-*O*-(α-L-idopyranosyluronic acid)-D-mannitol. Three monosulphated disaccharides, 2,5-anhydro-4-*O*-(β-D-glucopyranosyluronic acid)-D-mannitol 6-sulphate, 2,5-anhydro-4-*O*-(α-L-idopyranosyluronic acid)-D-mannitol-6-sulphate, and 2,5-anhydro-4-*O*-(α-L-idopyranosyluronic acid 2-sulphate)-D-mannitol were also identified. Low *N*-sulphate contents, mostly observed in the heparan sulphates, were associated with high ratios of mono-*O*-sulphated to di-*O*-sulphated (*N*-sulphated) disaccharide units. An alternative method for the separation of the same sulphated, but non-reduced disaccharides from porcine heparin and whale heparin has been reported.[325] A lower content of 2-acetamido-2-deoxy-D-glucose 6-sulphate was detected in the whale heparin than in the porcine hepatin. The proportion of 6-*O*-sulphates does not contribute to the anticoagulant activity of the heparin since the whale heparin has a higher activity than the porcine heparin. The sulphate residue in L-idopyranosyluronic acid 2-sulphate is more labile to dilute acid treatment than that in 2-amino-2-deoxy-D-glucose 6-sulphate, whereas the opposite holds for solvolysis.[326]

Heparin has been degraded by human blood platelets releasing oligosaccharides and products with low anticoagulant activity.[327] The binding of Acridine Orange to heparin is stabilized by dye–dye and dye–polymer interactions and by

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entropic factors.[328] The predominant interaction appears to be electrostatic, between the Acridine Orange cation and the heparin polyanion.

Heparin has been separated into fractions of constant molecular weight but containing a variable number of charged groups.[329] The anticoagulant activity varied with the anionic density, but an interpretation of the data in physical terms was not possible. By complexing heparin with Acridine Orange, a study of the electrostatic, as well as static and dynamic conformational aspects of this glycosaminoglycan *via* the thermodynamic and photochemical properties of the complexes, has been obtained.[330] The anticoagulant activity, co-operative binding constants, and fluorescence-fading rate parameters all show the same kind of dependence upon anionic density. Most, if not all, of the D-glucopyranosyluronic acid residues of heparin can be oxidized, without appreciable loss of anticoagulant activity.[331] In contrast, oxidation of non-sulphated L-idopyranosyluronic acid residues destroys this activity.

The interaction of heparin with human plasma low-density lipoprotein has been studied by gel permeation chromatography.[332] A fraction containing degradation products of fibrinogen formed in blood plasma after injection of thrombin also contains a complex of fibrinogen and heparin.[333] The rate of inactivation of thrombin and Factor X_a by anti-thrombin has been shown to be directly proportional to the chain-length of heparin.[334] The effects of heparin on the reactions catalysed by Factor X_a have been examined.[335] Heparin inhibits the ability of phospholipid to accelerate prothrombin activation in a mechanism which appears to involve the displacement of Factor X_a from the surface of the phospholipid.

Heparin fractions of different molecular weights and anticoagulant activities have been prepared by affinity chromatography on immobilized protamine.[336] A heparin fraction (mol. wt. 7.9×10^3) exhibited binding to anti-thrombin III with a stoicheiometry of 2:1, whereas two higher molecular weight fractions exhibited 1:1 binding. Similar results have been reported for two fractions from porcine heparin.[337] A heparin–anti-thrombin III model was proposed with binding on two sites on anti-(thrombin III), which can accommodate one large heparin molecule with high affinity or two smaller molecules with low affinity. Immobilized heparin has been used to show that anti-(thrombin III) and platelet

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anti-heparin proteins bind to different sites on the heparin molecule.[338] Active heparin species are quite heterogeneous with respect to anticoagulant activity and can be fractionated into 'active' and 'relatively inactive' forms (mol. wts. both approximately 6×10^3).[339] A detailed analysis of the fundamental properties of the two forms has included a quantitative evaluation of their ability to bind to anti-thrombin and to inactivate this protease inhibitor.

Heparin has been separated on columns of immobilized anti-thrombin into fractions with high anticoagulant activity and low anticoagulant activity.[340] Non-sulphated L-idopyranosyluronic acid residues appeared to be essential for anticoagulant activity of the heparin. They were located in larger amounts in, and concentrated in, the anti-thrombin binding regions of the heparin with high anticoagulant activity, amounting to approximately one residue per binding site. A tentative structure for the anti-thrombin binding site of pig mucosal heparin is suggested to involve an oligomer comprising twelve to sixteen monosaccharide residues. The continuous sequence represents the majority of the binding sites, whereas some isolated sugar units represent structural variants compatible with, but not required for, anticoagulant activity. These conclusions differ from those of other workers.[341] In the presence of heparin the rate of inhibition of thrombin-clotting activity by α_2-macroglobulin is decreased.[342] Heparin binds to thrombin, impairing the formation of a thrombin–α_2-macroglobulin complex. The data show that heparin, paradoxically, protects thrombin from inhibition by α_2-macroglobulin, whereas it increases the enzyme inhibition by anti-(thrombin III). The binding of heparin to human α-thrombin has been measured by evaluating the kinetic parameters for the enzyme with a synthetic tripeptide both in the presence and absence of heparin.[343] The binding of heparin to thrombin therefore enhances inactivation of the enzyme by anti-(thrombin III).

The effect of transformation by SV 40 virus on cultures of Swiss mouse 3T3 cells has been shown to involve differences in levels of sulphate content of heparan sulphate. The cells are not affected by changes in the molecular weight or protein content of the heparin sulphate.[344]

Cytochemical investigations have been used in the identification of heparan sulphate in glomerular basement membranes of rats.[345] Specific anionic sites can be removed by treatment of perfused kidney cells with heparan sulphate-lyase or nitrous acid. Sialoglycoproteins and other glycosaminoglycans do not represent major components of these sites. The isolated membranes on digestion

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with proteolytic enzymes released minor amounts of hyaluronic acid and major amounts of heparan sulphate as shown by analytical electrophoresis.[346]

A purified proteoglycan (mol. wt. 7.5×10^4) isolated from rat-liver membranes contains a core protein (mol. wt. $1.7–4.0 \times 10^4$) after removal of polysaccharide chains, which were identified as heparan sulphate.[347] Some of the heparan sulphate is released from the core protein on treatment with alkali.

Tetrasaccharides have been isolated from heparan sulphate by nitrous acid degradation.[348] Structural studies show that 2-acetamido-2-deoxy-D-glucosyl residues are preceded by a non-sulphated uronic acid residue that can be either D-glucopyranosyluronic acid or L-idopyranosyluronic acid, and are followed by a D-glucopyranosyluronic acid residue. Very few, if any, of the uronic acid residues appear to be sulphated.

Proteoglycans appear to be associated with the substrate-adhesion sites of normal and virus-transformed murine cells.[349] Cell-surface heparan sulphate is capable of cross-linking the cell-surface fibronectin and substrate-bound cold-insoluble globulin in an initial adhesion step.[350]

Compared with normal chondrocytes the accumulation of extracellular glycosaminoglycans in the cell layer of Rous sarcoma virus-transformed chondrocytes is reduced.[351] Virtually all of the secreted glycosaminoglycan of the transformed cells, most of which is hyaluronic acid, is deposited in a cell surface-associated compartment and then rapidly shed into the medium.

Full assignments of proton chemical shifts in high resolution ^{1}H n.m.r. spectra of hyaluronate, chondroitin 4- and 6-sulphates have been made.[352] Changes in chemical shifts were observed between neutral and alkaline solutions of all of the glycosaminoglycans, with hyaluronate giving anomalous shifts in alkali. Even-numbered oligosaccharides derived from hyaluronic acid with either D-glucuronic acid or 2-acetamido-2-deoxy-D-glucose in non-reducing terminal positions have been used as substrates for chondroitin lyase ABC and chondroitin lyase AC.[353] Chondroitin lyase ABC acts as an *endo*-lyase towards hyaluronic acid and requires the presence of at least a disaccharide on both reducing and non-reducing sides of the amino-sugar residue to be split. None of the amino-sugar linkages is split by the chondroitin lyase ABC. In contrast chondroitin lyase AC acts as an *exo*-glycanase towards hyaluronic acid and recognizes only the linkage between amino-sugar and uronic acid residues. Thus 2-acetamido-2-deoxy-D-glucopyranosyl and D-glucopyranosyluronic acid residues at both ends of the tetrasaccharide (15) are liberated.

β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*A

(15)

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Multiple forms of 2-deoxy-2-sulphamido-D-glucose sulphamidase (EC 3.10.1.1) from human placenta have been isolated by affinity chromatography on immobilized heparan sulphate.[353] They are distinguishable by K_m values and pH optima with respect to heparin and heparan sulphate.[354]

A 2-acetamido-2-deoxy-D-glucose 6-sulphate sulphatase from human urine is predominantly an *exo*-sulphatase and liberates sulphate from the non-reducing terminal 2-acetamido-2-deoxy-D-glucosyl 6-sulphate residue of a trisaccharide from heparan sulphate.[355] Enzyme activity is highest in kidney and cultured fibroblasts. The desulphation of sulphated glycosaminoglycans has been reported, using enzymes isolated from rat skin.[356] The enzymes were separated by chromatography and characterized as including a 2-acetamido-2-deoxy-D-glucose 6-sulphate sulphatase, a 2-acetamido-2-deoxy-D-galactose 6-sulphate sulphatase, a 2-acetamido-2-deoxy-D-galactose 4-sulphate sulphatase, and a 2-deoxy-2-sulphamido-D-glucose sulphamidase. In addition, an *O*-sulphatase active against suitably located sulphate groups on L-idopyranosyluronic acid residues of heparan sulphate and dermatan sulphate was identified. Diminished activity of any one of the enzymes may result in a specific storage disease. Chondroitin lyase AC, heparin lyase and heparan sulphate lyase from *Flavobacterium heparinum* have been separated by affinity chromatography on immobilized glycosaminoglycans.[357]

Rabbit connective tissues secrete at least three distinct neutral proteinases, capable of degrading collagen, gelatin, and proteoglycans, respectively.[358] A modified procedure for the preparation of Smith-degraded proteoglycans has been reported.[359] The product has been used in the assay of D-xylosyltransferase in cartilage extracts.

Biosynthesis. — Up to 56% of sulphated glycosaminoglycans synthesized by explant cultures of human and rabbit articular chondrocytes are located in the trypsin-digestible pericellular coat.[360] Sulphate is incorporated into both D-glucopyranosyluronic acid and L-idopyranosyluronic acid residues in hybrid glycosaminoglycans by monolayers of chick embryo arterial fibroblasts.[361] Several oligosaccharides, released on treatment with chondroitin lyase ABC and chondroitin lyase AC, have been characterized.

The development of embryos of the sea urchins, *Hemicentrotus pulcherrimus* and *Clypeaster japonicus*, is arrested at the blastula stage by treating them with reagents to inhibit proteoglycan synthesis.[362] The formation of completely assembled proteoglycan in sufficient amounts is a prerequisite for the events occurring at the end of the blastula stage.

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Short exposure of human skin fibroblasts to concanavalin A and wheat-germ agglutinin leads to the intra- and extracellular accumulation of sulphated glycosaminoglycans.[363] The intracellular accumulation is caused by an impaired degradation of the sulphated glycosaminoglycans, whereas the extracellular accumulation is ascribed to a lectin-mediated inhibition of endocytosis of these polysaccharides. In chick embryo fibroblasts, but not in chondrocytes, the synthesis of sulphated proteoglycans is inhibited by tunicamycin, with a resultant alteration in the cell shape and surface morphology.[364] The synthesis of hyaluronic acid and protein are only slightly affected by the antibiotic.

Components of chick embryos and of new born chickens and rats are capable of synthesis of sulphated glycosaminoglycans.[365] In rats up to two weeks old, only heparan sulphate could be detected, but in chick embryos and newborn chicks keratan sulphate as well as heparan sulphate was detected.

The production of hyaluronic acid during cell growth in embryonic chick tendon cell culture is inversely proportional to cell density, while levels of sulphated glycosaminoglycans remain constant.[366] Hyaluronic acid production remained constant during cell proliferation, although the synthesis of chondroitin sulphate increased rapidly during the actively growing phase and dermatan sulphate and heparan sulphate increased gradually.

Microsomal preparations of chick embryo epiphyseal cartilage synthesize two proteoglycan populations of different molecular weights.[367] The larger macromolecule may represent a precursor proteoglycan present at the site of synthesis of glycosaminoglycan chains. In the biosynthesis of heparin the formation of L-iduronic acid residues is accompanied by the exchange of the hydrogen at C-5 of the D-glucuronic acid precursor residues.[368] This observation forms the basis for an assay of the epimerase in which release of tritium from enzymically synthesized D-[5-^{3}H]glucopyranosyluronic acid residues in heparin is measured, thus allowing certain basic properties of the epimerase to be studied.

In the biosynthesis of hyaluronic acid by group A streptococci, no lipid intermediates appear to be involved.[369] Chain elongation of the hyaluronic acid proceeds by the alternate addition of monosaccharides from UDP-sugars by a membrane-bound synthase system followed by the release of completed hyaluronic acid chains.

The results of enzymic degradation of a hyaluronate analogue synthesized from 2-deoxy-2-fluoroacetamido-D-[1-^{14}C]glucose as a precursor suggests that the presence of fluoroacetamido groups may render the hyaluronate analogue more resistant to hyaluronidase.[370] A system for studying the enzymic sulphation of a

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high molecular weight glycoprotein and of a proteoglycan associated with pig brain membranes has been described.[371] Sulphate was transferred from adenosine 3 -phosphate 5'-*P*-dihydrogen phosphatosulphate (PAPS) to the proteoglycan, which has chemical and chromatographic characteristics of heparan sulphate. At least one *O*- and one *N*-sulphatotransferase appeared to be active in the membrane preparations.

Pathology. – Methods have been developed for the isolation of chondrocytes from Swarm rat chondrosarcoma.[372] When biosynthesis of proteoglycans and their secretion from the cells and assembly into aggregates was studied, the newly synthesized proteoglycans were found not to be bound to link-stabilized aggregates at the time of secretion. Oligomers of hyaluronic acid that are long enough to interact only with the hyaluronic acid-binding site of the proteoglycans retard, but do not prevent, link-stabilized aggregation. A human chondrosarcoma containing proteoglycans having two different sizes of keratan sulphate chains has been isolated.[373] The larger species of keratan sulphate, containing about 23 monosaccharide units, was isolated from the keratan sulphate-enriched region only, with a smaller species containing 13 monosaccharide units which was recovered from the proteoglycans released after treatment with proteolytic enzymes.

The pericellular sulphated glycosaminoglycans of a malignant glioma cell line showed faster turnover than those of normal glial cells.[374] Most of the sulphated glycosaminoglycan was released into the medium rather than being degraded.

Abnormalities in the distribution of glycosaminoglycans found in keratoconus cell cultures have been reported.[375] Normal stromal cells incorporate sulphate into sulphated glycosaminoglycans, which are located mainly within the cell layer. In contrast, the keratoconus cells produce most of the newly synthesized material into the growth medium with a much decreased amount within the cell layer.

A tissue-culture model of the breakdown of cartilage in rheumatoid arthritis has been made on the basis of quantitative aspects of the release of proteoglycans.[376] The findings support the view that a significant component of cartilage breakdown may be mediated by the chondrocytes.

Compared with normal human articular cartilage, osteoarthritic cartilage shows a significant reduction of dermatan sulphate, chondroitin 6-sulphate, keratan sulphate, and heparan sulphate.[377] The content of glycoprotein also appeared to be reduced. The levels of chondroitin 4-sulphate, keratan sulphate,

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and dermatan sulphate were increased considerably in osteoarthritic serum. Adult human articular cartilage contains almost exclusively chondroitin 6-sulphate, whereas normal young and arthrosic cartilage chondroitin sulphates are hybrid polymers of disaccharide units containing 4-sulphate and 6-sulphate distributed randomly along the polymer chain.[378]

A protein fraction of rat serum prevents the precipitation of lipoprotein by rat heparin and Ca^{2+}.[379] Phosphorylcholine is able to counteract the effect of the protein. The results have been discussed with reference to the possibility of a relationship between glycosaminoglycans, Ca^{2+}, lipoproteins, and arterial phospholipids in the pathogenesis of atherosclerosis.

Increased levels of sulphated glycosaminoglycans have been identified as a genetic characteristic in cell cultures of one variety of dominant dystrophic epidermolysis bullosa.[380] The major glycosaminoglycan accumulating in the liver of a patient with Morquio syndrome (type IV mucopolysaccharidosis), but not in normal liver, is keratan sulphate.[381] The heparan sulphate arising from the Morquio patient exhibited structural differences from that of the control in being composed mainly of disaccharide repeating units of 2-deoxy-2-sulphamido-D-glucose and uronic acid, with the almost total exclusion of repeating units of 2-acetamido-2-deoxy-D-glucose and uronic acid. Abnormal keratan sulphate has been detected in the urine of patients with Morquio syndrome only during childhood.[382] Abnormal excretion is also a feature of Kniest dysplasia and G_{M_1}-gangliosidosis and may be present in other bone and cartilage dysplasias of unknown aetiology.

The deficiencies of 2-acetamido-2-deoxy-D-glucose 6-sulphate sulphatase and 2-acetamido-2-deoxy-D-galactose 6-sulphate sulphatase causing an inability to degrade keratan sulphate and heparan sulphate in one case and keratan sulphate and chondroitin 6-sulphate in the other, have been discussed in relation to Morquio and Sanfilippo syndromes.[383] Glycosaminoglycans, lipids, and lysosomal enzymes have been measured in brain, spleen, and liver of a patient with Sanfilippo syndrome B.[384] All the tissues showed increased levels compared with controls, with the greatest occurring in the liver from an excessive accumulation of partially degraded heparan sulphate. Evidence has been presented for the existence of a third biochemical sub-type of Sanfilippo syndrome, designated as type C.[385] Excessive accumulation of heparan sulphate, which is characterized as containing unsubstituted amino-groups on some of the 2-amino-2-deoxy-D-glucosyl residues, occurred in cultured skin fibroblasts.

[378] Y. M. Michelacci, P. A. S. Mourao, J. Laredo, and C. P. Dietrich, *Calcif. Tissue Res.*, 1979, **7**, 29.
[379] S. Mookerjea, *Can. J. Biochem.*, 1978, **56**, 746.
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[383] N. Di Ferrante, L. C. Ginsberg, P. V. Donnelly, D. T. Di Ferrante, and C. T. Caskey, *Science*, 1978, **199**, 79.
[384] G. Constantopoulos, R. M. Eiben, and I. A. Schafer, *J. Neurochem.*, 1978, **31**, 1215.
[385] H. Kresse, K. Von Figura, and U. Klein, *Eur. J. Biochem.*, 1978, **92**, 333.

Glycosaminoglycans from urine and tissues of patients with I-cell disease (Mucolipidosis II) contain high levels of dermatan sulphate.[386] The increased urinary excretion of this glycosaminoglycan could reflect an abnormal metabolism of this glycosaminoglycan in connective tissue, as shown by others in I-cell cultured fibroblasts.

A fluorimetric assay, which employs 4-methylumbelliferyl α-L-idopyranosyluronic acid for the estimation of α-L-iduronidase activity has been reported for the detection of Hurler and Scheie syndromes.[387]

The heparan sulphate from patients with Hunter syndrome has a complex structure composed of nine different disaccharide units containing regions similar to normal heparan sulphate and regions similar to heparin.[388] In contrast to heparin, where most of the L-idopyranosyluronic acid residues are sulphated, 66% of those residues are non-sulphated in Hunter heparan sulphate.

Two types of ascites hepatoma cells, on treatment with trypsin, release carbohydrates from the plasma membranes.[389] A heparan sulphate proteoglycan was isolated from type AH66 cells, whereas type AH130FN cells released only glycopeptides. The heparan sulphate proteoglycan (mol. wt. 6×10^4) contained 10% protein, with high contents of L-glutamic acid, L-aspartic acid, L-proline, glycine, L-serine, and L-threonine.[390]

8 Cell and Tissue Glycoproteins

A book dealing with the complex carbohydrates of nervous tissue covers aspects of chemistry, structure, biosynthesis, metabolism, localization, and functional roles.[391]

The results of *X*-ray diffraction studies on frog sciatic nerve myelin have indicated that the major glycoprotein is inserted into the extracellular half of the myelin bilayer and that it extends into the extracellular space.[392] Various glycopeptides containing L-fucose and 2-acetamido-2-deoxy-D-glucose, but no neuraminic acid, have been isolated from garfish olfactory nerve.[393]

A light-microscopical, immunocytochemical method for detecting myelin-associated glycoproteins in myelin and myelin-forming cells of developing rat nervous systems has been reported.[394] When horse-radish peroxidase is activated with glutaraldehyde and then covalently coupled to wheat germ agglutinin, the conjugate is forty times more sensitive than the free horse-radish peroxidase in tracing the retrograde connections from the submandibular gland to the superior

[386] G. V. Coppa, A. Maiorana, O. Gabrielli, and S. Sani, *Clin. Chim. Acta*, 1979, **95**, 135.
[387] J. J. Hopwood, V. Muller, A. Smithson, and N. Baggett, *Clin. Chim. Acta*, 1979, **92**, 257.
[388] H. B. Nader, D. M. Cohen, and C. P. Dietrich, *Biochim. Biophys. Acta*, 1979, **582**, 33.
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[394] N. H. Sternberger, R. H. Quarles, Y. Itoyama, and H. D. Webster, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 1510.

cervical ganglion of the rat.[395] Solubilized glycoproteins of purified rat brain myelin bind to immobilized concanavalin A and other lectins.[396]

Examination of proteins for D-glucosyl-L-lysine in normal human lens, senile and diabetic cataracts shows that D-glucosylation does not appear to be a primary factor in cataract formation.[397]

A small molecular weight oligosaccharide (16) attached separately at two sites near the *N*-terminus of bovine rhodopsin has been examined by a combination of methylation analysis, acetolysis, and enzymic studies.[398] This structure is identical to a proposed intermediate in the biosynthesis of complex L-asparagine-linked oligosaccharides.[399] Three oligosaccharides have been released from bovine rhodopsin by hydrazinolysis.[400] One of the components has an identical structure to (16) except for the absence of the L-asparagine residue, and structures [(17) and (18)] were established for the other two oligosaccharides.

β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc-L-Asn
6
↑
1
α-D-Man*p*

(16)

β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc
6
↑
1
α-D-Man*p*
6/3
↑
1
α-D-Man*p*

(17)

β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc
6
↑
1
α-D-Man*p*-(1→3)-α-D-Man*p*
6
↑
1
α-D-Man*p*

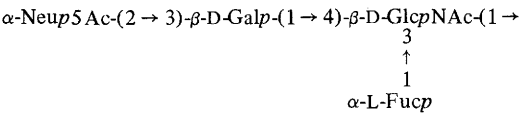
(18)

[395] N. K. Gonatas, C. Harper, T. Mizutani, and J. O. Gonatas, *J. Histochem. Cytochem.*, 1979, **27**, 728.
[396] R. H. Quarles, L. J. McIntyre, and C. F. Pasnak, *Biochem. J.*, 1979, **183**, 213.
[397] A. Pande, W. H. Garner, and A. Spector, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 1260.
[398] M. N. Fukuda, D. S. Papermaster, and P. A. Hargrave, *J. Biol. Chem.*, 1979, **254**, 8201.
[399] I. Tabas and S. Kornfeld, *J. Biol. Chem.*, 1978, **253**, 7779.
[400] C. J. Liang, K. Yamashita, C. G. Muellenberg, H. Schichi, and A. Kobata, *J. Biol. Chem.*, 1979, **254**, 6414.

Bovine D-galactosyltransferase (lactose synthase) has been used to label glycoproteins of mouse retina by transferring D-[^{14}C]galactose to non-reducing 2-acetamido-2-deoxy-D-glucosyl residues.[401] Information was obtained concerning changes in the patterns of glycoproteins during the development of mouse neuronal retina. Photoreceptor loss in mice with retinal degeneration was paralleled by the disappearance of certain glycoproteins.

Sixteen glycoproteins have been separated from rat brain by a combination of hydroxyapatite and lectin affinity chromatography.[402] A simple reproducible procedure for the fractionation of brain glycoproteins using hydroxyapatite-gel column chromatography has been developed.[403]

Whole rat brain on digestion with proteolytic enzymes releases an *N*-glycosidic glycopeptide fraction which has been purified using immobilized concanavalin A.[404] One of the glycopeptides has an unusual sugar sequence (19) that has been shown to be part of the determinant of the so-called X-antigen.



(19)

A glycoprotein antigen (mol. wt. 7.6×10^4) comprising three sub-units of identical size has been isolated from a cell-free extract of a human lung adenocarcinoma.[405] All the properties of this glycoprotein are very similar to those of another antigen purified from a separate human lung tumour.

A patient with an inherited deficiency of lysosomal N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl) hydrogen L-asparaginate amidohydrolase accumulated, in the liver, oligosaccharides whose structures are related to the inner-core region of a number of different glycoproteins.[406] They have been characterized by g.l.c.–m.s. of the appropriate derivatives as N^4-(acetamido-2-deoxy-β-D-glucopyranosyl) hydrogen L-asparaginate and N^4-[O^2-α-D-mannopyranosyl-(1 → 6)-O-β-D-mannopyranosyl-(1 → 4)-O-2-acetamido-2-deoxy-D-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl] hydrogen L-asparaginate.

The hepatic storage of glycopeptides and glycolipids in a cat affected by G_{M_1}-gangliosidosis has been reported.[407] Two major oligosaccharide chains, derived from the glycopeptides, had molecular weights of 1.8×10^3 and 1.35×10^3 and contained residues of D-galactose, D-mannose, and 2-acetamido-2-deoxy-D-glucose in the molar proportions 2.0 : 3.1 : 4.1 and 1.0 : 2.2 : 2.7, respectively.

[401] B. Wallenfels, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 3223.
[402] O. K. Langley, *J. Neurochem.*, 1979, **33**, 221.
[403] S. Marchesini, S. Sonnino, and G. Tettamanti, *Ital. J. Biochem.*, 1978, **27**, 211.
[404] T. Krusius and J. Finne, *Eur. J. Biochem.*, 1978, **84**, 395.
[405] S. A. Gaffar, J. A. Braatz, K. H. Kortright, G. L. Princler, and K. R. McIntire, *J. Biol. Chem.*, 1979, **254**, 2097.
[406] P. Maury, *J. Biol. Chem.*, 1979, **254**, 1513.
[407] E. W. Holmes and J. S. O'Brien, *Biochem. J.*, 1978, **175**, 945.

Mechanisms by which newly synthesized glycoproteins are transferred from the hepatocytes to the bile have been reported.[408]

The effects of the bacterial tripeptide, leupeptin, on the lysosomal degradation of glycoproteins in perfused rat liver have been examined.[409] Leupeptin inhibits digestion of endocytosed proteins such as asialofetuin within the lysosomes. A hydrophilic glycoprotein with insulin-binding activity has been isolated from rat liver by affinity chromatography on columns of immobilized insulin.[410] Treatment of the glycoprotein with either neuraminidase or trypsin caused a decrease in the insulin-binding activity. The glycoprotein and glycosaminoglycan content of liver in which hepatic cancer has been induced with 3′-methyl-4-dimethylaminoazobenzene show a gradual increase in quantity with time.[411]

Various structural features of proteins, such as polypeptide size and net charge, are known to influence their *in vivo* degradative rates. The presence of covalently bound carbohydrate has been shown to be a third feature of protein structure that correlates with protein half-lives within cells.[412] Glycoproteins have been found to be degraded more rapidly than unfractionated proteins or fractions enriched for non-glycoproteins in rat liver, muscle, brain, and kidney, and also in human fibroblasts grown in culture.

A DNA double-helix unwinding glycoprotein, isolated from roe-deer liver, contains residues of L-fucose, D-xylose, and D-mannose together with two unidentified sugars.[413] The glycoprotein is involved in the unwinding of adenine–thymine deoxyribose base pairs in DNA.

From results obtained using a sandwich technique with immunofluorescence, it appears that Tamm–Horsfall glycoprotein is present in hamster kidney slices only in the ascending limb of the loop of Henle and the distal convoluted tubule.[414] The glycoprotein may operate as a barrier to decrease the passage of water molecules by trapping them at the membrane of the cells. The structure of the oligosaccharide chains in Tamm–Horsfall glycoprotein has been studied using the native glycoprotein as an acceptor for calf kidney sialyltransferase (CMP *N*-acetylneuraminic acid:glycoprotein sialyltransferase) in competition studies with desialylized α_1-acid glycoprotein and fetuin.[415] The results indicate that the carbohydrate structures of the Tamm–Horsfall glycoprotein are most probably not identical with the *N*-glycosidically-bound oligosaccharide chains present in the other two glycoproteins.

Localization of glycoproteins in lysosomes and prelysosomes of a kidney epithelial cell line from monkey has been established by use of high resolution autoradiography after incorporation of L-[^{3}H]fucose and by a semicarbazide–

[408] B. M. Mullock and R. H. Minton, *FEBS Lett.*, 1979, **106**, 121.
[409] W. A. Dunn, J. H. La Badie, and N. N. Aronson, *J. Biol. Chem.*, 1979, **254**, 4191.
[410] T. Momoi, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 541.
[411] H. Masuda, S. Schichijo, and M. Takeuchi, *Int. J. Biochem.*, 1978, **9**, 829.
[412] F. Kalish, N. Chovick, and J. F. Dice, *J. Biol. Chem.*, 1979, **254**, 4475.
[413] J. Szopa and H. Janska, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 398.
[414] K. L. Sikri, C. L. Foster, F. J. Bloomfield, and R. D. Marshall, *Biochem. J.*, 1979, **181**, 525.
[415] W. Van Dijk, A. M. Lasthuis, and D. H. van der Eijnden, *Biochim. Biophys. Acta*, 1979, **584**, 129.

silver proteinate staining method after oxidation with periodate.[416] The glycoproteins appear to be transported through different components of the vacuolar system of the cell in a sequential fashion rather than *via* independent pathways. The majority of the glycoproteins in the lysosomal membranes do not have acid hydrolase activity.

A homogeneous sulphated glycoprotein composed of protein (40%), carbohydrate (53%), and sulphate (1.6%) and containing D-galactose, 2-amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-glucose, and neuraminic acid as the principal sugars, has been isolated from the small intestine of the rabbit.[417]

Resonance Raman scattering spectroscopy within the visible absorption band of uteroferrin, a glycoprotein of the porcine uterus, results in an intense Raman spectrum that is similar to that of Fe^{3+} transferrin, indicating similarities in the metal binding sites of the two macromolecules.[418]

Sulphated glycoproteins (mol. wts. $>2.4 \times 10^5$) are biosynthesized, at least in the uterine luminal epithelial cells of the rat, under the stimulation of oestrogen but not progesterone, which inhibits their biosynthesis.[419]

Glycoproteins having chemical and antigenic properties similar to those of carcinoembryonic antigen have been isolated from human prostate.[420] Carcinoembryonic antigen prepared by identical procedures from three different liver metastases has been shown to have different degrees of homogeneity on electrophoresis and crossed immunoelectrophoresis, and also has different antigenic activities.[421]

A minor isoenzyme of human prostatic acid phosphatase (pI 5.5) has been separated from the major isoenzyme (pI 4.9).[422] Immunological similarities between the two enzymes were demonstrated.

Ribonuclease isolated from HeLa cell lysosomes has been characterized as a glycoprotein on the basis of its binding to immobilized concanavalin A.[423] Glycoproteins associated with two rat ascites mammary adenocarcinoma cell lines differ in their behaviour towards lectins and their xenotransplantability.[424] Differences were observed in the complexity and size of their oligosaccharide chains. The maximum release of a sialoglycoprotein, but not a sialoglycolipid, from a murine tumour cell by neuraminidase from *Clostridium perfringens* was obtained only in the presence of physiological concentration of Ca^{2+}.[425]

Glycoproteins, isolated from granulation tissue and fractionated by gel filtration and ion-exchange chromatography, inhibit collagen synthesis in rat

[416] E. Knecht and J. Hernandez, *Cell Tissue Res.*, 1978, **193**, 473.
[417] H. Munakata and Z. Yosizawa, *J. Biochem. (Jpn.)*, 1978, **84**, 1587.
[418] B. P. Gaber, J. P. Sheridan, F. W. Bazer, and R. M. Roberts, *J. Biol. Chem.*, 1979, **254**, 8340.
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[421] H. Zawadzka, E. Lisowska, A. Harlozinska, Z. Albert, R. Richter, and M. Janusz, *Neoplasma*, 1979, **26**, 157.
[422] P. Vihko, *Invest. Urol.*, 1979, **16**, 349.
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[424] R. L. Buck, A. P. Sherblom, and K. L. Carraway, *Arch. Biochem. Biophys.*, 1979, **198**, 12.
[425] N. W. Barton and A. Rosenberg, *Biochim. Biophys. Acta*, 1979, **554**, 125.

embryo tendon cells.[426] The glycoproteins may function in the feedback regulation of granuloma development.

A glycopeptide, composed of thirty-nine amino-acid residues isolated from pig, ox, and sheep pituitary, exhibits a high degree of sequence conservation.[427] The glycopeptide arising from each source contains one oligosaccharide chain attached to L-asparagine at position 6 in the sequence and contains five residues of 2-amino-2-deoxy-D-glucose.

Part of the microheterogeneity shown by isoelectric focusing of glycoproteins from the electric organ of the eel *Electrophorus electricus* is attributable to variations in the content of neuraminic acid.[428] Treatment of the glycoproteins with neuraminidase reduces the number from ten to four. The microheterogeneity is due to post-translational modification of oligosaccharides on a common polypeptide backbone.

A glycoprotein (mol. wt. 1.5×10^5) has been isolated from whole human platelets by affinity chromatography on immobilized wheat-germ agglutinin.[429] Extraction of the whole platelets with lithium di-iodosalicylate released three major glycoprotein fractions.

A glycoprotein, designated thrombospondin, containing three disulphide-linked chains, each having a molecular weight of 1.4×10^5, is released from human blood platelets in response to thrombin.[430] Isolation of this glycoprotein was achieved by affinity chromatography on immobilized heparin. Its position in the cytoplasmic granule fraction of the platelets, from which it is released with known coagulation factors, suggests a physiological role related to the coagulation scheme.

A novel glycoprotein containing *N*-glycolylneuraminic acid has been isolated from the eggs of rainbow trout (*Salmo irideus*).[431] All of the carbohydrate chains, which contain residues of D-galactose and 2-acetamido-2-deoxy-D-galactose, are terminated by the disaccharide *O*-α-*N*-glycolylneuraminyl-(2→8)-*N*-glycolylneuraminic acid. The identity of this disaccharide has been confirmed by methylation and g.l.c.–m.s. analysis.[432] The embryos of the sea urchin (*Strongylocentrotus purpuratus*) synthesize several classes of sulphated glycoproteins during gastrulation.[433] During development of the embryos, glycoproteins containing both unsulphated and sulphated *N*-glycosidically-linked oligosaccharides are synthesized *via* lipid-linked intermediates. The biosynthesis of these molecules appears to be a prerequisite to the differentiation and morphogenesis that occurs during gastrulation.

The structures [(20)–(22)] of three glycopeptides, each having a high level of D-mannosyl residues and present in Chinese hamster ovary cells, have been determined.[434] Oligosaccharides with identical structures have been found in

[426] M. Aalto, M. Potila, and E. Kulonen, *Biochim. Biophys. Acta*, 1979, **587**, 606.
[427] D. G. Smith and D. E. Massey, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 1006.
[428] P. J. Marshall and L. E. Hokin, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 476.
[429] S. L. Marchesi and J. A. Chasis, *Biochim. Biophys. Acta*, 1979, **555**, 442.
[430] J. W. Lawler, H. S. Slayter, and J. E. Coligan, *J. Biol. Chem.*, 1978, **253**, 8609.
[431] S. Inoue and M. Iwasaki, *Biochem. Biophys. Res. Commun.*, 1978, **83**, 1018.
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[433] A. Heifetz and W. J. Lennarz, *J. Biol. Chem.*, 1979, **254**, 6119.
[434] E. Li and S. Kornfeld, *J. Biol. Chem.*, 1979, **254**, 1600.

R^1-α-D-Man*p*-(1 → 6)-α-D-Man*p*-(1 → 6)-β-D-Man*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 4)-D-Glc*p*NAc-L-Asn

3 3
↑ ↑
1 1
α-D-Man*p* α-D-Man*p*
| 2
R^2 ↑
1
α-D-Man*p*
↑
R^3

(20) $R^1 = R^2 = R^3$ = α-D-Man*p*-(1 → 2)

(21) $R^1 = R^3$ = α-D-Man*p*-(1 → 2), R^2 = H

(22) $R^1 = R^2 = R^3$ = H

other soluble glycoproteins such as Unit A glycopeptide of thyroglobulin, Taka-amylase (a microbial α-amylase), and ovalbumin. The structure (23) of the oligosaccharide portion of a low molecular weight lipid-linked intermediate, which is primarily involved in the assembly of a major lipid-linked oligosaccharide of Chinese hamster ovary cells, has been established.[435]

Antigens isolated from the eggs of *Schistosoma mansoni* contain at least six glycoproteins capable of binding to immobilized concanavalin A.[436] These glycoproteins are able to elicit lymphocyte blastogenic responses. The same glycoproteins can also be isolated by affinity chromatography on immobilized anti-(soluble egg antigen) antibody.[437]

An oligosaccharide, containing residues of D-glucose, D-mannose, and 2-acetamido-2-deoxy-D-glucose in the molar ratio 3 : 9 : 2, is the major or exclusive species transferred *en bloc* from the lipid-linked intermediate to proteins in intact, uninfected chick embryo fibroblasts.[438] The initial stages of processing of this oligosaccharide species after transfer involve the sequential removal of the three D-glucosyl residues.[439] The three D-glucosyl residues are attached as a trisaccharide to a D-mannosyl residue at one of the non-reducing termini of the oligosaccharide, and the 2-acetamido-2-deoxy-D-glucosyl residues are located at the reducing terminus.[440] Treatment with tunicamycin affects the binding and degradation of low-density lipoproteins by cultured human fibroblasts, probably by inhibition of glycosylation of the glycoprotein receptor for the lipoproteins.[441] The large-scale purification of neutral and acidic glycopeptides from human diploid fibroblasts has been reported.[442]

D-Mannose 6-phosphate has been identified as a constituent of glycoproteins that inhibit the assimilation of bovine testicular β-D-galactosidase by human skin

[435] E. Li and S. Kornfeld, *J. Biol. Chem.*, 1979, **254**, 2754.
[436] C. E. Carter and D. G. Colley, *J. Immunol.*, 1979, **122**, 2204.
[437] D. J. Harrison, C. E. Carter, and D. G. Colley, *J. Immunol.*, 1979, **122**, 2210.
[438] S. C. Hubbard and P. W. Robbins, *J. Biol. Chem.*, 1979, **254**, 4568.
[439] S. J. Turco and P. W. Robbins, *J. Biol. Chem.*, 1979, **254**, 4560.
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[441] S. Chatterjee, P. O. Kwiterovisch, and C. S. Sekerke, *J. Biol. Chem.*, 1979, **254**, 3704.
[442] P. Cammarata and C. Ceccarini, *J. Chromatogr.*, 1979, **168**, 395.

α- D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4/3)-β-D-Glc*p*NAc-(1 → 4)-D-Glc*p*NAc
6
↑
1
α-D-Man*p*

(23)

fibroblasts.[443] Phosphorylated oligosaccharides have been isolated from the inhibitor and D-mannose 6-phosphate appears to be associated with (1→2)-linked D-mannosyl residues in the glycoprotein. The recognition marker encompasses a structure larger than a single D-mannosyl 6-phosphate residue. D-Mannose 6-phosphate is present as a recognition marker in human spleen β-D-glucuronidase.[444]

Differentiation antigens and glycoproteins of lymphocytes have been the subject of a review.[445]

Human peripheral blood lymphocytes have been fractionated into sub-classes by an affinity chromatographic technique involving immobilized lentil lectin.[446] Two groups of cells were isolated, differing both in their surface properties and in certain *in vitro* functional capabilities.

Glycoproteins have been purified from rat thymocytes and spleen.[447] Monoclonal antibodies to these glycoproteins have been prepared in order to study the relationship between the thymocyte glycoproteins and rat I_a-antigens. Metabolic labelling of the carbohydrate residues of glycoproteins from mitogen-stimulated mouse lymphocytes has demonstrated that by using a combination of different labelled sugars, such as L-fucose, D-mannose, and D-galactose, it is possible not only to differentiate between activated T- and B-cells, but also between certain sub-populations.[448] The method has been used to discriminate early- and late-responding concanavalin A-reactive thymocytes.[449] The interactions of peanut agglutinin with human thymocytes, peripheral blood lymphocytes, and peripheral blood cells of various types of leukaemia have been investigated.[450] The majority of human thymocytes bind to the lectin. The validity of using the lectin as a marker of immature blood cells and its potential clinical application have been discussed.

Human fibroblast and leukocyte interferons show a strong affinity for the copper chelate of bis-carboxymethylaminoagarose.[451] Human leukocyte interferon adsorbs non-specifically to immobilized hyperimmune serum immunoglobulin G.[452] The interferon can be recovered by washing the adsorbent with 1,2-dihydroxyethane. An improved procedure for the isolation of interferons produced by mouse Ehrlich ascites tumour cells infected with Newcastle disease virus provides interferons of three size classes (mol. wts. 3.3×10^4, 2.6×10^4,

[443] J. Distler, V. Hieber, G. Sahagian, R. Schmickel, and G. W. Jourdian, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 4235.
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[445] A. F. Williams, W. R. McMaster, R. Standring, and C. A. Sutherland, *Trends Biochem. Sci.*, 1978, **3**, 272.
[446] D. H. Bolt and R. D. Lyons, *Cell. Immunol.*, 1979, **43**, 82.
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[450] Y. Reisner, M. Biniaminov, E. Rosenthal, N. Sharon, and B. Ramot, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 447.
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and 2.0×10^4).[453] The differences in size may be due, in part, to unequal glycosylation.

Glycosylation of interferon, produced by mouse L cells after induction by Newcastle disease virus, has been inhibited by tunicamycin with no loss of antiviral activity.[454]

Human lymphoid cells, when stimulated *in vitro* with concanavalin A or phytohaemagglutinin, release lymphotoxins, a group of cytologically active molecules.[455] They have been separated into various classes and sub-classes by gel and ion-exchange chromatography, and also by affinity chromatography on immobilized lectins, thus indicating their glycoprotein nature. Treatment of the glycoproteins with glycosidases has no effect on the antigenicity of these molecules and the carbohydrate moieties do not appear to be necessary for their *in vitro* lytic activity.

D-[^{14}C]Glucose has been incorporated into lipid-linked oligosaccharides containing up to eleven monosaccharide units by explants of lactating rabbit mammary glands.[456] Considerable redistribution of the label occurred into D-mannosyl and 2-acetamido-2-deoxy-D-glucosyl residues.

A hypothesis that has been presented may give some meaning to the various changes in carbohydrate attached to proteins and lipids in malignant cells.[457] Extensive changes in the populations of glycolipids and glycoproteins as seen in malignant cells may result in persistent cell division, decreased intercellular adhesiveness, altered transport, altered immunogenicity, and loss of specialized function.

9 Cell-surface and Membrane Glycoproteins

The continued interest in this field of study is reflected in the large number of review articles which have been published. The proceedings of an ICN–UCLA Symposium, in Colorado in 1977, have been published under the title 'Cell Surface Carbohydrates and Biological Recognition',[458] and the proceedings of another symposium on cell membranes of normal and neoplastic cells has appeared as 'Cell Surface Carbohydrate Chemistry'.[459] Cell-surface antigens, with special reference to the histocompatibility antigens, have been reviewed.[460] A comprehensive survey of the metabolism of cell-surface components in both eukaryotic and prokaryotic cells has been published.[461] Other reviews have

[453] B. Cabrer, H. Taira, R. J. Broese, T. D. Kempe, K. Williams, E. Slattery, W. H. Konigsberg, and P. Lengyel, *J. Biol. Chem.*, 1979, **254**, 3681.
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[457] L. Warren, C. A. Buck, and G. P. Tuszynski, *Biochim. Biophys. Acta*, 1978, **516**, 97.
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[459] 'Cell Surface Carbohydrate Chemistry', ed. R. E. Harmon, Academic Press, New York, 1978.
[460] M. J. Crumpton and D. Snary in 'MTP International Review of Biochemistry', ed. E. S. Lennox, University Park Press, Baltimore, 1979, p. 127.
[461] 'Synthesis, Assembly, and Turnover of Cell Surface Components', ed. G. Poste and G. L. Nicolson, N. Holland, Amsterdam, 1977, Vol. 4.

dealt with the structure, biosynthesis, and biological functions of cell-surface glycoproteins,[462] the purification of cell-membrane glycoproteins,[463] the role of fibronectin in malignant transformation,[463,464] fibronectin-adhesive glycoproteins of cell-surface blood,[465] and protein-bound oligosaccharides of cell membranes.[466]

The organization of the glycoproteins and glycolipids on the surface of plasma membranes and their participation in cell contacts, transformation, and regulation of the cell cycle have been discussed.[467] A model of intracellular recognition is presented and the role of the surface matrix of the cell is discussed.

Neuraminic acid residues of glycoproteins have been spin-labelled in order to study the effects of various binding processes upon the mobility and environment of the sugar in tissue sections from human colon and erythrocyte components.[468] Quantitative data obtained with fluorescent-substituted lectins is in agreement with data obtained for radioactively labelled lectins for the determination of cell-surface glycoconjugates.[469] A survey of the application of a lectin-based method to the light-microscopical localization of D-mannose-rich glycoproteins and a range of methods for the ultrastructural characterization of glycoconjugates on cell surfaces has been reported.[470]

Several differences, including the presence of an additional glycoprotein, have been noted on the surface labelling of hamster variant cell lines when compared to wild cells.[471] The accompanied resistance to the cytotoxic effect of concanavalin A provides support for the view that modified cell-surface glycoproteins are directly involved in the complex concanavalin A-resistant phenotype, which includes changes in some fundamental biological properties. Hamster-cell fibronectin contains two sub-units (mol. wt. 2.3×10^5 each) in a disulphide-bonded dimer.[472] The disulphide bonds, the single free thiol group and the carbohydrate units, are distributed randomly along domains that can be separated by proteolytic cleavage. A fragment (mol. wt. 2.0×10^5) containing all or most of the carbohydrate side chains, but relatively little L-cystine, contains the gelatin-binding site. Intact disulphide bonds are required for the binding of fibronectin to cells and to gelatin, and blockage of the free thiol groups prevents the binding to cells. Treatment of fibronectin with cyanogen bromide and subtilisin releases two fragments (mol. wts. 5.0×10^4 and 3.0×10^4),

[462] 'Cell Surface Glycoproteins: Structure, Biosynthesis, and Biological Functions' in 'Current Topics in Membranes and Transport', ed. R. L. Juliano and A. Rothstein, Academic Press, New York, 1978, Vol. 11.
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which have been isolated by affinity chromatography on immobilized gelatin.[473] Since the higher molecular weight fragment, which was derived from the *C*-terminus of the protein chain of the glycoprotein, also mediated the adhesion of fibroblasts to collagen, it contains both the collagen- and cell-binding sites in the fibronectin molecule. Similar results have been independently published.[474,475]

Fibronectin is a sensitive target for leukocyte proteases under physiological conditions.[476] Because of the sensitivity of fibronectin to neutral proteases from inflammatory cells and its apparent role in tissue organization, it is possible that the morphological and functional changes accompanying inflammation may reflect destruction of fibronectin in addition to other components of the connective tissue matrix. Plasma transglutaminase (Factor XIIIa) catalyses the cross-linking of fibronectin and collagen.[477] The reaction may be important in wound healing and tissue repair. Heparin induces the transition of fibronectin from a globular to an elongated form, capable of forming filamentous precipitates which adsorb native collagen.[478] This reaction is inhibited by hyaluronic acid and putrescine. Peritoneal macrophages are capable of the *in vitro* biosynthesis and secretion of a protein that by immunological, biochemical, and functional criteria is identical to fibronectin.[479]

A change in glycoprotein components of the plasma membrane in liver cells is part of the complex adaptation to diet containing high fat and high D-glucose levels.[480] Membranes from rats fed on a high fat diet bind significantly less [^{125}I]concanavalin A than do those from rats fed on a fat-free diet. Analysis of delipidated membranes show significantly lower values for neuraminic acid, 2-amino-2-deoxy-D-hexose, L-fucose, and D-mannose, but not D-galactose. Differences between glycoproteins from diabetic and normal rat-liver membranes have been described.[481]

The hepatic-binding protein, which binds to terminal D-galactosyl residues of asialoglycoproteins, and the receptors for *Ricinus communis* lectin have been localized simultaneously on the plasma membrane of isolated rat hepatocytes.[482]

The specificity of the combining site of the rabbit hepatic lectin has been explored by a competitive binding assay using a [^{3}H]-labelled precursor blood-group I substance with terminal non-reducing β-D-galactosyl residues and the

[473] L. I. Gold, A. Garciapardo, B. Frangione, E. C. Franklin, and E. Pearlstein, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 4803.
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lectin immobilized on macroporous agarose.[483] Data on the relative inhibiting power of various polysaccharides, glycoproteins, and oligosaccharides, and also monosaccharides and their glycosides have been recorded.

A recycling mechanism may exist wherein the receptor is not destroyed in the lysosomes and remains available for subsequent reinsertion into the hepatocyte plasma membrane as part of a continuing process for the clearance of D-galactosyl-terminated ligands in the circulation.[484] The existence and distribution on parenchymal and non-parenchymal hepatic cells of separate and distinct receptors for selectively modified proteins has been reported.[485] Whereas D-galactosyl-terminated glycoproteins were found preferentially in the hepatocytes, glycoproteins terminated in D-mannosyl or 2-acetamido-2-deoxy-D-glucosyl residues were recovered largely in the sinusoidal cells of the liver.

These findings have been substantiated by the observation that the selective hepatic uptake of the lysosomal glycosidase β-D-2-acetamido-2-deoxyhexosidase, which is a glycoprotein with terminal D-mannosyl residues, is by the sinusoidal cells.[486]

Isolated specific cell-surface components of rat hepatocytes involved in intracellular adhesion do not appear to be receptors for either fibronectin or heparin.[487] The endocytosis of human urine α-D-2-acetamido-2-deoxy-glucosidase is mediated by at least three different cell-surface receptors on rat hepatocytes, involving the recognition of D-galactosyl, D-mannosyl 6-phosphate, and 2-acetamido-2-deoxy-D-glucosyl residues.[488]

A binding protein exists in rat-liver microsomes with specificity for β-D-glucuronidase.[489] Once removed from the membrane, the protein undergoes self-aggregation forming large macromolecular complexes. The binding protein shows organ localization, being present in liver and kidney but not spleen. Lysolecithin has been used to solubilize the D-galactosyl- and 2-acetamido-2-deoxy-D-glucosyl-transferases from rat-liver microsomal membranes.[490]

A method, which is not dependent on cell fractionation, has been developed to analyse the sub-cellular location of membrane glycoproteins.[491] The method uses the conversion of glycoproteins containing neuraminic acid residues into forms having a differing mobility in a two-dimensional electrophoretic system when intact cells are treated with neuraminidase. Using this method, it is possible to determine whether or not a glycoprotein is present only on the plasma membrane or whether it is shared by membrane systems. Most of the glycoproteins of rat hepatoma tissue culture cells are shared by the plasma membrane and by an intracellular membrane system. Treatment of hepatoma

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[491] H. Baumann and D. Doyle, *J. Biol. Chem.*, 1979, **254**, 2542.

tissue cells with trypsin at a concentration that results in cell aggregation releases a glycopeptide (mol. wt. 5.5×10^4) from a membrane glycoprotein (mol. wt. 8.5×10^4).[492] The glycopeptide is much more resistant to further degradation by proteases and also accumulates in the medium during normal cell growth.

Plasma membranes of beef kidney glomeruli have been prepared and found to contain 9% carbohydrate.[493]

Laminin, a high molecular weight non-collagenous glycoprotein consisting of at least two polypeptide chains (mol. wt. 2.2×10^5 and 4.4×10^5) and joined by disulphide bonds, has been isolated from a mouse tumour.[494] Immunological studies have indicated that this glycoprotein is distinct from fibronectin and is produced by various cultured cells.[495] A glycopeptide consisting of fifteen amino-acids and two oligosaccharide units, one linked to L-hydroxylysine and the other to L-asparagine, has been isolated from bovine glomerular basement membrane.[496] The oligosaccharide side chains appear to be attached to the polypeptide backbone within a space of not more than twelve amino-acid residues.

A low apparent turnover in organ culture for most of the high molecular weight human intestinal brush border membrane glycoproteins has been reported.[497] Some of the glycoproteins co-migrate on polyacrylamide gel electrophoresis with enzymic activities of known intestinal glycosidases.

Rabbit intestinal brush borders incorporate [^{35}S]sulphate into glycoprotein and glycolipid as sulphate ester, which is the major contributor to the anionic character of the membrane.[498] D-Glucose is the predominant sugar component of the glycoprotein fraction, which also contains smaller amounts of D-mannose, D-galactose, L-fucose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose.[499] Biosynthetic studies have indicated that the glycoproteins and glycolipids are actively synthesized and degraded within the mature small intestinal enterocyte.

Colchicine, given intraperitoneally to rats before and after injection of L-[^{3}H]fucose, markedly inhibits the incorporation of labelled glycoprotein into microvillus membrane with a corresponding increase in incorporation into the lateral basal membrane.[500] By using specific immunochemical techniques in combination with two-dimensional electrophoresis, it is possible to identify a set of four glycoprotein sub-units present only in placental brush border membranes.[501] A group of seven placental glycoproteins was found to be common to liver and kidney membranes.

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A two-dimensional mapping procedure, involving isoelectric focusing and sodium dodecylsulphate electrophoresis, has been used in a comparative study of the surface anatomy of baby hamster kidney cells and three clones resistant to ricin.[502] Abnormal patterns were observed in all three mutant clones indicating different mechanisms of ricin resistance; glycoproteins, which may be involved in cellular reactions, were identified.

Tunicamycin causes selective inhibition of protein glycosylation in baby hamster kidney fibroblasts.[503] Membrane glycoproteins continue to be transported to the cell surface despite the absence of oligosaccharides linked to L-asparagine.

The localization of brain glycoprotein NSA3 has been studied by indirect immunofluorescence of sections of rat brain.[504]

Grey matter membranes from calf brain catalyse the transfer of *NN'*-diacetylchitobiose from dolichyl diphosphate to at least one membrane-associated polypeptide acceptor, *via* an *N*-glycosidic linkage.[505] Axolemma-enriched membranes from bovine myelinated axons catalyse the synthesis of dolichyl-linked derivatives of D-mannose and 2-acetamido-2-deoxy-D-glucose.[506] The lipid-mediated glycosylation involves *P'*-dolichyl phosphate D-mannose and a dolichyl phosphoryl oligosaccharide consisting of eight hexose units terminated at the reducing end with *NN'*-diacetylchitobiose.[507] The dolichyl-bound oligosaccharide is transferred to an L-asparagine residue in endogenous membrane polypeptide acceptors to give a glycoprotein (mol. wt. 2.4×10^4).

Evidence for the transfer of an oligosaccharide containing D-glucosyl, D-mannosyl, and two 2-acetamido-2-deoxy-D-glucosyl residues from exogenous glycosylated oligosaccharide lipid to endogenous membrane glycoproteins by a membrane preparation from calf brain has been reported.[508]

D-[^{14}C]Glucose is incorporated into protein acceptors of bovine retinal membranes.[509] Treatment of the resulting glycoprotein with α-amylase released maltose, indicating the presence of (1→4)-linked α-D-glucosyl residues.

The externally disposed polypeptides in chick synaptic plasma membranes are glycoproteins, containing non-reducing terminal D-galactosyl residues.[510] A myelin-associated glycoprotein on the surface of rat, bovine, and human myelin membranes is one of the principal receptors for concanavalin A.[511]

A complex glycoprotein I, consisting of two polypeptides (mol. wt. 2.1×10^5 and 1.5×10^5) has been isolated from human platelet membranes.[512] Glyco-

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[506] J. B. Harford, C. J. Waechter, R. Saul, and G. H. De Vries, *J. Neurochem.*, 1979, **32**, 91.
[507] J. B. Harford and C. J. Waechter, *J. Neurochem.*, 1979, **32**, 1707.
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calicin, a soluble loosely bound membrane glycoprotein related to the glycoprotein I system, was also purified. The two polypeptides of glycoprotein I produce essentially the same peptides on tryptic hydrolysis, but these are different from those of glycocalicin. Human platelet membranes contain sixteen glycoproteins having molecular weights ranging from 3.5×10^4 to 3.0×10^5, which have been resolved by polyacrylamide gel electrophoresis.[513] Essentially all of the carbohydrate is exposed to the exterior surface of the membrane.

A glycoprotein, with binding properties for the Fc fragment of immunoglobulin G, has been isolated from human platelets.[514] The Fc fragment binding-glycoprotein, which has a molecular weight of 2.55×10^5, and forms *in vitro* complexes with immunoglobulin G, dissociated into sub-units of molecular weight 5.0×10^4 upon reduction.

Wheat-germ agglutinin binds to the surface of human platelets and leads to their agglutination.[515] Unlike other aggregating agents, the clumping ability of platelets by the lectin is markedly reduced on removal of cell-surface neuraminic acid. Since the terminal neuraminic acid residues have been implicated in the clearance of platelets from the circulation, the lectin may prove to be a useful tool in the exploration of those clinical conditions in which platelet survival is shortened.

Wheat-germ agglutinin also stimulates platelets by a mechanism which closely mimics the action of thrombin, and is independent of intracellular cross-linking.[516] The lectin, which binds mainly to glycoprotein I on the platelet membrane, increases the subsequent binding of thrombin to fixed platelets. The platelets of patients with T_n syndrome contain abnormalities in the glycoproteins of the membranes.[517] These abnormalities may arise from the incomplete synthesis of oligosaccharide chains since the platelets also exhibit low T transferase (UDP-D-galactose : 2-acetamido-2-deoxy-D-galactose 3-β-D-galactosyltransferase) activity.

A complete absence of glycocaliacin and a significant reduction of membrane-bound glycoprotein I in the platelets of patients with Bernard–Soulier syndrome has been reported.[518] In the case of platelets from patients with Glanzmann's thrombasthenia, a significant reduction of glycoprotein II (mol. wt. 1.2×10^5) and glycoprotein III (mol. wt. 1.0×10^5) were observed, whereas patients with storage pool disease had increased levels of glycoprotein IV (mol. wt. 8.5×10^4). Glycoproteins isolated from membrane-enriched fractions of platelets from dogs with thrombobasthenic thrombopathia and von Willebrand's disease and from normal controls give patterns on polyacrylamide electrophoresis similar to those for the analogous human conditions.[519]

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The major surface glycoprotein of normal human blood granulocytes is markedly reduced in granulocytes from three patients with a chromosomal abnormality in all or most bone marrow mitoses.[520]

Solubilized surface proteins from normal human lymphocytes have been obtained by mild digestion with trypsin, and the binding of these components to labelled *Ricinus sanguineus* has been studied.[521] A method for the direct visualization of lymphocyte membrane receptors has made use of fluoresceinyl derivatives of glycosylated cytochemical markers such as bovine serum albumin covalently attached to either D-mannose, 2-acetamido-2-deoxy-D-galactose, L-fucose, or lactose.[522]

Glycoproteins from human or mouse lymphocytes and labelled either by the D-galactose oxidase or lactoperoxidase techniques have been isolated by affinity chromatography on the immobilized haemagglutinin from *Helix pomatia*.[523] The major cell surface glycoprotein (mol. wt. 1.5×10^5) from both normal and thymus-derived human lymphocytes was responsible for almost all the binding to the haemagglutinin. This was not observed with various B cells at different steps of differentiation. However, two of four B lymphoma lines and a myeloma line contained instead a glycoprotein (mol. wt. 2.0×10^5) capable of binding to the haemagglutinin. Studies on the mouse lymphocyte lines showed that the T-derived lymphocytes from both normal and malignant cells, but not from adult B-cells expressed a major glycoprotein (mol. wt. 1.3×10^5) capable of binding to *H. pomatia* haemagglutinin.

An affinity chromatographic method based on immobilized *Helix pomatia* haemagglutinin has been used to differentiate mouse natural killer cells from other lymphoid sub-populations.[524] A major membrane glycoprotein (mol. wt. 5.4×10^4) has been isolated from membrane preparations of human B-type lymphoid lines.[525] Its expression on cultured human cell lines and normal lymphocytes, as well as various types of leukaemic cells, has been determined. Mouse B- and T-cells differ in the relative amounts of different types of carbohydrate units present in their cell-surface glycoproteins.[526] The major oligosaccharide component in both cell types, which was labile to alkali, was identified as 2-acetamido-2-deoxy-3-*O*-β-D-galactosyl-D-galactose.

The surface glycoproteins of rat lymphoid cells have been labelled after periodate oxidation followed by reduction with sodium borotritide.[527] The

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[527] R. Standring, W. R. McMaster, C. A. Sunderland, and A. F. Williams, *Eur. J. Immunol.*, 1978, **8**, 832.

labelled glycoproteins were studied for binding to lentil lectin, and for immunoprecipitation or affinity chromatography/immunoadsorption with antibodies to rat cell-surface antigens. All of the labelled glycoproteins have been identified as previously characterized differentiation antigens of rat thymocytes or of B- and T-lymphocytes.

Direct evidence has been obtained for the binding of diphtheria toxin to specific glycoproteins from the plasma membranes of guinea-pig lymph node cells.[528] These glycoproteins also bind to immobilized lentil lectin and are not detectable in cell membranes of mouse L cells that are resistant to the toxin. A glycoprotein (mol. wt. 1.0×10^5), which binds to diphtheria toxin, has been isolated from the surface of lymph nodes and thymus cells of hamsters.[529] Lectins such as concanavalin A do not appear to block the binding of toxin to receptors on mammalian cells, although they appear to prevent the internalization of the toxin.[530] In studies on the effects of diphtheria toxin receptor on Chinese hamster cells, concanavalin A, wheat-germ agglutinin, and a glycopeptide from ovalbumin all acted as inhibitors of the cytotoxic effect.[531] A decreased affinity of toxin for a cell-surface receptor has been suggested, since a mutant was isolated having ten- to fifteen-fold more resistance to the toxin than wild-type cells.

Different types of leukaemic cells have been surface-labelled by sequential treatment with neuraminidase, D-galactose oxidase, and sodium borotritide.[532] Analysis of the surface glycoprotein profile by polyacrylamide gel electrophoresis has provided a method for the classification of leukaemic cells according to cell type and the stage of differentiation.

Specific changes in the pattern of surface glycoproteins of a human promyelocytic leukaemia cell line during morphological and functional differentiation have been observed.[533] The major surface glycoprotein (mol. wt. 1.6×10^5) after induced differentiation is replaced by another glycoprotein (mol. wt. 1.3×10^5) having properties similar to those of a major surface glycoprotein from human blood granulocytes. Surface glycoproteins, including the receptor for immunoglobulin E, from rat basophilic leukaemia cells, bind to immobilized lentil lectin.[534] The immobilization of *N*-acetylneuraminidase and its potential application in the modification of cell surfaces of leukaemia, mouse thymus, and spleen lymphocytes has been described.[535] Structural and

[528] R. L. Proia, D. A. Hart, R. K. Holmes, K. V. Holmes, and L. Eidels, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 685.
[529] R. L. Proia, L. Eidels, and D. A. Hart, *Infect. Immun.*, 1979, **25**, 786.
[530] J. L. Middlebrook, R. B. Dorland, and S. H. Leppla, *Exp. Cell Res.*, 1979, **121**, 95.
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[532] L. C. Andersson, C. G. Gahmberg, M. A. Siimes, L. Teerenhovi, and P. Vuopio, *Int. J. Cancer*, 1979, **23**, 306.
[533] C. G. Gahmberg, K. Nilsson, and L. C. Andersson, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 4087.
[534] R. M. Helm, D. H. Conrad, and A. Froese, *Int. Arch. Allergy Appl. Immunol.*, 1979, **58**, 90.
[535] E. R. Bazarian and L. B. Wingard, *J. Histochem. Cytochem.*, 1979, **27**, 125.

functional aspects of differentiation antigens and glycoproteins of lymphocytes have been reviewed.[536]

Serologically and biochemically pure preparations of HLA-A and -B antigens have been isolated from a series of immunoadsorption columns constructed from monoclonal antibodies having specificity for these antigens.[537] Similar methodology has been used in the preparation of the H-2K^k antigen, which is expressed at levels of 2×10^6 molecules/cell by a murine lymphoma.[538]

Purified preparations of the histocompatibility-Z antigen from mouse liver contain two moles of alloantigenic heavy chain per mole of β_2-microglobulin.[539] Denaturing conditions were required to remove the β_2-microglobulin component. Peptides have been isolated and the amino-acid composition recorded of peptides arising from tryptic and chymotryptic hydrolysis of the histocompatibility antigen HLA-B7.[540] The complete amino-acid sequence of the papain-solubilized heavy chain of the antigen consists of 271 amino-acid residues with a single glycan moiety linked at position 86 by an *N*-glycosidic bond.[541] Significant homology was observed between certain regions and immunoglobulin G-constant domains and β_2-microglobulin. Major differences between amino-acid sequences of murine H-2K^b antigen and HLA glycoprotein occur within delineated regions.[542]

Alveolar macrophages bind glycoconjugates having D-mannosyl, D-glucosyl, or 2-acetamido-2-deoxy-D-glucosyl residues in a non-reducing terminal position.[543] The interaction between α-macroglobulin–trypsin complexes and receptors on the surface of rabbit alveolar macrophages, and the dependency of the interaction on divalent cations has been described.[544] Proteases that bind to α-macroglobulin can be rapidly internalized *via* specific cell-surface receptors and then degraded, thus preventing potentially injurious proteolytic activity.[545] A relationship between rabbit macrophage plasma-membrane glycoproteins and rabbit lung lavages, which contain a glycoprotein inhibitor of phagocytosis, has been demonstrated immunoelectrophoretically.[546]

Five glycoproteins of adrenal chromaffin granules have been isolated by lectin affinity chromatography.[547] The D-galactose oxidase labelling technique has

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[539] O. Henriksen, E. A. Robinson, and E. Appella, *J. Biol. Chem.*, 1979, **254**, 7651.
[540] J. A. Lopez de Castro, H. T. Orr, R. J. Robb, T. G. Kostyk, D. L. Mann, and J. L. Strominger, *Biochemistry*, 1979, **18**, 5704.
[541] H. T. Orr, J. A. Lopez de Castro, D. Lancet, and J. L. Strominger, *Biochemistry*, 1979, **18**, 5711.
[542] J. E. Coligan, T. J. Kindt, B. M. Ewenstein, H. Uehara, J. M. Martinko, and S. G. Nathenson, *Mol. Immunol.*, 1979, **16**, 3.
[543] P. D. Stahl, J. S. Rodman, M. J. Miller, and P. H. Schlesinger, *Proc. Natl. Acad. Sci., USA*, 1978, **75**, 1399.
[544] J. Kaplan and M. L. Nielsen, *J. Biol. Chem.*, 1979, **254**, 7323.
[545] J. Kaplan and M. L. Nielsen, *J. Biol. Chem.*, 1979, **254**, 7329.
[546] R. S. Pratt and G. M. W. Cook, *Biochem. J.*, 1979, **179**, 305.
[547] E. Huber, P. Konig, G. Schuler, W. Aberer, H. Plattner, and H. Winkler, *J. Neurochem.*, 1979, **32**, 35.

revealed that at least the carbohydrate portion of the major glycoprotein is present on the inner side of the granule membranes.

Conjugates of horse-radish peroxidase with lectins or with cholera toxin have been used for the cytochemical detection of carbohydrates on the cell surfaces of cultured neurons and neuroblastoma cells.[548] The lectins and the toxin undergo endocytosis in the cisternae and vesicles of the Golgi–endoplasmic reticulum–lysosome complex. Treatment of neuroblastoma N2a cells with $N^6O^{2'}$-dibutyryl adenosine 3′,5′-cyclic monophosphate and theophylline results in changes in the overall degree of glycoproteins and changes in the turnover of glycoproteins (mol. wt. 1.0×10^5).[549]

The organization of binding sites on mouse neuroblastoma cells for fluorescein-labelled lectins has been reported.[550] Receptors for concanavalin A, wheat-germ agglutinin, and *Ricinus communis* lectin redistribute and become internalized, although the mode of redistribution differs from concanavalin A to the other two lectins. Solubilized receptors from mouse submaxillary glands have been adsorbed on immobilized concanavalin A and a ligand-binding assay performed on the immobilized receptor.[551]

Of three different groups of glycoproteins which are released from the surface of TA3-Ha murine adenocarcinoma cells, two groups contain epiglycanin with receptors for *Vicia graminea* lectin and are believed to be responsible for the ability of this line to kill allogeneic mice.[552] The cellular origin of these glycoproteins has been studied.[553] Unequivocal evidence for the presence in epiglycanin of 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactosyl units has been presented.[554]

Using an indirect immunoperoxidase technique an antigen, immunologically related to a glycoprotein of mouse mammary tumour virus, has been identified in sections of human breast cancer.[555] Cross-reactivity between the human and murine antigens is due to the polypeptide rather than the oligosaccharide component.[556]

The plasma membranes of AH66 hepatoma ascites cells contain a glycoprotein resembling glycophorin A from human erythrocyte membranes in having a high content of 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, D-galactose, and neuraminic acid.[557] In the TA-3 tumour system, the proportion of *N*-glycolylneuraminic acid in the neuraminic acid of the cell-

[548] N. K. Gonatas, *J. Histochem. Cytochem.*, 1979, **27**, 1165.
[549] A. G. Milenkovic, M. Rachmeler, and T. C. Johnson, *Biochem. J.*, 1978, **176**, 695.
[550] P. Maher and R. S. Molday, *J. Supramol. Struct.*, 1979, **10**, 61.
[551] E. Nexø, R. A. Hock, and M. D. Hollenberg, *J. Biol. Chem.*, 1979, **254**, 8740.
[552] D. K. Miller and A. G. Cooper, *J. Biol. Chem.*, 1978, **253**, 8798.
[553] D. K. Miller, A. G. Cooper, and M. C. Brown, *J. Biol. Chem.*, 1978, **253**, 8804.
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[556] T. Ohno, R. Mesa-Tejada, I. Keydar, M. Ramanarayanan, J. Bausch, and S. Spiegelman, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 2460.
[557] S. Nakajo, K. Nakaya, and Y. Nakamura *Biochim. Biophys. Acta*, 1979, **579**, 88.

surface glycoproteins of an ascites cell line correlates directly with the proportion of *N*-glycosyl-linked carbohydrate chains in the glycoprotein.[558]

The role of cell-surface glycoproteins in malignant transformation has been discussed.[559] An outline of the teratoma system in relation to cell-surface antigens of mouse embryonal carcinoma cells has been published.[560]

A membrane-associated glycoprotein has been solubilized by proteolytic digestion of membranes of rat mammary carcinoma cells.[561] The material was not further characterized other than by its affinity for *Lens culinaris* lectin. A class of glycoproteins recognized as cell-surface markers by lectins and antibody appears to be expressed on the surface of embryonal carcinoma cells.[562] Because of their unusual structural characteristics and abundance, the carbohydrate chains may play a role in the early stages of embryogenesis.

High molecular weight D-galactosyl glycoproteins that are characteristic of early embryonic carcinoma cells are at least partly exposed to the cell surface and they contain binding sites for peanut agglutinin.[563]

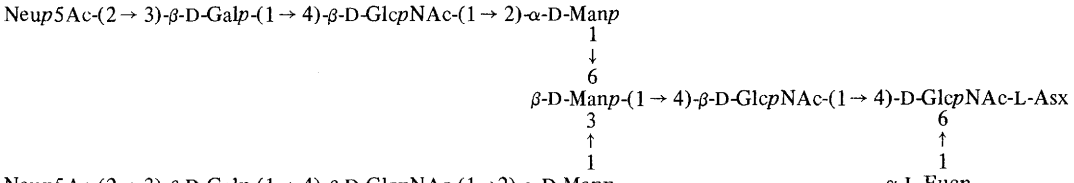
Several ^{125}I-labelled plasma-membrane glycoproteins of HeLa cells have been isolated by affinity chromatography on wheat-germ agglutinin immobilized on agarose.[564]

An abnormal membrane glycoprotein that is associated with malignancy in a wide range of different tumours binds to both concanavalin A and to wheat-germ agglutinin.[565] Several physical parameters of this glycoprotein resemble those of the insulin receptor.

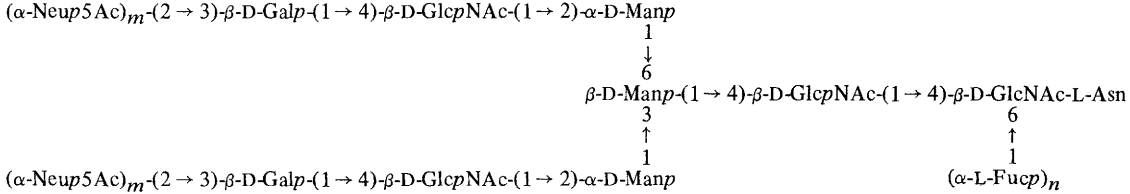
A major group of glycoproteins, which have a high molecular weight and stain only faintly with Coomassie Blue, but bind to concanavalin A, have been identified in the plasma membranes of Chinese hamster fibroblasts.[566] Their presence is only revealed by two-dimensional electrophoresis and not by conventional electrophoretic techniques. The alteration of membrane components of hamster-kidney fibroblasts transformed by Rous sarcoma virus is expressed in an increase in the levels of three glycoproteins, when compared with those of control cells.[567] The structure (24) of the major cell-surface glycoprotein from hamster-embryo fibroblasts has been established.[568]

The structure of a surface glycoprotein from virus-transformed hamster-kidney cells has only been partially characterized and may well prove to have the same structure as that of the embryo fibroblast glycoprotein.[569] Normal human

[558] J. F. Codington, G. Klein, C. Silber, K. B. Linsley, and R. W. Jeanloz, *Biochemistry*, 1979, **18**, 2145.
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[562] T. Muramatsu, G. Gachelin, and F. Jacob, *Biochim. Biophys. Acta*, 1979, **587**, 392.
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[564] R. H. Kramer and E. S. Canellakis, *Biochim. Biophys. Acta*, 1979, **551**, 328.
[565] M. E. Bramwell and H. Harris, *Proc. R. Soc. London, Ser. B*, 1978, **201**, 87.
[566] M. N. Horst, G. Baumbach, and R. M. Roberts, *FEBS Lett.*, 1979, **100**, 385.
[567] M. C. Glick, *Biochemistry*, 1979, **18**, 2525.
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[569] U. V. Santer and M. C. Glick, *Biochemistry*, 1979, **18**, 2533.



(24)



(25) $m = 0$ on α-sub-units; $m = 1$ on β-sub-units; $n = 0$ or 1

fibroblasts contain two classes of binding sites on the surface membrane for concanavalin A.[570] The membrane receptor on human fibroblasts for epidermal growth factor is associated with a glycoprotein or glycolipid containing D-mannosyl, D-galactosyl, and 2-acetamido-2-deoxy-D-glucosyl residues.[571] At least half of the membrane glycoproteins of the rough and smooth microsomes and Golgi vesicles from rat liver are associated with the cytoplasmic surface of the membranes.[572]

The cell-surface receptor of cultured rat basophilic leukaemia cells for immunoglobulin E has been purified by affinity chromatography on immobilized immunoglobulin E.[573] The purified receptor exists in monomeric (mol. wt. 4.0×10^4) and polymeric (mol. wt. 1.7×10^5) forms, both of which are capable of binding to immunoglobulin E.[574]

Chinese hamster ovary plasma cell membranes contain a glycoprotein (mol. wt. 1.0×10^5), which precipitates with wheat-germ agglutinin, and a glycoprotein (mol. wt. 1.3×10^5) which precipitates with concanavalin A.[575] The advantages of such a lectin immunoprecipitation technique over affinity chromatography for the isolation of glycoproteins is discussed. The biosynthesis of a secretory protein and a transmembrane viral glycoprotein, when compared by different experimental approaches, appear to involve an initially common pathway.[576]

10 Hormonal Glycoproteins

Studies on the sub-units of human glycoprotein hormones in relation to reproduction have been reviewed.[577] One volume of a treatise on hormonal proteins and peptides has been devoted to thyroid hormones.[578] The ectopic production of human chorionic gonadotrophin (hCG) and its α- and β-sub-units has been discussed.[579]

Like thyroid-stimulating hormone (TSH) human chorionic gonadotrophin interacts with a receptor that has a ganglioside or ganglioside-like structure, and undergoes a change in conformation upon interacting with its receptor.[580] The α- and β-sub-units of human chorionic gonadotrophin each contain two *N*-glycosidically linked oligosaccharide chains with structures (25) similar to

[570] M. Feller, D. Behnke, and E. Gruenstein, *Biochim. Biophys. Acta*, 1979, **586**, 315.
[571] G. Carpenter and S. Cohen, *Biochem. Biophys. Res. Commun.*, 1977, **79**, 545.
[572] E. L. Appelkvist and G. Dallner, *Biochim. Biophys. Acta*, 1978, **513**, 173.
[573] A. Kulczycki and C. W. Parker, *J. Biol. Chem.*, 1979, **254**, 3187.
[574] A. Kulczycki, B. L. Hempstead, S. L. Hofmann, E. W. Wood, and C. W. Parker, *J. Biol. Chem.*, 1979, **254**, 3194.
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[577] C. Hagen, *Scand. J. Clin. Lab. Invest., Suppl. 148*, 1977, **37**, 1.
[578] 'Hormonal Proteins and Peptides, Vol. 6, Thyroid Hormones', ed. C. H. Li, Academic Press, New York, 1978.
[579] P. Franchimont, A. Reuter, and U. Gaspard, in 'Current Topics in Experimental Endocrinology', ed. L. Martini and V. H. T. James, Academic Press, New York, 1978, Vol. 3.
[580] G. Lee, S. M. Aloj, R. O. Brady, and L. D. Kohn, *Biochem. Biophys. Res. Commun.*, 1976. **73**, 370.

those of many other *N*-glycosidic carbohydrate units of animal glycoproteins.[581, 582]

The precise location and structure (26) of the four *O*-glycosidically-linked oligosaccharide units in the *C*-terminal peptide of β-sub-unit chains of human chorionic gonadoptrophin and a revised amino-acid sequence for this peptide have been reported.[583]

α-Neu*p*5Ac-(2 → 3)-β-D-Gal*p*-(1 → 3)-D-Gal*p*NAc-(1 → L-Ser
6
↑
2
(α-Neu*p*5Ac)$_n$

(26)

The chorionic gonadotrophin excreted by patients with hydatidform mole resembles that from urine of normal pregnant women in amino-acid composition, but differs in having a lower content of D-mannosyl and 2-acetamido-2-deoxy-D-glucosyl residues.[584] Any biological role for the carbohydrate moiety of human chorionic gonadotrophin must also involve the protein moiety since a glycopeptide fraction prepared from human chorionic gonadotrophin exhibited no cross-reactivity with anti-(human chorionic gonadotrophin) antiserum.[585] Likewise there was no competition of this glycopeptide with human chorionic gonadotrophin for testicular receptors.

First trimester placental mRNA directs the synthesis of a pre-protein form of the α-sub-unit of human chorionic gonadotrophin, containing a segment of twenty-four amino-acid residues at its *N*-terminus.[586] In the presence of microsomal membranes, the pre-protein is cleaved into products with a homogeneous *N*-terminus. Evidence is submitted to suggest that this processing step is unlikely to account for the heterogeneity that has been observed previously in the structure of this region of the sub-unit.

An improved method for the purification of radiolabelled glycoprotein hormones is based on their competitive elution from immobilized concanavalin A with methyl α-D-glucopyranoside.[587] The method separates free iodine, damaged hormone, and radiolabelled bovine serum albumin.

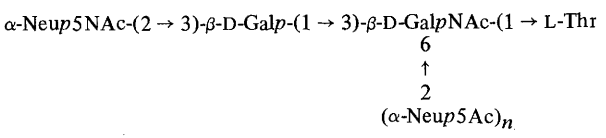
After removal of five amino-acid residues from the α-sub-unit of bovine luteinizing hormone (LH) with carboxypeptidase, followed by covalent cross-linking of the product, no significant changes in conformation have been detected by c.d. measurements although the biological activity is almost com-

[581] M. J. Kessler, M. S. Reddy, R. H. Shah, and O. P. Bahl, *J. Biol. Chem.*, 1979, **254**, 7901.
[582] Y. Endo, K. Yamashita, Y. Tachibana, S. Tojo, and A. Kobata, *J. Biochem. (Jpn.)*, 1979, **85**, 669.
[583] M. J. Kessler, T. Mise, R. D. Ghai, and O. P. Bahl, *J. Biol. Chem.*, 1979, **254**, 7909.
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[585] D. Puett, R. Benvenido, A. Kenner, and D. Rabinowitz, *Horm. Metab. Res.*, 1979, **11**, 165.
[586] S. Birken, J. Fetherston, J. Desmond, R. Canfield, and I. Boime, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 1247.
[587] N. Patritti-Laborde, Y. Yoshimoto, A. Wolfsen, and W. D. Odell, *Clin. Chem.*, 1979, **25**, 163.

pletely abolished.[588] The cell-free synthesis of bovine pituitary proteins from total cellular pituitary RNA has been studied.[589] The products include the α-sub-unit of bovine luteinizing hormone, which was identified by immunoprecipitation and sodium dodecyl sulphate-gel electrophoresis. Increases in the extent of covalent cross-linking between α- and β-sub-units of bovine luteinizing hormone with carbodiimide results in a concomitant decrease in the receptor binding activity of the non-dissociable product.[590] Perturbations in sub-unit interactions presumably due to the presence of the cross-links have been examined.[591]

11 Milk Glycoproteins

Approximately half of the casein micelle from bovine milk binds to wheat-germ agglutinin *via* neuraminic acid moieties of κ-casein.[592] The structures of a trisaccharide (27) and a tetrasaccharide (28) linked *O*-glycosidically to L-threonine residues in bovine κ-casein have been reported.[593]



(27) $n = 0$
(28) $n = 1$

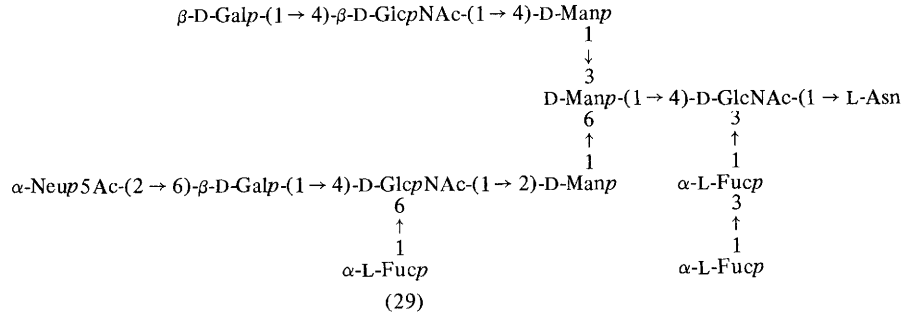
α-Lactalbumin and casein have been measured by radioimmunoassay in rat mammary gland at various stages of gestation and lactation.[594] Rabbit α-lactalbumin contains five residues of 2-amino-2-deoxy-D-glucose per mole of protein, as well as other sugars.[595] The amino-acid sequence has been determined for the 122 amino-acids and includes a single carbohydrate moiety, probably at L-asparagine-45.

A protein that is antigenically identical to the soluble glycoprotein of the milk fat globule membrane has been identified in whey.[596] By indirect elimination, the major protein components of whey (β-lactoglobulin, α-lactalbumin, and immunoglobulin G) were shown not to be the anti-(soluble glycoprotein) antibody.

[588] T. F. Parsons and J. G. Pierce, *J. Biol. Chem.*, 1979, **254**, 6010.
[589] T. D. Landfeld, *J. Biol. Chem.*, 1979, **254**, 3685.
[590] J. A. Weare and L. E. Reichert, *J. Biol. Chem.*, 1979, **254**, 6964.
[591] J. A. Weare and L. E. Reichert, *J. Biol. Chem.*, 1979, **254**, 6972.
[592] M. Yoshikawa, K. Takahata, R. Sasaki, and H. Chiba, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 1923.
[593] B. Fournet, A. M. Fiat, C. Alais, and P. Jollès, *Biochim. Biophys. Acta*, 1979, **576**, 339.
[594] H. L. Nakhasi and P. K. Qasba, *J. Biol. Chem.*, 1979, **254**, 6016.
[595] T. P. Hopp and K. R. Woods, *Biochemistry*, 1979, **18**, 5182.
[596] C. Kanno and K. Yamauchi, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 1697.

The effects of tunicamycin on the glycosylation of lactating rabbit-mammary glycoproteins has been reported.[597] Although lipid-linked sugars are not involved in the glycosylation of casein, which is probably glycosylated by direct sequential transfer of monosaccharides to protein, they serve as intermediates in the glycosylation of a group of mammary polypeptides.

The carbohydrate chains of the free secretory component from human milk have the general composition and structural features (29) of *N*-glycosidically linked oligosaccharides of numerous other mammalian glycoproteins.[598] Two unusual features are the di-L-fucosyl disaccharide unit and the single 2-acetamido-2-deoxy-D-glucosyl residue instead of the usual two amino-sugar residues at the reducing end of the molecule.



(29)

Multiple forms of β_2-microglobulin have been detected in human colostrum.[599] One form (mol. wt. 4.8×10^4), identical to lactollin, is a tetrameric form of β_2-microglobulin, and three others (mol. wt. 1.2×10^4) are separable by electrophoresis.

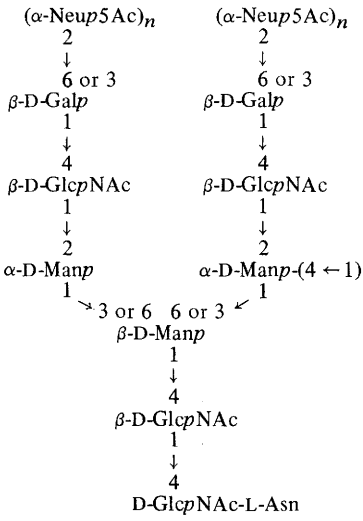
12 Plasma and Serum Glycoproteins

The sera of certain animal species contain substances which are not antibodies, although they bind to hepatitis B surface antigen.[600] They are probably glycoproteins and can be selectively removed from sera by reaction with purified hepatitis B surface antigen.

A heterogeneous population of serum glycoproteins, which bind to immobilized *Ricinus communis* lectin, have been isolated and shown to account for the inhibition of the binding of desialylized glycoprotein to the hepatocyte membrane.[601] These glycoproteins are present in small amounts in normal human serum, but in increased levels in serum from patients with cirrhosis.

[597] B. K. Speake and D. A. White, *Biochem. J.*, 1979, **180**, 481.
[598] S. Purkayastha, C. V. N. Rao, and M. E. Lamm, *J. Biol. Chem.*, 1979, **254**, 6583.
[599] N. Tanahashi, Y. Watanabe, and F. Yamada, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 1263.
[600] I. Millman and J. C. McMichael, *Infect. Immun.*, 1978, **21**, 879.
[601] J. S. Marshall and S. Williams, *Biochim. Biophys. Acta*, 1978, **543**, 41.

[^{3}H]-Labelled raffinose has been covalently attached to a variety of serum glycoproteins and used in detecting the tissue and cellular sites of catabolism of long-lived circulating glycoproteins.[602]



n = 0, 1

(30) R = H

(31) R = α-Neu*p*5Ac-(2 → 6)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc

A method using metal chelate chromatography has been developed for the large-scale purification of two major plasma proteinase inhibitors, α_1-acid glycoprotein and α_2-macroglobulin, from pooled plasma under non-denaturing conditions.[603] A solid-phase enzyme-linked immunosorbent assay (ELISA) for the assay of α_1-acid glycoprotein has been developed, using alkaline phosphatase covalently attached to the glycoprotein and α_1-acid glycoprotein-specific antibody coated to polystyrene tubes.[604] No differences in the carbohydrate composition of normal and variant human α_1-proteinase inhibitors have been observed.[605] The glycoprotein (mol. wt. 5.4×10^4) contains three carbohydrate chains, two of which contain the biantennary structure (30) and one the triantennary structure (31).[606]

[602] J. Van Zile, L. A. Henderson, J. W. Baynes, and S. R. Thorpe, *J. Biol. Chem.*, 1979, **254**, 3547.
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The major proelastase 2 binding factor in human plasma is α_1-protease inhibitor.[607] Proelastase 2 reacts directly with the inhibitor *via* a partial active site in a manner similar to that of *endo*-peptidases.

Guinea-pig α_1-microglobulin, isolated from plasma or urine, is homologous to human α_1-microglobulin, on the basis of molecular weight, carbohydrate composition, and cross-reaction with antisera.[608]

Traces of concanavalin A have been detected in samples of purified α_1-antitrypsin after affinity chromatography on immobilized concanavalin A.[609] The interfering lectin may be removed by adsorption of the α_1-antitrypsin on thiol-activated macroporous agarose.

Unequivocal evidence has been presented for the existence of a recognizable pattern in the complex multiple interactions between trypsin and human serum which allows the classification of human α_2-macroglobulin into seven distinct categories.[610] The molecular interpretation of the binding of trypsin with α_2-macroglobulin and the potential clinical value in recognizing the nature of such binding are discussed.

The native tetramers of human plasma α_2-macroblogulin are reported to exist in two forms that differ in electrophoretic mobility as well as in other properties.[611] The irreversible transition of the two forms is associated with the binding of proteases. Fragmentation of human α_2-macroglobulin (mol. wt. 1.85×10^5) has been achieved by heating in the presence of sodium dodecyl sulphate and dithiothreitol, two fragments (mol. wt. 1.2×10^5 and 6.2×10^4 being produced).[612] On the basis of amino-acid sequencing studies, the native glycoprotein is composed of four identical polypeptide chains.[613]

The contents of Zn^{2+} in human α_2-macroglobulin in normal, hyperzincemic, and hypozincemic sera have been reported.[614] In cystic fibrosis patients the plasma α_2-macroglobulin contains normal amounts of total hexose, but as much as 40% decreased amounts of neuraminic acid.[615] Compared with control preparations, the binding of these preparations to concanavalin A and wheat-germ agglutinin is markedly reduced. Alterations in the carbohydrate moiety of the glycoprotein in cystic fibrosis may be due to a defective post-translational process.

An α_2-acute phase macroglobulin has been isolated from rat under conditions avoiding damage to biological activity.[616] The molecular weight and carbohydrate composition of the glycoprotein were similar to those of α_2-macroglobulins from other sources.

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[613] R. P. Swenson and J. B. Howard, *J. Biol. Chem.*, 1979, **254**, 4452.
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[615] Y. Ben-Yoseph, C. L. De Franco, and H. L. Nadler, *Clin. Chim. Acta*, 1979, **99**, 31.
[616] W. Niewenhuizen, J. J. Emeis, and J. Hemmink, *Biochim. Biophys. Acta*, 1979, **580**, 129.

A pregnancy-associated α_2-glycoprotein (mol. wt. 3.65×10^5) dissociates into two sub-units (mol. wt. 1.44×10^5).[617] Physicochemical similarities, but no antigenic similarities, have been demonstrated between this glycoprotein and human α_2-macroglobulin.

Pregnancy-specific β_1-glycoprotein has been measured using an enzyme-linked immunoassay,[618] and a radioactive immunoassay system.[619]

An agglutinin, identified by physical and serological methods as β_2-glycoprotein, has been isolated from human serum by affinity chromatography on immobilized anti-(β_2-glycoprotein) antibody.[620]

A treatise on chemistry, biology, and clinical applications of the carcinoembryonic proteins, α-foetoprotein, isoferritin, pregnancy-specific β_1-glycoprotein, and carcinoembryonic antigen has been published.[621] Studies on carcinoembryonic antigen and related glycoproteins as tumour markers have been reviewed.[622]

The chemical and immunological properties of carcinoembryonic antigen purified after sodium chloride extraction are almost identical to those of the glycoprotein purified after perchloric acid treatment.[623] Glycopeptides have been isolated from carcinoembryonic antigen after its treatment with neuraminidase and digestion with proteolytic enzymes.[624] Large peptide fragments are required to demonstrate the antigenicity of the molecule. The glycoprotein has been deglycosylated by treatment with hydrogen fluoride revealing a single polypeptide chain.[625] The polypeptide, which retains 15% of its original antigenic activity and is able to inhibit up to 85% of the binding of intact carcinoembryonic antigen to anti-(carcinoembryonic antigen) antibody, has been sequenced through the first thirty amino-acid residues. An improved indirect and direct radioimmunoassay for carcinoembryonic antigen has been developed.[626] Blood group A activity has been observed in two preparations of carcinoembryonic antigen even after passage of these preparations through columns of immobilized anti-A, -B, and -H antibodies.[627]

Normal human bile contains a major carcinoembryonic antigen-cross-reactive glycoprotein, biliary glycoprotein I, which can be purified by a procedure similar to that used for carcinoembryonic antigen and non-specific cross-reacting antigen.[628] The same sugars and amino-acids were found in similar molar proportions in the three glycoproteins.

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The structure of the component proteins of complement have been reviewed.[629] All nine components of the human classical complement pathway bind to immobilized Cibacron Blue F3GA, and are eluted without contamination of substrates by albumin or immunoglobulin G.[630] The sub-component $C1_q$ of the first component of bovine complement shows many similarities to the corresponding human and rabbit component.[631] Almost all of the L-hydroxylysine residues are substituted with D-galactosyl-D-glucosyl disaccharide units. These glycosyl L-hydroxylysine residues are only detected in the collagen-like regions of the molecule.[632] Bovine sub-component $C1_q$ contains hexose, aminosugar mainly as 2-amino-2-deoxy-D-glucose, and neuraminic acid.[633] The carbohydrate is associated with the heavy chain in this glycoprotein.

Like human component C4 of complement, the corresponding bovine glycoprotein (mol. wt. 2×10^5) contains three polypeptide chains, α, β, and γ (mol. wts. 9.8×10^4, 8.2×10^4, and 3.2×10^4, respectively).[634] The carbohydrate is located in the α- and β-chains.

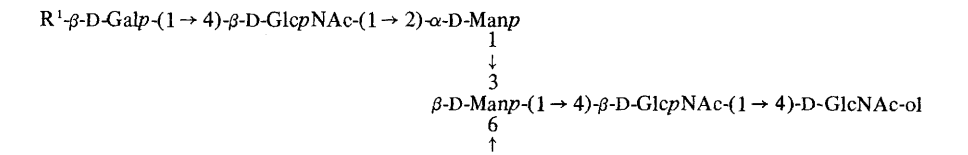
Erythropoietin has been isolated by affinity chromatography on immobilized wheat-germ agglutinin,[635] and on immobilized phytohaemagglutinin.[636]

Human plasma cold-insoluble globulin, which appears to exist in a slightly modified form (fibronectin) on the cell surface of fibroblasts, has been purified by affinity chromatography on immobilized gelatin,[637] as well as by chromatography on either immobilized heparin or immobilized anti-(cold-insoluble globulin) antibody.[638] The glycoprotein agglutinates trypsin-treated erythrocytes from certain species, and the agglutination is inhibited by polyamines, basic amino-acids, non-acetylated amino-sugars, and antibodies to the glycoprotein.[639] The structure and stability of cold-insoluble globulin have been investigated by ultracentrifugation, c.d., and by fluorescence measurements.[640]

Bovine plasma cold-insoluble globulin contains six *N*-glycosidic chains per dimer molecule.[641] These have been released from the polypeptide backbone by hydrazinolysis followed by reduction with sodium borohydride, and their structures [(32)–(35)] established using conventional methods.

The preparation and properties of anti-freeze glycoproteins from Arctic and Antarctic fish blood have been reviewed.[642] In a c.d. spectroscopic study of

[629] R. R. Porter in 'MTP International Review of Biochemistry', ed. E. S. Lennox, University Park Press, Baltimore, 1979, p. 177.
[630] A. P. Gee, T. Boros, and M. D. P. Boyle, *J. Immunol. Methods*, 1979, **30**, 119.
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(32) R^1 = H, R^2 = α-Neu*p*5Ac-(2 → 6)

(33) R^1 = α-Neu*p*5Ac-(2 → 4 or 6), R^2 = α-Neu*p*5Ac-(2 → 6 or 4)

protein and glycoprotein anti-freezes, two glycoproteins appeared to have an unordered conformation in solution, whereas two proteins had a right-handed α-helical conformation.[643] Thus the freezing-point depressing activity of the anti-freezes does not require them to have a particular secondary structure.

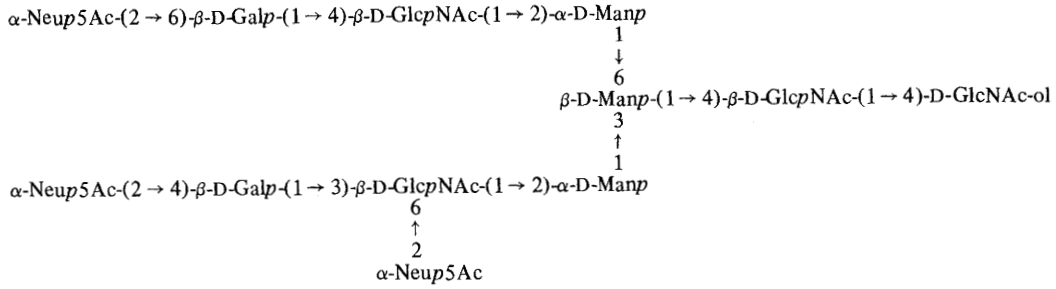
Factor VIII and fibrinogen can be removed from normal human plasma by affinity chromatography on immobilized rabbit anti-Factor VIII.[644] In human Factor VIII neither the size distribution nor its ristocetin cofactor activity is related to the carbohydrate content.[645] The binding affinity of bovine Factor VIII for concanavalin A is gradually decreased with increasing aggregate size, suggesting an impaired accessibility of reactive sugar residues in the large aggregates of the bovine glycoprotein. Factor VIII-related protein aggregates are multimers of pairs of basic sub-unit chains.[646] When prepared from lipid-poor plasma, the size heterogeneity ranges from 6.0×10^5 to 2.0×10^7.[647] Heparin and dextran sulphate can bind to Factor VIII, an essential factor for the maintenance of weak interplatelet bonds, and either inhibits or reverses platelet aggregation.[648]

The Ca^{2+}-binding characteristics of the products of Factor IX activation are similar to those of the native zymogen.[649] A low α-helical content and a high level of β-structure and random coil conformations were the main conformational features of all the products as shown by c.d. spectroscopic studies.

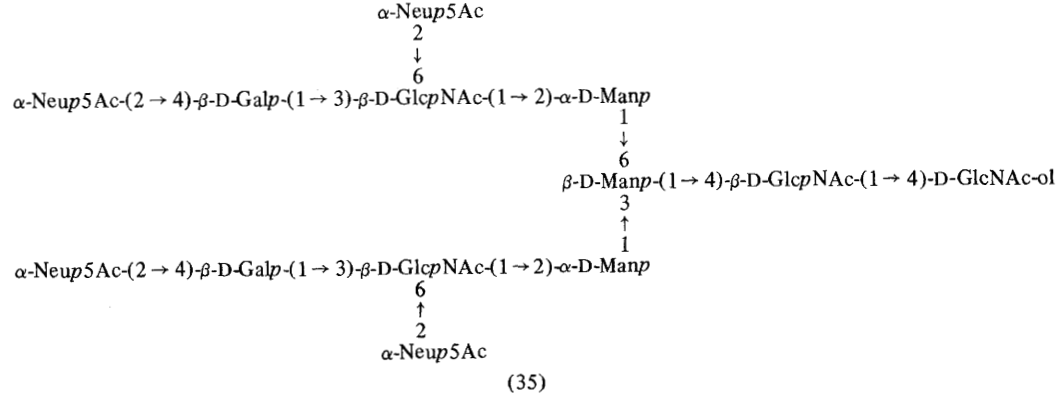
Factor V is cleaved by thrombin to form either an activation intermediate or a stable form of fully activated Factor V.[650] Both the intermediate and the activated Factor V can be dissociated by chelating agents.

Bovine anti-thrombin is cleaved by thrombin at only one site, which is at the peptide bond in the L-arginyl-L-serine sequence near the *C*-terminal end of the chain.[651] The modified anti-thrombin thus formed loses all or most of its ability to inhibit thrombin, and has a reduced affinity for heparin.[652] Two polypeptide fragments (mol. wt. 5.0×10^4 and 5.0×10^3) are formed. Limited proteolysis of

[643] J. A. Raymond, W. Radding, and A. L. De Vries, *Biopolymers*, 1977, **16**, 2575.
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α-Neup5Ac-(2 → 6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 2)-α-D-Manp
1
↓
6
β-D-Manp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-D-GlcNAc-ol
3
↑
1
α-Neup5Ac-(2 → 4)-β-D-Galp-(1 → 3)-β-D-GlcpNAc-(1 → 2)-α-D-Manp
6
↑
2
α-Neup5Ac

(34)


α-Neup5Ac
2
↓
6
α-Neup5Ac-(2 → 4)-β-D-Galp-(1 → 3)-β-D-GlcpNAc-(1 → 2)-α-D-Manp
1
↓
6
β-D-Manp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-D-GlcNAc-ol
3
↑
1
α-Neup5Ac-(2 → 4)-β-D-Galp-(1 → 3)-β-D-GlcpNAc-(1 → 2)-α-D-Manp
6
↑
2
α-Neup5Ac

(35)

anti-thrombin by thrombin may be involved in formation of an inactive complex.[653] The inhibitor may therefore exist in the complex as a proteolytically modified two-chain glycoprotein. Subsidiary forms of the anti-thrombin–thrombin complex may arise as a result of complex formation between anti-thrombin and autolysed thrombin.[654]

Bovine prothrombin contains three *N*-glycosidically linked chains per molecule and these have been isolated from the glycoprotein by hydrazinolysis.[655] One of the oligosaccharide chains has the typical complex-type widely found in other glycoproteins, however, the other two are unique in possessing a 2-acetamido-2-deoxy-3-*O*-β-D-galactosyl-D-glucosyl disaccharide moiety in the outer chains.

The covalent modification of human α-thrombin with pyridoxal 5′-phosphate involves phosphopyridoxylation at two sites.[656] Attachment of the cofactor to the first site was accompanied by a loss in the fibrinogen clotting activity of the thrombin, whereas attachment of the cofactor to the second site resulted in a decrease in the sensitivity to heparin in the anti-(thrombin III)–thrombin reaction.

The oligosaccharide side chain of fragment E of rat plasma fibrinogen contains residues of neuraminic acid, D-galactose, D-mannose, and 2-amino-2-deoxy-D-glucose (in the molar ratio 1:1:2:2).[657] Removal of the neuraminic acid residue has no effect on the clearance rate of fragment E from the circulation and so no role is played by the oligosaccharide chain in the clearance of fibrinogen degradation products.

The carbohydrate composition of two human plasminogen variants has been reported.[658] Plasminogen 1 possesses two oligosaccharide units, one of which is similar to the single 2-acetamido-2-deoxy-D-galactosyl-containing oligosaccharide in plasminogen 2, but the second chain of residues consists of neuraminic acid, D-galactose, D-mannose, and 2-acetamido-2-deoxy-D-glucose (in the molar ratio 1:2:3:4). A D-mannosyl-containing oligosaccharide attached to L-asparagine at residue 288 was shown to have a structure identical to that of oligosaccharide chains proposed for glycopeptides isolated from transferrin and thyroxine-binding globulin.[659] Both variants contain tri- and tetra-saccharide units linked to L-threonine with structures identical to those of *O*-glycosidically linked oligosaccharides (27 and 28) of κ-casein.[660]

Greater structural heterogeneity apparently exists in the carbohydrate moiety of human transferrin than previously envisaged.[661] This heterogeneity can be

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[660] M. L. Hayes and F. J. Castellino, *J. Biol. Chem.*, 1979, **254**, 8777.
[661] M. W. C. Hatton, L. März, L. R. Berry, M. T. Debanne, and E. Regoeczi, *Biochem. J.*, 1979, **181**, 633.

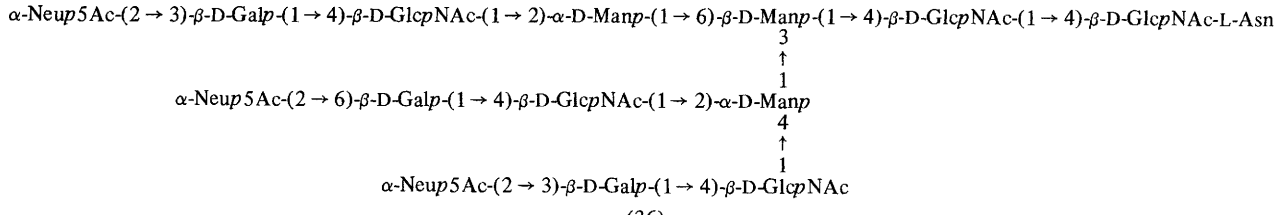
reflected in several molecular forms, which after desialylization differ significantly in their affinities for the hepatic lectin. Three types of asialotransferrin have been isolated after affinity chromatography of human transferrin on immobilized rabbit-liver lectin specific for the asialoglycopeptides.[662] Type 1 contains two biantennary glycans, but Types 2 and 3 are assumed to possess one biantennary and one triantennary glycan. Bovine transferrin contains only one heterosaccharide chain per molecule.[663] The asialoglycopeptide from bovine transferrin has been compared with asialoglycopeptides from other serum proteins for affinity to rat-liver hepatic lectin. Rabbit transferrin contains two glycopeptide segments structurally identical to those from plasminogen and thyroxine-binding globulin.[664]

Mouse α-foetoprotein is heterogeneous with respect to binding to concanavalin A owing to the derivation of the glycoprotein from the yolk sac and the liver.[665] The glycoprotein derived from the yolk sac, and therefore the major α-foetoprotein before birth, does not bind to the lectin. The major α-foetoprotein, which is synthesized in the liver after birth binds to concanavalin A. Human α-foetoprotein from cord serum has been purified to homogeneity after affinity chromatography on immobilized concanavalin A followed by polyacrylamide gel electrophoresis.[666] Two variants of bovine α-foetoprotein with different affinities for concanavalin A show antigenic identity suggesting that the polypeptide chain rather than the carbohydrate moiety is the antigenic determinant.[667] The technique of supplementation counter-immunoelectrophoresis is more sensitive than immuno-diffusion, but less sensitive than radioimmunoassay, for the detection of α-foetoprotein.[668]

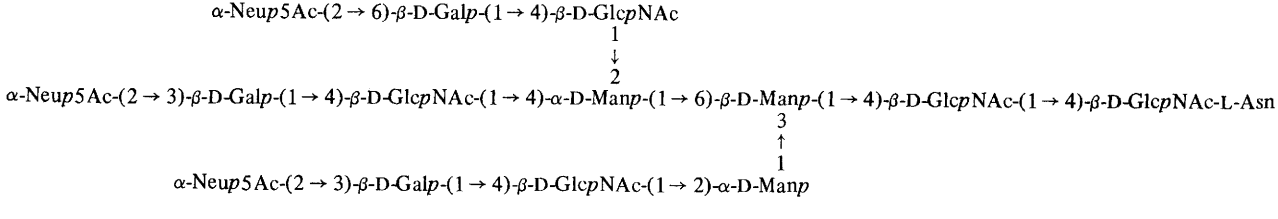
The *O*-glycosically linked carbohydrate chains of fetuin have been cleaved from the protein by alkaline borohydride treatment.[669] Structural analysis revealed the existence of tri- and tetra-saccharides linked *O*-glycosidically to L-serine or L-threonine and having structures identical to those previously identified for bovine κ-casein and human chorionic gonadotrophin [(27) and (28)]. The *N*-glycosidically linked chains of fetuin have been assigned structures (36)[669] or (37).[670]

Glycosylated haemoglobins have been isolated following ion-exchange chromatography of haemolysed erythrocytes.[671] Bound hexoses, after release from the protein by hydrolysis with oxalic acid, were measured as 5-hydroxy-

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α-Neup5Ac-(2 → 3)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 2)-α-D-Manp-(1 → 6)-β-D-Manp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-β-D-GlcpNAc-L-Asn
3
1
α-Neup5Ac-(2 → 6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 2)-α-D-Manp
4
1
α-Neup5Ac-(2 → 3)-β-D-Galp-(1 → 4)-β-D-GlcpNAc

(36)


α-Neup5Ac-(2 → 6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc
1
2
α-Neup5Ac-(2 → 3)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-α-D-Manp-(1 → 6)-β-D-Manp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-β-D-GlcpNAc-L-Asn
3
1
α-Neup5Ac-(2 → 3)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 2)-α-D-Manp

(37)

methylfurfural derivatives. Four glycosylated minor components, possessing unique functional properties, have been isolated from human adult haemoglobin.[672] Two of these components are low affinity haemoglobins similar to certain rare human haemoglobin variants but present in every human haemolysate. Rhesus monkey haemoglobin A_{1c} contains a carbohydrate content identical to that of human haemoglobin A_{1c}.[673] Heterogeneity of the major haemoglobin component HbA_0 was demonstrated, reflecting a slow non-enzymic glycosylation at sites other than the *N*-terminus of the β-chain.[674] The non-enzymic glycosylation of haemoglobin in normal and diabetic subjects has been reviewed.[675]

Non-enzymically D-glucosylated albumin represents approximately 6–15% of total serum albumin from normal adults.[676] The post-translational modification appears to occur by a process analogous to that responsible for D-glucosylation of haemoglobin A through Schiff base formation and Amadori rearrangement to a ketoamine derivative. Elevated levels of D-glucosylated albumin have been detected in the sera of diabetic patients.[677] The level of D-glucosylated albumin may be a sensitive indicator of moderate hyperglycemia and of early D-glucose intolerance.[678]

13 Immunoglobulins

Reviews dealing with the structure of immunoglobulins,[679] the organization of immunoglobulin genes,[680] the antibody-combining site,[681] and the structural basis of antibody complementarity[682] have been published.

Marine anti-[high α-(1→3)-content dextran] antibody production by hybrid cells has been reported.[683] Binding studies of methyl 6-*O*-benzoyl-β-D-galactopyranoside to the Fab′ fragment of immunoglobulin J539 have indicated that the phenyl group appears to participate in interactions with the antibody.[684]

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[681] See reference 679, p. 71.
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Immunogens have been prepared from various oligosaccharides covalently attached to edestin in order to study the specificities of the anti-carbohydrate antibodies that they elicit in rabbits.[685] The radioimmunoassay method used to define the specificity of the antibodies offers a sensitive means for detecting specific carbohydrate linkages in various complex carbohydrates. Several oligo-D-mannosides and glycoproteins have been tested for their ability to inhibit the binding of D-[^{3}H]mannotetraitol (38) to anti-D-mannan antibodies.[686] The antibodies are highly specific for the 3-*O*-α-D-mannosyl-D-mannose units and react poorly with 2-*O*- and 6-*O*-α-D-mannosyl-D-groups. Antisera with specificities for chito-oligosaccharides have been obtained by immunization of animals with the oligosaccharides covalently attached to bovine serum albumin or to cytochrome *c* as carrier proteins.[687] Differences in the electrophoretic mobility of serum lysozyme from humans may be due in some cases to its interaction with anti-lysozyme antibody.[688]

α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-D-Man-ol
(38)

By monitoring various fluorescent properties of both a constant and a variable region in γ- and L-chains, reassociation studies have identified a two-stage transition of immunoglobulin chains.[689] There is a fast step involving the two polypeptides and a slow step confined to the variable region. In MPCH (immunoglobulin G_{2b}) and MOPC (immunoglobulin G_1) cells, the initial step in the formation of intermolecular disulphide bonds between heavy and light, or heavy and heavy, chains occurs to a substantial extent on nascent heavy chains as well as on the completed chains.[690] The nascent heavy chains must have molecular weights of approximately 3.8×10^4 before light chains become covalently bound. Some immunoglobulin molecules have neuraminic acid-containing oligosaccharides linked to variable segments of the light chains in addition to their Fc fragments.[691] These Fab oligosaccharides are potential determinants of antibody specificity for the formation of immunoglobulin G–immunoglobulin G complexes.

The lack of binding of the pFc′ fragment (corresponding to the C_H3 domain of immunoglobulin G) or of the Facb derivative (which is derived from C_H3) of rabbit immunoglobulin to staphylococcal protein A, suggests that the locus of protein A binding is at the interface between the C_H2 and C_H3 domains.[692]

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[687] P. R. Anderas, D. Paul, J. T. Tomita, and B. Anderson, *Mol. Immunol.*, 1979, **16**, 341.
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The interpretation of ^{13}C n.m.r. spectroscopic results agrees with *X*-ray data in considering that the carbohydrate moiety of human immunoglobulin G is as immobile as the protein itself.[693] In contrast the results of spin-labelling of the carbohydrate moiety imply mobile carbohydrate in the Fc fragment. The carbohydrate content of immunoglobulin G, the apoprotein of low density lipoprotein, and normal sera from mucolipidosis II and III patients show no differences in the relative ratios and total amounts of D-mannose, D-galactose, neuraminic acid, and 2-acetamido-2-deoxy-D-glucose.[694] If the postulated post-translational defect in these disorders involves changes in the carbohydrate composition, it is not a general defect with glycosylation of these glycoproteins, but may be specific for the lysosomal hydrolases.

In addition to their Fc oligosaccharides, some immunoglobulin molecules have oligosaccharides linked to variable segments of heavy or light chains, making the Fab fragments potential determinants of antibody specificity. Examination of the immunoglobulin anti-globulin antibody from a patient with immunoglobulin G–immunoglobulin G complexes showed that a neuraminic acid-containing oligosaccharide on the light chain of the anti-globulin antibody is required for the binding action.[695] Horse-radish peroxidase has been coupled to immunoglobulin G with retention of high enzymatic and immunological activity.[696]

Immunoglobulins G from antisera specific for human chorionic gonadotrophin, carcinoembryonic antigen, α-foetoprotein, and casein have been isolated by affinity chromatography on columns of immobilized staphylococcal protein A, before being themselves immobilized on supports.[697] The resulting coupled antibodies provide a greater sensitivity and convenience in radioimmunoassay studies than the conventional double antibody-precipitation methods.

A two-point kinetic turbidimetric technique using commercially available monospecific antisera has been used for the determination of immunoglobulins G, A, and M in sera.[698] The accuracy of the method when measuring idiotypic monoclonal proteins is greater than that of the radial diffusion method.

Polystyrene particles coated with immunoglobulin G are agglutinated by guinea-pig and human skin collagen.[699] The interaction appears to be specific for the Fc region of the immunoglobulin and also for native collagen, which is unexpected since other workers have suggested that the *C*-terminal regions of the $C1_q$ sub-units of complement and not the collagen-like segments react with immunoglobulins.

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[695] A. J. Hymes, G. L. Mullinax, and F. Mullinax, *J. Biol. Chem.*, 1979, **254**, 3148.
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The murine anti-(insulin myeloma immunoglobulins A47N and E109) antibodies have an affinity for the D-fructofuranosyl residues of inulin, with the D-glucosyl residues contributing little if at all to the binding of the whole polysaccharide to the immunoglobulins.[700]

The specific binding of [^{125}I]-protein A to the Fc region of rabbit immunoglobulin G anti-immunoglobulin E, has been used in the determination of specific anti-immunoglobulin E antibody bound to immobilized immunoglobulin E.[701]

A synthetic immunogen, poly-(L-tyrosine-L-glutamic acid)-poly-DL-alanine-poly-L-lysine has been used to raise immunoglobulin M in rabbits and cattle.[702] The immobilized antigen has then been used in the purification of the immunoglobulin.

Immobilized concanavalin A and immobilized *Ricinus communis* lectin have been used in the separation of Fc from Fab fragments of a myeloma immunoglobulin M.[703] The Fc fragment binds to both lectins, but the Fab fragment only interacts with concanavalin A.

N-Glycosidically-linked oligosaccharides having a high proportion of D-mannosyl residues have been identified in two glycopeptides (I and II) isolated from a human immunoglobulin M myeloma protein.[704] The basic structure (39) of the oligosaccharide has been established. The larger oligosaccharides present have additional α-D-mannosyl groups linked (1→2) to terminal D-mannosyl residues. Glycopeptide I contains D-*manno*-pentaosyl and D-*manno*-hexaosyl species, whereas glycopeptide II contains D-*manno*-hexaosyl and D-*manno*-octaosyl species. Glycopeptide I also contains four minor oligosaccharides which are all related to the basic structure (39).[705]

In contrast to human membrane-derived immunoglobulins M and D, those from murine lymphocyte membrane display different affinities for lentil lectin.[706]

Some properties of a cell-bound homogeneous murine immunoglobulin M anti-dextran have been reported.[707] Immunoglobulin antibodies invoked by *Mycoplasma pneumoniae* are cross-reactive with native erythrocyte antigens, which share determinants with *M. pneumoniae*.[708]

α-D-Man*p*-(1→6)-α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→4)-D-Glc*p*NAc-L-Asn

3 3

↑ ↑

1 1

α-D-Man*p* α-D-Man*p*

(39)

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14 Erythrocyte Glycoproteins

Residues of neuraminic acid bearing *O*-acetyl groups have been detected in glycoproteins from murine and rat erythrocyte ghosts, but not in similar preparations from human, rabbit, or guinea-pig erythrocytes.[709] A modified periodate–Schiff reagent has been developed for the removal of these *O*-acetyl groups. Two sialoglycoproteins, glycophorin B and C, have been isolated from erythrocyte membranes by extraction with lithium di-iodosalicylate.[710] Glycophorin B is found in two forms, corresponding to monomer and dimer, on electrophoresis of normal erythrocyte membranes. The amino-acid sequence of thirty-six residues of the *C*-terminal segment and nineteen residues of an internal glycosylated segment of porcine glycophorin A have been shown to differ from the corresponding human glycoprotein.[711]

The behaviour of glycophorin in aqueous solution and some of its structural characteristics have been studied by ^{1}H n.m.r. spectroscopy.[712] Carbohydrate chains were found to be conformationally mobile and no evidence was found for tight carbohydrate–protein interactions. Despite its extensive glycosylation (60%), the carbohydrate moieties of glycophorin A contribute little to the c.d. spectrum.[713] The native glycoprotein contains 27% helix, 10% β-form, and 63% unordered form. This transmembrane glycoprotein has three distinct chemical domains; the heavily glycosylated *N*-terminal portion located externally, a central portion thought to span the lipid bilayer, and a *C*-terminal portion consisting of a heavily charged, L-proline-rich segment that extends into the cytoplasm.

The human leukaemic cell line K562 synthesizes a surface membrane which is identical to or closely similar to glycophorin A.[714] Anti-glycophorin A antiserum precipitated the glycoprotein from both surface-labelled and metabolically-labelled K562 cells. The biosynthesis of glycophorin A by this cell line resembles that described for viral membrane glycoproteins.[715]

X-Ray diffraction studies on sheep erythrocyte ghost membranes, which are prepared by agglutination of the ghosts with phytohaemagglutinin M, have revealed side-to-side packed transmembrane α-helices.[716]

The content of 2-amino-2-deoxy-D-galactose in human erythrocyte membranes of the A, B, and AB blood groups is higher than that of the O group.[717] Similarly significant differences in the amounts of D-galactose in the membranes derived from different pairs (A/O, B/O, AB/O, A/B, A/AB) of blood groups were observed. Basic differences in the core oligosaccharide chains may exist between the different erythrocyte membranes.

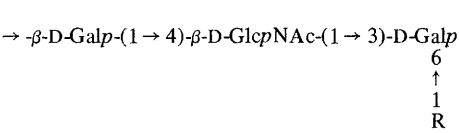
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[715] M. Jokinen, C. G. Gahmberg, and L. C. Andersson, *Nature (London)*, 1979, **279**, 604.
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[717] D. Bladier, G. Perret, J. Baudelot, P. Cornillot, and F. Gourbeault, *Z. Phys. Chem.*, 1979, **360**, 501.

An abnormal blood group S-s active sialoglycoprotein has been detected in the membrane of Miltenberger class III, IV, and V human erythrocytes.[718] Interactions among the major sialoglycoproteins and peripheral proteins of human erythrocytes have been studied by fluorescence energy transfer.[719] Divalent cations are necessary for the direct association between membrane sialoglycoproteins and spectrin.

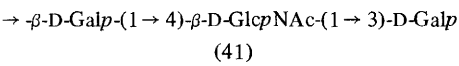
Vesicles, enriched in glycophorin, are obtained by selective extraction of human erythrocyte membranes with a non-ionic detergent.[720]

Glycoproteins of the human erythrocyte membrane, including glycophorin A and 'band 4.5' have been identified as two major classes of methyl-acceptor polypeptides for the enzyme, protein methylase II (*S*-adenosylmethionine: protein carboxy *O*-methyl transferase, EC 2.1.1.24).[721]

The structure of band 3 glycopeptide, prepared from erythrocytes of normal adult (blood group OI) contains the branched repeating unit (40), whereas the corresponding glycopeptide from an i adult variant who fails to develop I antigen contains a linear repeating unit (41).[722] The lack of a branching enzyme in the i individual may give rise to the differences in the structures. Under appropriate conditions, band-3 glycoprotein can be crosslinked with Cu^{2+}-2-phenanthroline primarily to give a dimer with the resultant loss of only one reactive thiol group per band-3 monomer.[723] The formation of one disulphide bond precludes the formation of others, suggesting that a conformational change occurs in the glycoprotein on cross-linking.



(40) R = glycosyl residue



(41)

Changes in the cell-surface carbohydrates of erythrocytes during their life span include changes in the neuraminic acid, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and D-galactose contents of the membranes.[724] Extensive heterogeneity exists in the cell-surface carbohydrates of the circulating

[718] D. J. Anstee, W. J. Mawby, and M. J. A. Tanner, *Biochem. J.*, 1979, **183**, 193.
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[724] A. Baxter and J. G. Beeley, *Biochem. Biophys. Res. Commun.*, 1978, **83**, 466.

population of erythrocytes. Several surface components of human erythrocytes are sensitive to hydrolysis by *endo*-β-D-galactanase.[725]

The removal of residues of neuraminic acid from the glycoproteins of human erythrocytes decreases the degree of solubilization of membrane glycoproteins by non-ionic detergents.[726] Solubility of these glycoproteins with these detergents may be achieved by addition of borate, implying that a charge on the membrane protein is an important consideration.

Human erythrocytes having blood group I antigen have been surface-labelled with ^{125}I, and with ^{3}H by treatment with D-galactose oxidase–sodium borotritide.[727] Using human serum containing monoclonal anti-blood group I antibody, the immunoprecipitates having predominant ^{125}I-label were in band-3 protein (mol. wt. 0.9×10^5–1.0×10^5), whereas the tritium label was in a diffusely migrating component (mol. wt. 4.0×10^4–7.0×10^4) on electrophoresis. The tritium-labelled component has a similar mobility to a sub-population of erythrocyte poly(glycosyl) ceramides with blood group I activity. Human erythrocyte membranes have been labelled with a photosensitive hydrophobic probe, 1-azido-4-iodo-[^{3}H]benzene.[728] Characterization of purified tryptic and chymotryptic peptides show that the probe is covalently attached only to the transmembrane region of the glycoprotein.

The aggregation of bovine erythrocytes by concanavalin A is highly sensitive to the level of functionally identical concanavalin A receptors.[729] Threshold densities of these receptors are required for aggregation to occur.

Glycoproteins isolated from the erythrocytes of rhesus monkeys inhibit haemagglutination by measles virus.[730]

15 Salivary and Mucous Glycoproteins

The structure and biosynthesis of mucins have been reviewed.[731]

Mucin glycoproteins and protein components of human-lung musosal gel have been resolved.[732] In contrast to meconium, the purified lung mucin glycoproteins are capable of aggregation and gel formation without either addition of albumin or of the lower molecular weight proteins of lung mucosal gel.

Purified human asthmatic bronchial mucin has a minimum molecular weight of 1.8×10^6 with aggregates of 1×10^7 or greater.[733] Oligosaccharide chains are linked *N*-glycosidically *via* 2-acetamido-2-deoxy-D-galactosyl residues to L-serine or L-threonine residues, which account for approximately one of every five amino-acids in the protein.

[725] T. J. Mueller, Y. T. Li, and M. Morrison, *J. Biol. Chem.*, 1979, **254**, 8103.
[726] R. S. Pratt and G. M. W. Cook, *Biochem. J.*, 1979, **179**, 299.
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[733] P. A. Feldhoff, V. P. Bhavanandan, and E. A. Davidson, *Biochemistry*, 1979, **18**, 2430.

Human bronchial mucous glycoproteins have been fractionated by ion-exchange and gel permeation chromatography.[734] The glycoproteins (mol. wt. 4.0×10^5) have an extended rod-like polypeptide conformation with about one amino-acid residue in three bearing a glycosidic side-chain attached through an L-serine- or L-threonine linkage.

The major component of goblet cell mucin in human intestinal scrapings contains a polydisperse glycoprotein component containing residues of D-galactose, L-fucose, 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, and neuraminic acid.[735] A minor monodisperse glycoprotein component contains the same sugar residues with the exception of neuraminic acid.

The extraparotid saliva of Macaque monkeys contains a glycoprotein (mol. wt. 9.6×10^3) capable of inhibition of the precipitation of calcium phosphate.[736] The major glycoproteins obtained from Bonnet monkey cervical mucous of the peri-ovulatory and of the pre-menstrual phases differ mainly in the point of linkage of the terminal neuraminic acid residues and of the substitution of the 2-acetamido-2-deoxy-D-galactosyl residues linked to the protein chain.[737] Differences in the levels of heterogeneity and in N-terminal amino-acid residues were also detected in the two groups of glycoproteins.

On the basis of the structural identity of the newly formed oligosaccharide unit arising from the *in vitro* D-galactosylation of desialylized ovine submaxillary mucin, the porcine submaxillary gland galactosyltransferase has been defined as a UDP-D-galactose:α-D-2-acetamido-2-deoxygalactosyl-protein 3-β-D-galactosyl transferase.[738]

Sulphated glycoproteins from porcine fundic mucosa inhibit pepsin activity by becoming bound to proteins.[739] The physiological role of these sulphated glycoproteins is speculated to be in the protection of the components of the gastric gland from peptic digestion.

Canine tracheal pouch secretions contain glycoproteins (mol. wt. $\geqslant 3.0 \times 10^6$), which dissociate into three sub-units on treatment with reducing agents.[740] Sub-fractions containing 87% carbohydrate, high levels of L-serine and L-threonine, but variable amounts of 2-acetamido-2-deoxy-D-glucose may then be isolated by ion-exchange chromatography. Similarities were observed between the properties of the canine tracheal pouch preparations and tracheobronchial secretions from human patients.

[734] G. Lamblin, N. Lhermitte, P. Degand, P. Roussel, and H. S. Slayter, *Biochimie*, 1979, **61**, 23.
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[738] D. H. Van Den Eijnden, R. A. Barneveld, and W. E. C. M. Schiphorst, *Eur. J. Biochem.*, 1979, **95**, 629.
[739] Y. Mikuni-Takagaki and K. Hotta, *Biochim. Biophys. Acta*, 1979, **584**, 288.
[740] T. H. Liao, O. O. Blumenfeld, and S. S. Park, *Biochim. Biophys. Acta*, 1979, **577**, 442.

The presence of hydrophobic sites in fetuin, ovine submaxillary mucin, and two canine trachael mucins has been established by a fluorescence probe technique which involves the interaction between the glycoproteins and sodium mansate (*N*-methyl-2-anilinonaphthalene-7-sulphonate).[741] In each case the interaction is accompanied by an enhancement in fluorescence and a shift in the fluorescence maximum to a shorter wavelength. A tracheal mucin (mol. wt. 5.8×10^5) containing 80% carbohydrate exhibited the highest fluorescence enhancement of all the glycoproteins and contained the largest number of binding sites for these fluorescent probes.

A mucin isolated from rat tracheal transplants contained protein (16.5%) and had D-galactose, 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, and neuraminic acid as the principal sugars.[742] The presence of *O*-glycosidic linkages was established by β-elimination in alkaline borohydride solution.

Cleavage of the *O*-glycosidic chains of armadillo submandibular mucin releases 30% disaccharide (42) and 75% monosaccharide (43) linked to L-serine or L-threonine.[743] Although similar oligosaccharide chains are found in the salivary glycoproteins of other species, those glycoproteins also contain a considerable proportion of oligosaccharides of larger size.

α-Neu*p*5Ac-(2 → 6)-α-D-Gal*p*NAc-(1 → L-Ser or L-Thr

(42)

α-D-Gal*p*NAc-(1 → L-Ser or L-Thr

(43)

16 Urinary Glycoproteins

Gel filtration has been used in conjunction with specific automated analyses for the separation of high and low molecular weight urinary material with a new potential for diagnosis based on the elution profiles obtained.[744]

An increased urinary excretion of the D-glucosyl tetrasaccharide (44) has been detected in patients with Duchenne muscular dystrophy.[745] This oligosaccharide was originally reported in the urine of a patient with glycogen storage disease type II (Pompe's disease).

A glycoprotein composed of sub-units (mol. wts. 1.5×10^4) and exhibiting marked gastric antisecretory activity has been isolated from human urine.[746]

[741] G. P. Sachdev, J. M. Zodrow, and R. Carubelli, *Biochim. Biophys. Acta*, 1979, **580**, 85.
[742] J. N. Clark and A. C. Marchok, *Biochim. Biophys. Acta*, 1979, **588**, 357.
[743] A. M. Wu, A. Slomiany, A. Herp, and B. L. Slomiany, *Biochim. Biophys. Acta*, 1979, **578**, 297.
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[745] A. Lundblad, S. Svensson, I. Yamashina, and M. Ohta, *FEBS Lett.*, 1979, **97**, 249.
[746] G. Carrea, G. Lugaro, R. Niada, P. Vecchini, and E. Antonini, *FEBS Lett.*, 1979, **104**, 393.

High yields of this inhibitor have been recovered by sub-unit exchange chromatography, in which protein sub-units are immobilized on a solid matrix and interact with sub-units in solution.

An increased urinary excretion of free *N*-acetylneuraminic acid has been detected in patients with Salla disease, which is a newly reported type of lysosomal storage disorder.[747] Patients appear to lack an *N*-acetylneuraminyl lyase or some lysosomal enzyme responsible for the degradation of *N*-acetylneuraminic acid.

The urine of a fucosidosis patient contained large amounts of L-fucosyl glycopeptides.[748] By use of a combination of gel chromatography, methylation analysis, and digestion with (*exo*)-glycoside hydrolases, the structures [(45)–(49] have been established for these glycopeptides. The related L-fucosyl glycopeptide analogue (50) has also been characterized from the urine of a patient with fucosidosis.[749]

The structures of twenty-two urinary glycopeptides from a fucosidosis patient have been established.[750] All contain one L-fucosyl residue at either O-3 or O-6 of the 2-acetamido-2-deoxy-D-glucosyl residue linked to L-asparagine. The accumulation of glycopeptides in the urine may be caused by the inability of human *endo*-β-D-2-acetamido-2-deoxy-glucanase to cleave the L-asparagine-linked sugar chains that bear an L-fucosyl residue on the innermost 2-acetamido-2-deoxy-D-glucosyl residue.

Although homogeneous on gel filtration, human urinary β_2-microglobulin can be separated into three zones on gel electrophoresis.[751] The partial sequences of amino-acids in rabbit and rat urinary β_2-microglobulins have been reported.[752]

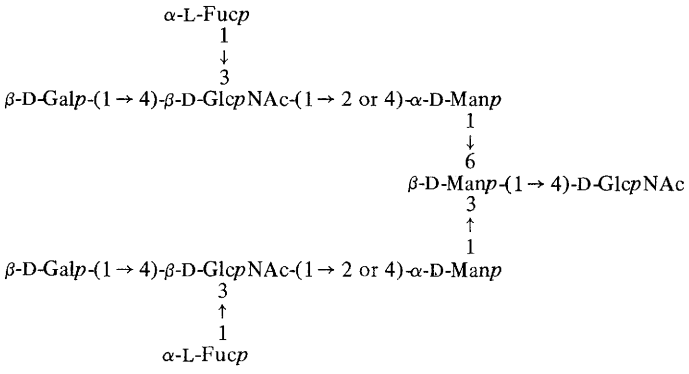
Tamm–Horsfall glycoprotein has been isolated from calf urine.[753] Mild acid hydrolysis cleaved the glycoprotein into two lower molecular weight fragments (mol. wts. 6.6×10^4 and 5.1×10^4).

By the theoretical addition of the structural sequences [(51) and (52)] to the oligosaccharides isolated from human urine from mannosidosis, fucosidosis, or sialidosis patients, numerous structures of L-asparaginyl glycans can be proposed.[754] It is postulated that these structures, which have not yet been characterized, pre-exist in glycans of human glycoproteins, probably in the cytoplasm and/or the cell membrane, and that they are the products of the action of *endo*-β-D-2-acetamido-2-deoxy-glucanases and accumulate in the cells and then in the urine.

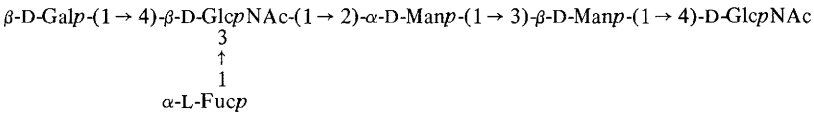
[747] M. Renlund, M. A. Chester, A. Lundblad, P. Aula, K. O. Raivio, S. Autio, and S. L. Koskela, *Eur. J. Biochem.*, 1979, **101**, 245.
[748] M. Nishigaki, K. Yamashita, I. Matsuda, S. Arashima, and A. Kobata, *J. Biochem. (Jpn.)*, 1978, **84**, 823.
[749] N. M. K. Ng Ying Kin and L. S. Wolfe, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 696.
[750] K. Yamashita, Y. Tachibana, S. Takada, I. Matsuda, S. Arishima, and A. Kobata, *J. Biol. Chem.*, 1979, **254**, 4820.
[751] N. Tanahashi, Y. Watanabe, and F. Yamada, *Agric. Biol. Chem (Jpn.)*, 1979, **43**, 1707.
[752] M. D. Poulik and O. Smithies, *Mol. Immunol.*, 1979, **16**, 731.
[753] W. Van Dijk, A. M. Lasthuis, and W. Ferwerda, *Biochim. Biophys. Acta*, 1979, **584**, 121.
[754] G. Strecker and J. Montreuil, *C.R. Hebd. Seances Acad. Sci., Ser. D*, 1979, **287**, 887.

α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 4)-α-D-Glc*p*-(1 → 4)-D-Glc

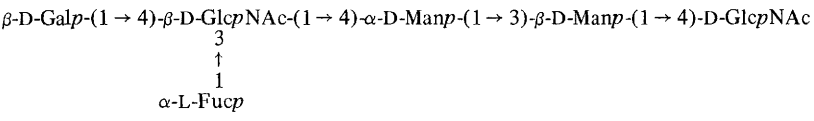
(44)



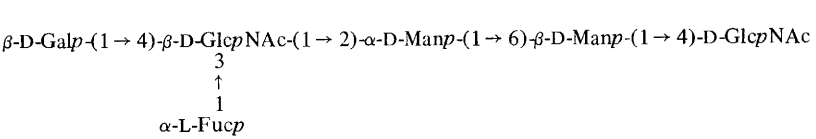
(45)



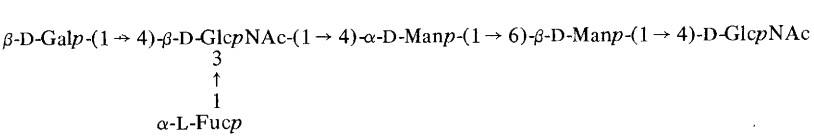
(46)



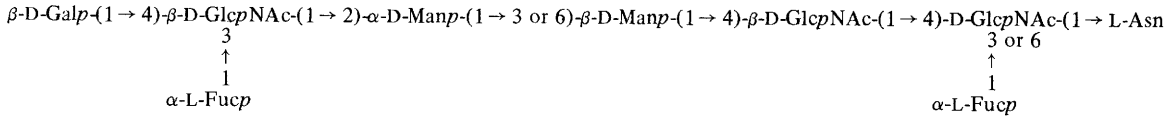
(47)



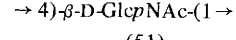
(48)



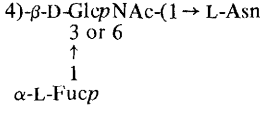
(49)


β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 2)-α-D-Manp-(1 → 3 or 6)-β-D-Manp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-D-GlcpNAc-(1 → L-Asn
3
↑
1
α-L-Fucp
3 or 6
↑
1
α-L-Fucp

(50)


→ 4)-β-D-GlcpNAc-(1 → Asn

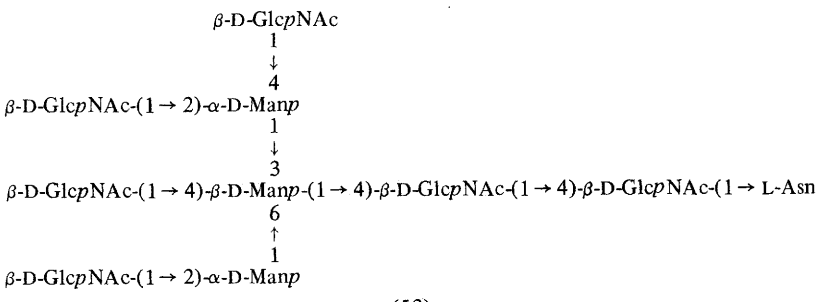
(51)


→ 4)-β-D-GlcpNAc-(1 → L-Asn
3 or 6
↑
1
α-L-Fucp

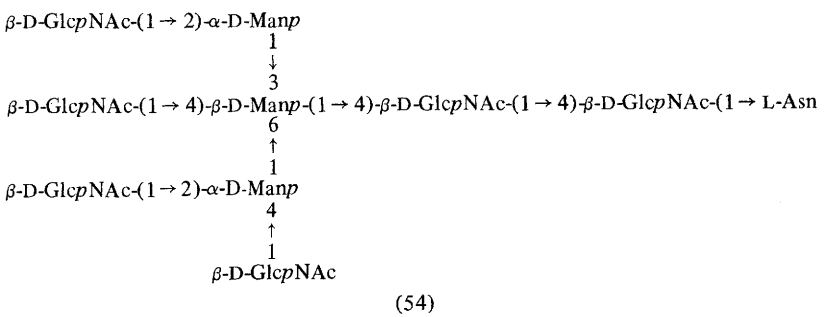
(52)

17 Avian Glycoproteins

Interactions of an ovalbumin glycopeptide with concanavalin A have been measured using solvent proton relaxation rates over a wide range of magnetic fields.[755] The binding of the glycopeptide to the lectin reduces the solvent proton relaxation rates in the same manner, but with a lower magnitude, as simple saccharides such as methyl α-D-glucopyranoside. Data from a Raman difference spectroscopic investigation have led to the suggestion that the conversion of ovalbumin into the heat-stable form S-ovalbumin involves a conformational change in a small part (3–4%) of the protein from α-helix to antiparallel β-sheet geometry.[756] This small difference in the three dimensional arrangement of the peptide chain may contribute to the large difference in the thermodynamic stabilities of the two forms.



(53)



(54)

Two structures [(53) and (54)] are proposed for the unique glycan of hen egg-white ovotransferrin.[757] A comparative study of this glycan with those of human serotransferrin and lactotransferrin reveals profound differences that could form the basis for the specificity of recognition of target cells by these glycoproteins.

[755] C. F. Brewer, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 117.
[756] S. Kint and Y. Tomimatsu, *Biopolymers*, 1979, **18**, 1073.
[757] G. Spik, B. Fournet, and J. Montreuil, *C.R. Hebd. Seances Acad. Sci., Ser. D.*, 1979, **288**, 967.

In addition, the structures of the four avian glycoproteins of egg white (ovotransferrin, ovalbumin, ovomucoid, and ovomucin) are distinct even though they are all synthesized by the oviduct. It is assumed that each of them is formed in specialized cells containing different glycosyltransferases. The protein moiety may also exert a control over the activity of the glycosyltransferases through the mechanism of polypeptide signals specifically recognized by each of these enzymes.

18 Miscellaneous Glycoproteins and Chitin

A heterogeneous polysaccharide, containing residues of D-galactose, D-mannose, L-fucose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-galactose, which activates the classical complement pathway has been isolated from tropical ant (*Pseudomyrmex* species) venom.[758] A low molecular weight fraction (3×10^3), which may be a cleavage product of a larger polysaccharide, was isolated by gel chromatography.

A glycoprotein (mol. wt. 4.5×10^4) composed of carbohydrate (36%) and protein (53%) has been isolated from the articular lubricating fraction of bovine synovial fluid.[759] It is likely that this glycoprotein is synthesized by the joint tissues and is not derived from serum.

Crab chitin, and chitin from the diatom *Thalassiorira fluviatilis* dissolves in hexafluoro-2-propanol; far-u.v. and c.d. spectra of solutions, gels, and films of the chitin have been recorded.[760] A trans-amide conformation and intermolecular hydrogen bonding are proposed as the important determinants of the observed solid state c.d. spectra.

Chitins and chitosans from a variety of sources have simple e.s.r. spectra, provided they have been isolated and purified under controlled conditions to avoid excessive oxidative oxidation.[761] The primary product of the thermal degradation of chitin, 2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose, has been detected and isolated on a preparative scale.[762]

Chitin has been treated with isocyanate or acid chloride derivatives of pesticides to produce controlled-release polymeric pesticide systems.[763]

The action of lysozyme on partially deacetylated chitin and chitin oligosaccharides has been studied.[764] Chitin was deacetylated to an extent of about 70% of its 2-acetamido-2-deoxy-D-glucosyl residues, and lysozyme digests of the deacetylated chitin were fractionated by gel chromatography, paper chromatography, and paper electrophoresis.

[758] D. R. Schultz, P. I. Arnold, M. C. Wu, T. M. Lo, J. E. Volanakis, and M. Loos, *Mol. Immunol.*, 1979, **16**, 253.
[759] D. A. Swann and G. Mintz, *Biochem. J.*, 1979, **179**, 465.
[760] L. A. Buffington and E. S. Stevens, *J. Am. Chem. Soc.*, 1979, **101**, 5159.
[761] R. A. A. Muzzarelli, F. Tanfani, G. Scarpini, and M. G. Mozarelli, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 706.
[762] P. Koll and J. Metzger, *Agnew. Chem., Int. Ed. Engl.*, 1978, **17**, 754.
[763] C. L. McCormick and D. K. Lichatowich, *J. Polym. Sci. Polym. Lett.*, 1979, **17**, 479.
[764] K. I. Amano and E. Ito, *Eur. J. Biochem.*, 1978, **85**, 97.

19 Analysis of Glycoproteins

Wheat-germ agglutinin has been immobilized in the presence of chito-oligosaccharides to give an affinity support for the purification of glycoproteins.[765] Although the resulting immobilized lectin has a high capacity for glycoproteins, possible hydrophobic interactions as well as non-specific adsorption effects may limit the yield and degree of purification achieved with this support.

Glycoproteins, after electrophoresis in polyacrylamide or agarose gels, have been visualized after incubation with fluorescein-labelled concanavalin A.[766] The method has been used to detect less than 100 ng of hexose bound to fibrinogen. Glycoproteins have been detected in polyacrylamide gels using sequential treatment which includes treatment with thymol and staining with alcoholic solutions of H_2SO_4.[767] Colours are produced in proportion to the carbohydrate content of the glycoprotein.

A laser light scattering technique has been used for the measurement of the concentration of glycoproteins when complexed with lectins.[768] A high degree of sensitivity has allowed less than 25 pmol glycoprotein or 4.5 pmol lectin to be detected.

Methods have been reported for the ultrastructural localization of complex carbohydrates, including glycoproteins, in rat macrophages and monocytes, by employing the reactivity of vicinal glycols to periodate ions and the presence of carboxylate and sulphate ester groups.[769]

The neutral sugar components in human serum glycoproteins have been identified and determined by hydrolysis of the glycoprotein with ion-exchange resin followed by g.l.c. as the alditol acetates.[770] Hydrolysis conditions were optimized for cleavage of glycosidic bonds with only minimal destruction of L-fucose, D-mannose, and D-galactose. Tritium-labelled alditol acetates, derived from monosaccharides released by acid hydrolysis of glycoproteins and lipopolysaccharides, have been subjected to g.l.c. analysis.[771] By trapping the gas effluent on silicone-coated glass-beads and determining the level of radioactivity, 0.2 nmol of monosaccharide have been detected with an accuracy of ±10–15%.

After methanolysis of glycoproteins and *O*-trimethylsilylation of the monosaccharides, re-*N*-acetylation of amino-sugars has been performed directly on gas liquid chromatographic columns, by injecting acetic anhydride together with the sample.[772] Quantitative *N*-acetylation occurs whilst the *O*-trimethylsilyl ethers remain intact.

By using a two column system on a commercially available analyser, amino-acids and the α- and β-anomers of 2-amino-2-deoxy-D-glucose and 2-amino-2-

[765] G. Mintz and L. Glaser, *Anal. Biochem.*, 1979, **97**, 423.
[766] M. Furlan, B. A. Perret, and E. A. Beck, *Anal. Biochem.*, 1979, **96**, 208.
[767] D. Racusen, *Anal. Biochem.*, 1979, **99**, 474.
[768] E. Kottigen, C. Bauer, and W. Gerok, *Anal. Biochem.*, 1979, **96**, 391.
[769] P. L. Sannes, M. Eguchi, and S. S. Spicer, *J. Histochem. Cytochem.*, 1979, **27**, 1180.
[770] C. W. Gehrke, T. P. Waalkes, E. Borek, W. F. Swartz, T. F. Cole, E. C. Kuo, M. Abeloff, D. S. Ettinger, N. Rosenshein, and R. C. Young, *J. Chromatogr.*, 1979, **162**, 507.
[771] P. Prehm and A. Scheid, *J. Chromatogr.*, 1978, **166**, 461.
[772] A. A. Akhrem, G. V. Avvakumov, D. V. Sviridov, and O. A. Strelchyonok, *J. Chromatogr.*, 1978, **166**, 123.

deoxy-D-galactose have been separated in the 100–1000 pmol range.[773] The separation of the anomeric forms of the amino-sugars is critically dependent on pH. In a method used for glycoprotein analysis, amino-sugars and several L-cysteine derivatives have been resolved on a single column amino-acid analyser.[774]

Monosaccharides, derived from polysaccharides, glycolipids, and glycoproteins, have been converted into the corresponding 1-amino-1-deoxy-glycitols by treatment with ammonia and sodium cyanoborohydride, prior to determination using an amino-acid analyser.[775]

The content of L-fucose and D-mannose in glycoproteins has been determined by h.p.l.c. of the perbenzoyl-L-[^{3}H]fucitol and D-[^{3}H]mannitol derivatives.[776] Since D-galactitol and D-glucitol cannot be separated from D-mannitol using this system, samples containing these polyols must be subjected to electrophoresis prior to h.p.l.c.

The direct quantitative comparison of sugars and amino-acids by h.p.l.c. has been reported.[777] Stepwise elution systems have been developed for the ion-exchange liquid chromatographic separation of these components.

A modified screening method using t.l.c. has been reported for the rapid diagnosis of glycoprotein storage disorders which are identifiable by their secretion of excess oligosaccharides.[778] The method has been used in the diagnosis of mannosidosis, G_{M_1}-gangliosidosis, mucopolysaccharidoses type I and type III, aspartylglycosaminuria, and fucosidosis.

Oligosaccharides derived from glycoproteins have been separated by t.l.c.[779] Apart from its diagnostic value in detecting glycoprotein-derived storage oligosaccharides in glycohydrolase deficiency diseases, the system may be used in the analysis of oligosaccharides released from glycoproteins or glycolipids by either chemical or enzymic methods.

Oligosaccharides partly degraded at the reducing end can be separated from low molecular weight peptide degradation products of glycoproteins which have been treated with mixtures of trifluoroacetic anhydride and trifluoroacetic acid.[780] Carbohydrate chains attached to the protein by *N*-glycosyl linkages are cleaved by transamidations of the N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl) hydrogen L-asparaginate-linkage and carbohydrate chains attached by *O*-glycosyl linkages are cleaved, probably by acid-catalysed elimination, from L-serine or L-threonine residues.

Radiolabelled glycopeptides have been analysed by polyacrylamide gel electrophoresis as their borate complexes.[781] Heterogeneity, which is not

[773] U. Del Valle and J. E. Shively, *Anal. Biochem.*, 1979, **96**, 77.
[774] M. Fauconnet and J. Rochemont, *Anal. Biochem.*, 1978, **91**, 403.
[775] S. Hara, H. Ikegami, A. Shono, T. Mega, T. Ikenaka, and Y. Matsushima, *Anal. Biochem.*, 1979, **97**, 166.
[776] P. F. Daniel, *J. Chromatogr.*, 1979, **176**, 260.
[777] M. M. Tikhomirov, A. Ya Khorlin, W. Voelter, and H. Bauer, *J. Chromatogr.*, 1978, **167**, 197.
[778] A. C. Sewell, *Clin. Chim. Acta*, 1979, **92**, 411.
[779] E. W. Holmes, and J. S. O'Brien, *Anal. Biochem.*, 1979, **93**, 167.
[780] B. Nilsson and S. Svensson, *Carbohydr. Res.*, 1979, **72**, 183.
[781] S. Weitzman, V. Scott, and K. Keegstra, *Anal. Biochem.*, 1979, **97**, 438.

apparent after gel filtration, is observed for glycopeptides derived from immunoglobulin G and Sindbis virus glycoprotein.

An analytical method, applicable to the assay of glycoprotein glycosyltransferases, has been developed.[782]

20 Biosynthesis of Glycoproteins

The thyroid contains a microsomal enzyme that can selectively release D-glucose from dolichyl-linked D-gluco-D-manno-oligosaccharides.[783] This D-glucosidase may carry out the first step in a series of processing reactions of the kind which have been proposed for vesicular stomatitis viral glycoprotein.[784] Since the thyroid D-glucosidase can act on lipid-linked as well as protein-bound oligosaccharides, it may have an additional physiological function involving the regulation of the amount of appropriate oligosaccharide-lipid donors available for the glycosylation of proteins. The transfer of the D-glucosyl oligosaccharide from its lipid carrier to endogeneous proteins *via* an L-asparagine linkage is carried out by a calf thyroid particulate enzyme classified as a dolichyl pyrophosphoryl oligosaccharide:protein oligosaccharide transferase.[785] Although the entire D-glucosyl oligosaccharide moiety is initially transferred to the protein, a selective loss of D-glucosyl residues for the newly biosynthesized glycoprotein occurs shortly afterwards owing to the D-glucosidase, which may represent the first of a series of processing enzymes.

In glycoprotein biosynthesis, two D-glucosidases from rat-liver microsomes are responsible for the cleavage of D-glucosyl residues from the pre-formed oligosaccharides (55).[786] The transfer of D-glucose, from UDP-D-glucose to dolichyl D-glucopyranosyl phosphate, a glucosylated oligosaccharide lipid and at least one D-glucoprotein, catalysed by membranes of central nervous tissue has been reported.[787]

$$(\text{D-Glc}p)_n\text{-}(\text{D-Man}p)_9\text{-D-Glc}p\text{NAc}$$

(55) $n = 1, 2, \text{or } 3$

Amphomycin specifically inhibits the formation of dolichyl D-mannosyl phosphate in pig aorta microsomal preparations, but does not inhibit the further transfer of D-mannose from dolichyl D-mannosyl phosphate or from lipid-linked oligosaccharides.[788] However, some of the D-mannosyl residues in the lipid-linked oligosaccharides come directly from GDP-D-mannose without the involvement of dolichyl D-mannosyl phosphate.

The enzyme responsible for the transfer of oligosaccharide from lipid-linked intermediates to endogenous proteins of rat liver is a structural component of

[782] A. Baxter and J. P. Durham, *Anal. Biochem.*, 1979, **98**, 95.
[783] R. G. Spiro, M. J. Spiro, and V. D. Bhoyroo, *J. Biol. Chem.*, 1979, **254**, 7659.
[784] S. Kornfeld, E. Li, and I. Tabas, *J. Biol. Chem.*, 1978, **253**, 7771.
[785] M. J. Spiro, R. G. Spiro, and V. D. Bhoyroo, *J. Biol. Chem.*, 1979, **254**, 7668.
[786] L. S. Grinna and P. W. Robbins, *J. Biol. Chem.*, 1979, **254**, 8814.
[787] M. G. Scher, A. Jochen, and C. J. Waechter, *Biochemistry*, 1977, **16**, 5037.
[788] M. S. Kang, J. P. Spencer, and A. D. Elbein, *J. Biol. Chem.*, 1978, **253**, 8860.

the rough and smooth liver microsomes.[789] Rat-liver mitochondria contain the highest specific activity of glycosyltransferase activity for D-mannose, D-glucose, and 2-acetamido-2-deoxy-D-glucose 6-phosphate from nucleotide donors to dolichol monophosphate in vesicles derived from rough and smooth endoplasmic reticulum and mitochondria.[790] In the smooth vesicles, the D-mannosyltransferase and D-glucosyltransferases are located at both the inner and outer faces of the membrane, whereas in the rough vesicles and mitochondria the two enzymes occur only at the outer face.

The primary structural requirements for the enzymic formation of *N*-glycosidic bonds in glycoproteins have been investigated.[791] Much of the primary structure of the acceptor protein is not required for an oligosaccharide transferase to interact with the acceptor site. The tripeptide (56) is the minimal peptide active as an acceptor of oligosaccharide from oligosaccharide-lipid and only if both its *N*- and *C*-termini are blocked. The accessibility of this sequence to the oligosaccharide transferase is probably the predominant structural feature of a protein that determines whether or not it is glycosylated. Low molecular weight synthetic peptides, containing the sequence (57) and larger peptides, can be *O*-glycosylated with a crude preparation of UDP-2-acetamido-2-deoxy-D-galactose:mucin polypeptide 2-acetamido-2-deoxy-D-galactosyltransferase from porcine submaxillary glands and UDP-2-acetamido-2-deoxy-D-galactose.[792] The peptides have sequences similar to the region of L-threonine-98 of bovine basic myelin protein.

L-Asn-X-L-Ser/L-Thr
(56)

L-Thr-L-Pro-L-Pro-L-Pro
(57)

Cancer-associated D-galactosyltransferase (GT-II) and the normal isoenzyme (GT-I) have been purified from pooled effusions from patients with various cancers.[793] The purified cancer-associated enzyme appears to be structurally and kinetically distinct from the normal isoenzyme.

Optimal conditions for the determination of UDP-D-galactosyltransferase in the Golgi system of rat liver have been reported.[794] In contrast to other reports the enzyme is not stimulated by non-ionic detergents.

[789] V. Idoyaga-Vargas, M. Perelmuter, O. Burrone, and H. Carminatti, *Mol. Cell Biochem.*, 1979, **26**, 123.
[790] T. Coolbear, S. Mookerjea, and F. W. Hemming, *Biochem. J.*, 1979, **184**, 391.
[791] G. W. Hart, K. Brew, G. A. Grant, R. A. Bradshaw, and W. J. Lennarz, *J. Biol. Chem.*, 1979, **254**, 9747.
[792] J. D. Young, D. Tsuchiya, D. E. Sandlin, and M. J. Holroyde, *Biochemistry*, 1979, **18**, 4444.
[793] D. K. Podolsky and M. M. Weiser, *J. Biol. Chem.*, 1979, **254**, 3983.
[794] G. N. Anderson and L. C. Eriksson, *Biochim. Biophys. Acta*, 1979, **570**, 239.

The α-2- and α-3-L-fucosyltransferase activities of human cells and plasma are inhibited by *N*-ethylmaleimide, but activities are restored by treatment with dithiothreitol, indicating the necessity of a free thiol group for activity.[795]

CMP-*N*-Acetylneuraminate : D-galactosyl-glycoprotein *N*-acetylneuraminyltransferase (sialyltransferase) activity is released in large amounts by two hepatoma cell lines.[796] The enzyme has been resolved into two forms (mol. wts. 6.5×10^4 and 8.0×10^4) by gel chromatography. Sialyltransferase activity has been studied in renal cortex tissue using asialofetuin and Tamm–Horsfall glycoproteins as exogenous acceptors and rat asialo-glomerular basement membrane as an endogenous acceptor.[797] The activities of sialyltransferase and D-galactosyltransferase of rat-liver Golgi membranes are enhanced to different levels by binding to wheat-germ agglutinin.[798] The stimulation of the transferase activities is similar to that reported for concanavalin A. Differences in behaviour of the transferases support earlier conclusions that the two enzymes occupy distinct membrane environments.

The substrate requirements, linkage specificity, and kinetic mechanism of an α-2,3-sialyltransferase have been compared with an α-2,6-sialyltransferase from porcine submaxillary mucin.[799] The former enzyme will specifically modify glycoproteins and glycolipids which contain 2-acetamido-2-deoxy-3-*O*-β-D-galactosyl-D-galactose sequences. The only acceptor substrates reported for the α-2,6-sialyltransferase are those glycoproteins containing the structure (58).[800]

R-(1→3)-D-Gal*p*NAc-α-(1→L-Thr/Ser

(58) R = H or β-D-Gal*p*

[795] D. Kessel and T. H. Chou, *Biochem. J.*, 1979, **181**, 767.
[796] C. K. Liu, R. Schmied, and S. Waxman, *Clin. Chim. Acta*, 1979, **98**, 225.
[797] P. Bardos, M. Lacord-Bonneau, J. P. Muh, and J. Weill, *Int. J. Biochem.*, 1979, **10**, 289.
[798] M. M. Mitranic, J. M. Sturgess, and M. A. Moscarello, *Can. J. Biochem.*, 1979, **57**, 1008.
[799] J. I. Rearick, J. E. Sadler, J. C. Paulson, and R. L. Hill, *J. Biol. Chem.*, 1979, **254**, 4444.
[800] J. E. Sadler, J. I. Rearick, and R. L. Hill, *J. Biol. Chem.*, 1979, **254**, 5934.

6

Enzymes

BY J. F. KENNEDY

1 Introduction

General Aspects and Nomenclature. – Current developments in chemical and biochemical disciplines are so rapid and so numerous that keeping abreast of the current scientific literature is an almost full-time task. One subject showing most rapid growth is immobilization: a technique useful for a vast range of properties of molecules, and including the immobilization of enzymes, enzyme co-factors, antigens, antibodies, proteins, glycoproteins and (other) molecules exhibiting bio-affinity phenomena. Advances in this area are of considerable interest and practical importance to biochemists, to chemists, and to workers in numerous medically oriented laboratories in universities, polytechnics, research institutes, and industry. One of the features of *Enzyme and Microbial Technology*, a new multi-purpose journal which features reviews, original papers, international comment, industrial comment, patent reports, and conference reports is the provision of a literature survey which will provide rapid access to the literature on immobilization methods and phenomena as a whole, rather than to isolated parts such as enzyme immobilization.[1-4] The first four surveys, covering much of the work appearing in the literature during recent months, have been presented. For simplicity and clarity the information is arranged in three tables. The first concerns the immobilization of enzymes, the second the immobilization of proteins, glycoproteins, and polysaccharides and the third the immobilization of other molecules. The substance immobilized, the support material used, and the method of coupling adopted are outlined. Also included are brief comments about the applications of the immobile product as discussed in the original paper. Whereas the tables are indexed under the name of the molecule immobilized, searches for references on particular support matrices or applications may be conducted conveniently by running down the second and third columns respectively of the tables.

The recommendations (1978) of the Nomenclature Committee of the International Union of Biochemistry on the nomenclature of enzymes has been published.[5] This publication is a revision of the 1972 Recommendations of the International Union of Pure and Applied Chemistry and the International Union

[1] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Tech.*, 1979, **1**, 53.
[2] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Tech.*, 1979, **1**, 129.
[3] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Tech.*, 1979, **1**, 210.
[4] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Tech.*, 1979, **1**, 290.
[5] Enzyme Nomenclature: by The International Union of Biochemistry Nomenclature Committee, Academic Press, New York, 1979.

of Biochemistry (see Vol. 7, Part II, Chapter 6, p. 356), which was itself a revision of the 1964 Recommendations of the International Union of Biochemistry. Following a historical introduction, and a chapter on the classification and nomenclature of enzymes, there follows a comprehensive list of enzymes giving the recommended trivial name, the systematic name, the basis of classification, the reaction catalysed by the enzyme, the Enzyme Commission number and reference, and a comprehensive index to the list, which covers not only names in current use, but also disused and incorrect names. The list of enzymes contains 2122 entries, compared with 1770 entries in the 1972 edition, and includes some 100 entries for carbohydrate hydrolases and lyases.

A third edition of the well-known book 'Enzymes' by Dixon and Webb reflects the changes and developments which have occurred in the area of enzyme chemistry.[6] Enzyme techniques, isolation, kinetics, classification, specificity, mechanisms, inhibition and activation, co-factors, structure, biosynthesis, and biology are all covered. Most of the material has been rewritten but, despite the wealth of new material included, the book retains the general form of the previous editions.

Three further volumes of the well-known series 'Methods in Enzymology' deal with the following aspects of biomembranes: Biological Oxidations – Specialized techniques;[7] Bioenergetics – Oxidative phosphorylation;[8] and Bioenergetics – Biogenesis of mitochondria, organization, and transport.[9]

A two part compendium in the 'Monographs in Human Genetics' series deals with inborn errors of metabolism in man and covers carbohydrate, amino-acid, and lipid metabolism[10] and connective tissues and purine metabolism and endocrinology.[11]

Methods of Assay. – The Technicon basic autoanalyser sampler system has been modified for simultaneous sampling of glycoside hydrolases and substrate-buffer solutions.[12] This inexpensive modification allows performance of automated enzyme analysis and enzyme kinetic studies with minimal consumption of substrate and/or enzyme.

Properties. – A book entitled 'Fundamentals of Enzyme Kinetics' covers all of the enzyme kinetics likely to be required by a student, and provides sufficient additional material to serve as a useful introduction to the subject for research workers coming to biochemistry from other disciplines.[13] The practical aspects of the subject are emphasized and the author discusses methods of purifying

[6] 'Enzymes', 3rd edition, M. Dixon, E. C. Webb, C. J. R. Thorne, and K. F. Tipton, Longman, 1979.
[7] 'Methods in Enzymology', vol. 52, ed. S. Fleischer and L. Packer, Academic Press, New York and London, 1978.
[8] 'Methods in Enzymology', vol. 53, ed. S. Fleischer and L. Packer, Academic Press, New York and London, 1978.
[9] 'Methods in Enzymology', vol. 54, ed. S. Fleischer and L. Packer, Academic Press, New York and London, 1978.
[10] Monographs in Human Genetics, vol. 9, ed. O. Sperling and A. de Vries, 1977.
[11] Monographs in Human Genetics, vol. 10, ed. O. Sperling and A. de Vries, 1977.
[12] R. Sarber, A. Langnas, and G. W. Jourdain, *Anal. Biochem.*, 1978, **91**, 361.
[13] 'Fundamentals of Enzyme Kinetics', A. Cornish-Bowden, Butterworths, London, 1979.

and assaying enzymes and of planning and analysing enzyme kinetic experiments. Modern techniques for studying fast reactions are described and there are detailed explanations of all mathematical derivations. Problems at the end of each chapter will increase the usefulness of the book to the student.

Structures. – A further volume in the well-known series 'Methods in Enzymology' deals with enzyme structure and covers molecular weight determinations and related procedures, interactions, and conformations and transitions.[14]

Functions. – A survey of which enzymes are lacking in cases of genetic hyperglycosaminoglycanuria (mucopolysaccharidoses) has been made.[15] A novel mechanism has been demonstrated for the introduction of hydrolytic enzymes into the lysosomes of fibroblasts in cell culture by a secretion and capture process.

Applications. – An article[16] on Biopolymers makes the following statement 'Biopolymers – naturally occurring macromolecules – are a case apart from the other polymers. The old slogan, 'what man can do in chemistry has already been done by nature in a more elegant way' bears repetition. Man-made polymers are really attempts to simulate the properties of natural polymers. The great activity in the field of polymer synthesis can be summed up as 'attempts to generate materials (which possess the properties or functions of natural molecules) by short-cut routes that avoid the expense and low availability of natural materials, and circumvent the lengthy time necessary for their isolation.' Examples drawn upon include enzymes and their immobilization.

A book entitled 'Enzymic and Non-enzymic Catalysis' provides a coherent and timely account of the new propsects for more powerful and diverse catalysis which are developing at the interfaces between chemistry, enzymology, and engineering.[17] The contributing authors discuss *inter alia* the present knowledge of enzyme catalysis, the current status of enzyme technology, enzymes in synthetic organic chemistry, the design and development of enzyme analogues, micellar catalysis, factors influencing performance of enzyme and non-biological catalyst reactions, asymmetric homogeneous catalysis, and the polymer-attached homogeneous catalysts.

An edited series entitled 'Polysaccharides in Food' is the first comprehensive review of the science behind the technology of the use of polysaccharides in food.[18] Papers discussing starch in food processing, the use of polysaccharides in food product formulation, the structure of new polysaccharides, *etc.* also deal with the enzymes which act upon these polysaccharides.

[14] 'Methods in Enzymology', vol. 61, ed. C. H. Hirs and S. N. Timasheff, Academic Press, New York, 1979.
[15] F. Neuffeld, Trends in Biochemical Sciences, vol. 2, 1977.
[16] 'Chemistry in Britain', 1978, **14**, 436.
[17] 'Enzymic and Non-enzymic Catalysis', ed. P. Dunnill, A. Wiseman, and N. Blakebrough, Ellis Horwood Ltd., Chichester, 1979.
[18] 'Polysaccharides in Food', ed. J. M. V. Blanshard and J. R. Mitchell, Butterworths, Sevenoaks, Kent, 1979.

Enzyme replacement therapy of lysosomal storage diseases has been investigated in some detail and a recently published book deals with lysosomes in applied biology and therapeutics.[19]

One of the Tate and Lyle Carbohydrate Chemistry Award Lectures[20] entitled 'Transition-metal Oxide Chelates of Carbohydrate-directed Macromolecules' concludes that 'it may be stated that hydrous oxides of titanium and zirconium in various forms are effective matrices for immobilization. Their advantages include their low cost, their convenient preparation (which may be conducted in any location without specialized facilities), the absence of any need for pre-preparation, their ability to couple enzyme at neutral pH, the high retentions of specific activity of the enzyme on immobilization, and the ability of modification to exert microenvironmental effects on, and thereby alter the characteristics of, the immobilized enzyme. The success of the preparation and activity of the matrix is guaranteed and, unlike the majority of immobilization matrices, does not require use of spectroscopy, titration, *etc.* to confirm the reactivity of the preparation in hand. The form of the hydrous oxide may be modified with ease to give high or low density, magnetic or non-magnetic, products of a variety of sizes, shapes, and uses. Although much of the work conducted hitherto has employed (carbohydrate-directed) enzymes as models, on account of the additional, biological testing parameter afforded thereby, it is clear that the hydrous metal oxide matrices are equally suitable for the immobilization, removal, and recovery of antibodies, antigens, affinants, amino-acids, antibiotics, carbohydrates, cells, enzymes, glycoproteins, lectins, peptides, *etc.* This necessarily gives rise to a number of possible investigations of the properties of such compounds, *etc.* in the fields of pure, biological, and clinical chemistries.'

A review on polysaccharide chemistry includes a section on water-insoluble polysaccharide immobilization of enzymes.[21]

The use of polymers for the immobilization of enzymes and other biologically-active molecules has been discussed.[22] The advantages of polymeric support materials and rules for their selection according to the type of use have been discussed. A review of various types of polymers which can serve as support matrices has been given. They are, *e.g.*, polymeric carbohydrate derivatives, poly(allyl carbonate) and poly(allyl alcohol), polymers of acrylamidosalicylic acids, polyacrylamide derivatives, *etc.* Some examples of the use of immobilized enzymes and other biologically-active molecules were mentioned.

2 β-D-2-Acetamido-2-deoxygalactosidases, β-D-2-Acetamido-2-deoxyglucosidases, and β-D-2-Acetamido-2-deoxyhexosidases

Intravenously administered ^{125}I-labelled human β-D-2-acetamido-2-deoxyhexosidase A was rapidly cleared from the circulation of rats and accumulated in the

[19] J. P. Lloyd and P. A. Griffths, 'Lysosomes in Applied Biology and Therapeutics', vol. 6, North Holland Publishing Co., Amsterdam, 1979, p. 517.
[20] J. F. Kennedy, *Chem. Soc. Rev.*, 1979, **8**, 221.
[21] J. F. Kennedy and C. A. White, in 'Comprehensive Organic Chemistry', Pergamon, Oxford, 1979, p. 755.
[22] C. A. White and J. F. Kennedy, *Polimery*, 1980, **25**, 1.

liver, particularly in the sinusoidal cells but also in the hepatocytes.[23] Clearance was inhibited by the simultaneous infusion of D-mannan but not by a D-galactose-terminated glycoprotein. The kinetics of uptake of β-D-2-acetamido-2-deoxyhexosidase A and D-mannose-terminated orosomucoid by sinusoidal cells were shown to be similar. These findings suggested that the hepatic uptake of the lysosomal enzyme is mediated by a receptor on sinusoidal cells which recognizes and binds D-mannose-terminated glycoproteins.

β-D-2-Acetamido-2-deoxyhexosidase has been purified from brain and liver of a patient of type O G_{M_2} gangliosidosis (Sandhoff's disease).[24] Brain β-D-2-acetamido-2-deoxyhexosidase S was further purified by preparative polyacrylamide gel electrophoresis. The pH optimum of the purified liver and brain enzyme was 5.0 and K_m values were 0.8–0.9 mM and 0.3–0.4 mM with 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-galacto- and -gluco-pyranosides respectively. β-D-Acetamido-2-deoxyhexosidase S was thermolabile, losing most of its activity after 50 min at 50 °C. The apparent molecular weights of the purified liver and brain were 1.54×10^5 and 1.52×10^5, respectively. 2-Amino-deoxyhexoses activated β-D-2-acetamido-2-deoxyhexosidase S whereas the isoenzymes A and B were inhibited. The glycoprotein nature of β-D-2-acetamido-2-deoxyhexosidase S was suggested by its affinity towards agarose-immobilized concanavalin A.

A pool of 'acid' hydrolases exists within the acellular lining material of the alveoli and distal airways of the lungs.[25] These extracellular hydrolases, obtained using pulmonary lavage procedures, appear to be of a selected variety insofar as some hydrolases (β-D-2-acetamido-2-deoxyglucosidase and α-D-mannosidase) are highly active whereas others (β-D-glucuronidase and arylsulphatase) are barely detectable. The origins of these hydrolases were investigated. Neither leakage of serum nor cell damage can account for the presence of the extracellular hydrolases in lavage effluents. Electrophoretic mobilities on acrylamide gels indicate that the extracellular hydrolases generally differ from those found in serum. Cytoplasmic soluble enzymes such as lactate dehydrogenase were used to monitor cell damage and show that the extracellular hydrolases did not originate from cell leakage during the lavage procedure. Hydrolases similar to those found extracellularly are associated with highly purified lysosome-free lamellar bodies isolated from homogenates of lung. The extracellular hydrolases are probably secreted by the type 2 cells of the pulmonary alveolar epithelium during their secretion of lamellar bodies.

β-D-2-Acetamido-2-deoxyglucosidase isolated from the secretions of fibroblasts of mucolipidosis-II and -III patients has been shown to be internalized by cultured non-parenchymal rat-liver cells.[26] The rate of endocytosis compared with that of β-D-2-acetamido-2-deoxyglucosidase from control fibroblasts was 11 and 19% for the enzyme from mucolipidosis-II and -III patients respectively. The inhibition of endocytosis by D-mannan indicates that the β-D-acetamido-2-

[23] C. J. Steer, J. W. Kusiak, R. O. Brady, and E. A. Jones, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2774.
[24] M. Potier, J. Teitelbaum, S. B. Melancon, and L. Dallaire, *Biochem. Biophys. Acta*, 1979, **566**, 80.
[25] G. E. R. Hook, *Biochemistry*, 1978, **17**, 520.

deoxyglucosidase from mucolipidosis-II and -III patients is recognized by cell-surface receptors specific for D-mannose.

The carbohydrate composition of human placental β-D-2-acetamido-2-deoxyhexosidases A, B, and heat-converted B was determined by g.l.c. Similar quantities of D-mannose, 2-acetamido-2-deoxy-D-glucose, and D-galactose are present in the A and B isoenzymes, whereas *N*-acetylneuraminic acid is found in significant amount in only the A isoenzyme.[27] The heat-converted β-D-acetamido-2-deoxyhexosidase B also contains only trace amounts of *N*-acetylneuraminic acid, but is about 1.5-fold richer in D-mannose and 2-acetamido-2-deoxy-D-glucose, and nearly two-fold richer in D-galactose than native β-D-2-acetamido-2-deoxyhexosidase B. Since native and converted B are thought to be composed of four identical protein chains, these results suggest that there may be variable glycosylation of these chains.

Antibodies against human placental β-D-2-acetamido-2-deoxyhexosidase A and kidney α-sub-units were raised in rabbits after cross-linking the antigens with glutaraldehyde.[28] Anti-(α_n-sub-unit) antiserum (anti-α_n) precipitated β-D-2-acetamido-2-deoxyhexosidase A but not β-D-2-acetamido-2-deoxyhexosidase B, whereas anti-(β-D-2-acetamido-2-deoxyhexosidase A) antiserum precipitated both β-D-2-acetamido-2-deoxyhexosidases A and B. Specific anti-(β-D-2-acetamido-2-deoxyhexosidase A) antiserum was prepared by absorbing antiserum with β-D-2-acetamido-2-deoxyhexosidase B. Both anti-α_n and anti-(β-D-acetamido-2-deoxyhexosidase A) antisera precipitated the cross-reacting material from eight unrelated patients with Tay–Sachs disease. Immunotitration, immunoelectrophoresis, double-immunodiffusion, and radial-immunodiffusion techniques were used to demonstrate the presence of cross-reacting material. The cross-reacting-material–antibody complex was enzymically inactive. Antiserum raised against kidney or placental β-D-acetamido-2-deoxyhexosidase A, without cross-linking with glutaraldehyde, failed to precipitate the cross-reacting material, implying that treatment of the protein with glutaraldehyde exposes antigenic determinants that are hidden in the native protein. Since anti-(β-D-2-acetamido-2-deoxyhexosidase B) anti-serum did not precipitate the cross-reacting material during the immunoelectrophoresis of Tay–Sachs liver extracts, it is suggested that altered α-sub-units do not combine with β-sub-units. By using immunotitration it was shown that there is competition between the β-D-2-acetamido-2-deoxyhexosidase B-free Tay–Sachs liver extract and β-D-2-acetamido-2-deoxyhexosidase A for the common binding sites on monospecific anti-(cross-linked β-D-2-acetamido-2-deoxyhexosidase A) antiserum. The amount of cross-reacting material in the liver samples from seven cases of Tay–Sachs disease was found to be in the same range as theoretically calculated α-sub-units in normal liver samples. Similar results were obtained by the radial-immunodiffusion studies. The present studies therefore suggests that Tay–Sachs disease is caused by a structural-gene mutation.

[26] K. Ullrich and K. von Figura, *Biochem. J.*, 1979, **182**, 245.
[27] H. Freele, B. Geiger, and A. L. Miller, *Biochem. J.*, 1979, **177**, 749.
[28] S. K. Srivastava, N. H. Ansari, L. A. Hawkins, and J. E. Wiktorowicz, *Biochem. J.*, 1979, **179**, 657.

Decreased binding by the lectins concanavalin A and wheat-germ agglutinin was found for a number of 'acid' hydrolases (β-D-2-acetamido-2-deoxyhexosidase, α-L-fucosidase, α- and β-D-glucosidases, β-D-galactosidase, and α-D-mannosidase) from skin fibroblasts of three unrelated patients with mucolipidosis II.[29] This decreased binding as compared with normal controls was demonstrated by titration of hydrolase activities with increasing amounts of immobilized lectins. Neuraminidase treatment slightly improved the binding of enzymes from mucolipidosis-II patients, in contrast with the diminished binding found for hydrolases from control cell-lines. The abnormality in binding by lectins of hydrolases of mucolipidosis-II patients was observed for enzymes with various degrees of intracellular deficiency as well as for enzymes with normal intracellular activities. These findings suggest a generalized alteration of fibroblast acid hydrolase molecules in mucolipidosis-II.

The effects of chloroquine and D-mannose 6-phosphate on the secretion and uptake of the lysosomal enzyme β-D-2-acetamido-2-deoxyglucosidase, by human fibroblasts, have been compared.[30] There was a reciprocal relationship between intracellular depletion, and extracellular accumulation, of enzyme at chloroquine concentrations ranging from 5 μM to 100 μM. A loss of enzyme activity from the system (intra- plus extra-cellular activities) with increasing concentrations of chloroquine was due to inhibition of the β-D-2-acetamido-2-deoxyglucosidase. At a concentration of 50 μM, chloroquine elicited a three-fold increase in the extracellular accumulation of β-D-2-acetamido-2-deoxyglucosidase in 24 h, whereas the addition of 5 mM D-mannose 6-phosphate (a competitive inhibitor of receptor-mediated uptake) resulted in only a 13% increase. Uptake of β-D-2-acetamido-2-deoxyglucosidase by enzyme-deficient fibroblasts was completely inhibited by 5 mM D-mannose 6-phosphate. In the presence of chloroquine there was also no uptake of enzyme; however, there was a marked decrease in the residual activity of the cells. The results suggest that the effect of chloroquine on fibroblasts is to stimulate secretion rather than to inhibit uptake as previously reported. The isoenzyme pattern of the β-D-2-acetamido-2-deoxyglucosidase from normal culture was compared with that accumulating in the medium following exposure of the cells to 50 μM chloroquine. In the presence of chloroquine, there was an increase in the A isoenzyme; however, the activity was eluted in a broad peak which probably represents several closely related forms of the enzyme. There was an almost total loss of the A isoenzyme of β-D-2-acetamido-2-deoxyglucosidase from fibroblasts cultured in the presence of chloroquine.

Normal human leucocytes present only one molecular form of β-D-2-acetamido-2-deoxygalactosidase, corresponding to α-D-galactosidase form II, which is the residual activity in patients with Fabry disease, an α-D-galactosidase deficiency.[31]

[29] R. Rousson, Y. Ben-Yoseph, M. B. Fiddler, and A. L. Nadler, *Biochem. J.*, 1979, **180**, 501.

[30] P. Willcox and S. Rattray, *Biochim. Biophys. Acta*, 1979, **586**, 442.

[31] R. Salvayre, A. Maret, A. Nègre, and L. Douste-Blazy, *C. R. Hebd. Seances Acad. Sci. Ser. D.*, 1979, **288**, 995.

In normal human leucocytes only one molecular form of α-D-2-acetamido-2-deoxygalactosidase corresponding to the form II of α-D-galactosidase (or α-D-galactosidase B) is present.[32] In leucocytes from patients with Fabry disease the deficient α-D-galactosidase activity corresponds to α-D-galactosidase forms I and IV, whereas the residual activity corresponds to form II. This single residual form seems to be identical to the normal α-D-2-acetamido-2-deoxygalactosidase. In leucocytes the molecular forms of α-D-galactosidase and α-D-2-acetamido-2-deoxygalactosidase from normal subjects and patients with Fabry disease can be divided into two groups on the basis of their enzymatic and genetic properties. One group is made of form I (isoenzyme A) and of the major form IV α-D-galactosidase: heat-labile, with close K_m values for 4-methylumbelliferyl α-D-galactopyranoside (K_mI = 2.1×10^{-3} M and K_mIV = 1.9×10^{-3} M), inhibited by α-D-galactosides and *meso*-inositol, and inactive in Fabry disease (coded by the same X-linked gene). The other group only concerns α-D-galactosidase form II (isoenzyme B), or α-D-2-acetamido-2-deoxygalactosidase. This form II from normal subjects has the same properties as the residual one from Fabry disease: heat-stable, close K_m values for 4-methylumbelliferyl α-D-galactopyranoside (K_mII = 5.1×10^{-3} M and K_mF = 5.5×10^{-3} M) and for 4-nitrophenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (K_m normal = 1.7×10^{-3} M and K_m Fabry = 2.0×10^{-3} M), inhibited by α-D-galactosides and by 2-acetamido-2-deoxy-D-galactosides. This form is coded by a gene different from the X-linked gene.

A search for lysomal hydrolases and related enzymes has been made in haemolysates from human and rabbit red cells.[33] Apart from acid phosphatases, significant activities were found only for α-D-mannosidase, neutral α-D-glucosidase, and β-D-2-acetamido-2-deoxyhexosidase.

A comparative study of the properties of human and rat β-D-2-acetamido-2-deoxyhexosidases has been reported.[34] There are four β-D-2-acetamido-2-deoxyhexosidases isoenzymes in rat serum; one is thermostable and the remaining three are thermolabile. Human serum contains three isoenzymes; two are thermostable and one is thermolabile. In general, the β-D-2-acetamido-2-deoxyhexosidases isoenzymes in rat serum are more thermolabile than those in human serum. In contrast, the kinetic properties of the β-D-2-acetamido-2-deoxyhexosidase isoenzymes in serum from the two species are similar. Rat liver and human placenta contain two β-D-2-acetamido-2-deoxyhexosidase isoenzymes, one of which is thermolabile and the other thermostable. These tissue isoenzymes from the two species have similar physical and kinetic properties.

Investigation of the binding characteristics of urinary 'acid' β-D-galactosidase, β-D-2-acetamido-2-deoxyglucosidase, α-D-galactosidase and α-L-fucosidase from patients with mucolipidosis II and mucolipidosis III to agarose-immobilized concanavalin A revealed a two to ten-fold decrease in the proportion of enzyme (that adsorbed on the lectin) activities from patients with mucolipidoses II and III.[35] Neuraminidase treatment of the unabsorbed enzyme fraction did not

[32] R. Salvayre, A. Maret, A. Nègre, and L. Douste-Blazy, *Eur. J. Biochem.*, 1979, **100**, 377.

[33] L. Poenaru and J. C. Drefus, *Biochim. Biophys. Acta*, 1979, **566**, 67.

[34] M. Drogin, S. Nakagawa, and H. M. Nitowsky, *Int. J. Biochem.*, 1977, **8**, 849.

[35] B. C. Kress and A. L. Miller, *Biochem. J.*, 1979, **177**, 409.

significantly increase the proportion of enzyme activities that bound to the agarose-immobilized concanavalin A. Characterization of 'acid' α-D-galactosidase from the absorbed and unabsorbed enzyme fractions of mucolipidosis II and mucolipidosis III patients demonstrated identical apparent K_m values of 0.22 mM with respect to 4-methylumbelliferyl β-D-galactopyranoside, altered pH–activity profiles, and heterogeneous isoelectric focusing patterns. The results of this study support the suggestion of an alteration of a post-translational modification (possibly glycosylation) occurring in mucolipidosis II and mucolipidosis III, common to the lysosomal hydrolases that affect the D-mannose-related properties of these enzymes.

Adsorptive endocytosis of β-D-2-acetamido-2-deoxyglucosidase from human urine by isolated rat hepatocytes is inhibited by glucoproteins, polysaccharides, and sugars that are known to bind to cell-surface receptors specific for either terminal D-galactose or 2-acetamido-2-deoxy-D-galactose residues, terminal D-mannose residues or D-mannose 6-phosphate residues.[36] Recognition of β-D-2-acetamido-2-deoxyglucosidase by a cell-surface receptor specific for terminal D-galactose or 2-acetamido-2-deoxy-D-galactose residues is supported by the observations (*a*) that neuraminidase pretreatment of the enzyme enhances endocytosis, (*b*) that β-D-galactosidase treatment decreases endocytosis, and (*c*) that neuraminidase pretreatment of hepatocytes decreases β-D-2-acetamido-2-deoxyglucosidase endocytosis. Recognition of β-D-2-acetamido-2-deoxyglucosidase *via* receptors recognizing D-mannose 6-phosphate residues is lost after treatment of the enzyme with alkaline phosphatase and *endo*-β-D-2-acetamido-2-deoxyglucanase H. The effect of *endo*-β-D-2-acetamido-2-deoxyglucanase H supports the view that the D-mannose 6-phosphate residues reside in *N*-glycosidically linked oligosaccharide side-chains of the high-D-mannose type. The weak inhibition of endocytosis produced by compounds known to interact with cell-surface receptors specific for D-mannose residues suggests that this recognition system plays only a minor role in the endocytosis of lysosomal β-D-2-acetamido-2-deoxyglucosidase by hepatocytes.

The characteristics of bovine mammary gland β-D-2-acetamido-2-deoxyglucosidase have been studied.[37]

β-D-2-Acetamido-2-deoxyglucosidase activities have been determined in homogenates of marmoset kidney, in serum, and in urine by using the 4-methylumbelliferyl substrate.[38] The enzyme activity was separated into several components by ion-exchange chromatography, starch-gel electrophoresis and isoelectric focusing. The kidney contained two major forms of the enzyme, A and B, which had similar pH optima and K_m values, but pI values of 5.0 and 7.64 respectively. The isoenzymes also differed in heat stability, the B-form being the more stable. Serum contained B-form activity and, in addition, two intermediate forms (I_1 and I_2). Serum from a pregnant marmoset contained a form which may be analogous to the human P-isoenzyme. Urine contained only a small amount of B-form activity, the majority being present in the A-form. The

[36] K. Ullrich, R. Bassner, V. Gieselmann, and K. von Figura, *Biochem. J.*, 1979, **180**, 413.
[37] B. J. Kitchen, C. J. Masters, and D. J. Winzor, *Proc. Aust. Biochem. Soc.*, 1979, **12**, 26.
[38] R. J. Pierce, R. G. Price, and J. S. L. Fowler, *Biochem. J.*, 1978, **175**, 859.

kidney A- and B-forms both had mol. wts. of $9.60–10.00 \times 10^4$ and the activity was predominantly lysosomal. Partial purification of the kidney A isoenzyme was undertaken. Immunoprecipitation studies indicated a relationship between marmoset kidney A-form and human liver A-form activity.

Seven renal glycoside hydrolases have been measured in four sub-cellular fractions prepared from highly inbred aglycosuric (*AV*-line) and glycosuric (*XA*-line) Chinese hamsters (*Cricetulus griseus*).[39] No significant difference was found in β-D-2-acetamido-2-deoxyglucosidase levels in any of the sub-cellular fractions of *AV* and *XA* animals.

L-Thyroxine has been shown to evoke an increase in β-D-2-acetamido-2-deoxyhexosidase activity in adult rat liver.[40] This increase occurred solely in the form of 'A-like' heat labile components of the activity, the activity of the form 'B-like' heat stable components being unaffected by the agent.

The separation and purification of the β-D-acetamido-2-deoxyhexosidase activities from tumours induced by 1,2-dimethylhydrazine in the rat colon and from colonic mucosa of tumour-bearing animals are reported.[41] Mucosa contained β-D-2-acetamido-2-deoxyhexosidase A and B, as well as a third form whose properties with regard to electrophoretic mobility and thermostability lay between those of A and B. Tumours contained only β-D-2-acetamido-2-deoxyhexosidase A and B activities. Each form possessed both β-D-2-acetamido-2-deoxyglucosidase and β-D-2-acetamido-2-deoxygalactosidase activities, which could not be separated by a variety of techniques. The alteration of the ratio of the two specific activities in each form during purification, together with differences in the kinetic inhibition constants and behaviour inactivation by various reagents or a temperature of 50 °C, supported the belief that each form contains the two enzyme activities, β-D-2-acetamido-2-deoxy-gluco and -galacto-sidases, at separate active sites. This model is in contrast to that reported for these activities from a number of other sources. A variety of treatments reported to cause the conversion of form A into a form resembling B failed to produce such an effect on the rate colonic β-D-2-acetamido-2-deoxyhexosidases.

β-D-2-Acetamido-2-deoxyglucosidase in the submandibular gland of mice was found to be androgen-dependent; the specific activities in males, females, and castrated males were 0.25, 0.11, and 0.11 unit mg^{-1} protein, respectively.[42] The activities in females and castrated males were increased to the level of normal male mice by testosterone injection. Injections of progesterone and 17β-oestradiol hardly affected the activity in males. In both males and females, the enzyme activity was detected in the convoluted tubular cells, not in acinous cells. The results of isoelectric focusing have shown that one enzyme having an isoelectric point of 9.0 is present in the glands of both sexes, indicating that the enzyme remains after castration and that the increases caused by testosterone

[39] A. Y. Chang and C. S. Perry, *Comp. Biochem. Physiol. B*, 1979, **62**, 557.
[40] L. V. Oberkotter, C. Horowitz, P. M. Coates, M. J. Palmieri, and O. Koldovsky, *Int. J. Biochem.*, 1979, **10**, 209.
[41] N. Mian, D. G. Herries, D. M. Cowen, and E. A. Batte, *Biochem. J.*, 1979, **177**, 319.
[42] K. Hosoi, S. Kobayashi, M. Hiramatsu, N. Minami, and T. Ueha, *J. Biochem. (Tokyo)*, 1979, **85**, 1483.

represent the same molecular species. In addition, it was shown that the saliva from both sexes contained significant activity of β-D-2-acetamido-2-deoxyglucosidase which also changed depending on the androgenic state of the animals. Most of the salivary activity was shown to originate from the submandibular gland, since the extirpation of this gland resulted in a significant decrease of the salivary activity.

The effects of several hormones (progesterone, diethylstilbestrol) on chick oviduct β-D-2-acetamido-2-deoxy-D-glucosidase isoenzymes have been studied.[43] The total activity increased fourfold upon hormone withdrawal and upon treatment with progesterone alone or with progesterone plus diethylstilbestrol.

Two β-D-2-acetamido-2-deoxyhexosidase isoenzymes (A and B) from the gastropod *Turbo cornutus* have been separated and purified by ion-exchange chromatographies, affinity chromatography on agarose-immobilized concanavalin A, and gel filtration.[44] The two isoenzymes, free from all other common glycoside hydrolase contaminations, were homogeneous on polyacrylamide gel electrophoresis, with the A form having a greater anodic migration rate. The isoenzymes (mol. wts. 1.0×10^5, pH optima, 4.0, K_m's for 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside 2.9 and 3.2 mM for A and B isoenzymes respectively) were stimulated by F^-, Cl^-, Br^-, I^-, and NO_3^- and inhibited by Hg^+, Ag^+, Fe^{3+}, and 2-acetamido-2-deoxy-D-galactose and -glucose.

When exponentially growing *Dictyostelium discoideum* amoebae were harvested from growth medium and suspended in a nutrient-free phosphate buffer, the cells released 20 to 30% of their total β-D-2-acetamido-2-deoxyglucosidase and α-D-mannosidase activities.[45] This release was inhibited by sodium azide but was stimulated (to a level of 90% release) by various saccharides when added to the phosphate buffer at millimolar concentrations. Various salts, amino-acids, and nucleotides were unable to stimulate the release process. Non-metabolizable sugars were more stimulatory than metabolizable sugars, but no clear distinction related to structure, ability to be transported, or ability to be metabolized was found to account directly for the various effects of the sugars on glycosidase release. The rate and amount of glycosidase release was dependent on the sugar concentration but not on the cell density. The results presented suggest that sugars stimulate a specific release of a variety of lysosomal enzymes and the relationship of this release to *D. discoideum* morphogenesis was discussed.

β-D-2-Acetamido-2-deoxyglucosidase has been extracted from malted barley and purified *via* affinity chromatography on a 4-aminophenyl 2-acetamido-2-deoxy-γ-D-glucopyranoside derivative of agarose.[46] The purified enzyme (pH optimum 5.0, activity range pH 3.0–6.5, K_m 0.25 mM for 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside, mol. wt. 9.2×10^4 in presence of sodium dodecyl sulphate, 4.65 S) was devoid of other glycoside hydrolyase activities.

[43] J. J. Lucas, *Arch. Biochem. Biophys.*, 1979, **197**, 96.
[44] K. Yeung, A. J. Owen, and J. A. Dain, *Comp. Biochem. Physiol. B*, 1979, **63**, 329.
[45] E. V. Crean and E. F. Rossamando, *J. Gen. Microbiol.*, 1979, **110**, 315.
[46] E. D. Mitchell, C. W. Houston, and S. B. Latimer, *Phytochemistry*, 1976, **15**, 1869.

β-D-2-Acetamido-2-deoxyglucosidase has been found in the germinated seeds of mung bean, *Phaseolus aureus*, accompanied by α-D-mannosidase and α- and β-D-galactosidases. β-D-2-Acetamido-2-deoxyglucosidase was active in the cotyledon at the initial stage of germination and subsequently in hypocotyl and plumule.[47] The purification of the enzyme was achieved by ammonium sulphate fractionation, ion-exchange chromatography, and gel filtration. Two forms, F_1 (mol. wt. 9.0×10^4, pI 4.7) and F_2, were obtained. Forms F_1 and F_2 similarly hydrolysed 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside as well as 4-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside and the ratio of both activities was constant throughout the course of purification. Forms F_1 and F_2 showed similar enzymatic properties on the hydrolysis of 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and 4-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside. The optimum pH of the enzymes was about 4.6–5.2 and the enzyme forms were stable in the range of pH 5–8, but they were unstable at 60 °C or more. The K_m values of F_1 for 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and 4-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside were 0.64 mM and 0.55 mM and those of F_2 were 0.71 mM and 0.54 mM, respectively.

β-D-2-Acetamido-2-deoxyhexosidase has been purified from an edible mushroom, *Tremella fuciformis*, to a single protein band (mol. wt. 1.25×10^5 by gel filtration) on disc gel electrophoresis.[48] The enzyme (pH optimum 5.0, K_m 0.19 and 0.31 mM for 4-nitrophenyl 2-acetamido-2-deoxy-β-D-galacto and -gluco-pyranosides respectively) was also active on *N*-acetyl-chito-oligosaccharides (DP 2–5).

β-D-2-Acetamido-2-deoxyglucosidase which coexists with 'Takadiastase' (from *Aspergillus oryzae*) has been shown to consist of two sub-units.[49]

The cytochemical localization of β-D-2-acetamido-deoxyglucosidase in hyphae of *Mucor racemosus* has been reported.[50]

The influence of carbon and nitrogen sources on the release of β-D-2-acetamido-2-deoxyglucosidase during autolysis of *Neurospora crassa* has been examined.[51]

Homogeneous preparations of pesticin, a bacteriocin produced by *Yersinia pestis*, neither significantly inhibited net synthesis of deoxyribonucleic acid, ribonucleic acid, or protein in *Escherichia coli* nor caused detectable degradation of deoxyribonucleic acid *in vivo*.[52] Accordingly, its mode of action does not resemble that of colicin E2 as suggested by others. However, incorporation of cell wall-specific label ([^{14}C]diaminopimelic acid) into trichloroacetic acid-insoluble material of growing cells was inhibited by pesticin which also promoted release of such radioactivity from both resting cells and purified mureinlipoprotein. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis of reaction mixtures containing appropriately labelled mureinlipoprotein showed

[47] A. Ohtakara and K. N. Tranth, *J. Agric. Chem. Soc. Jpn.*, 1978, **52**, 589.
[48] Y. Sone and A. Misaki, *J. Biochem. (Tokyo)*, 1978, **83**, 1135.
[49] T. Mega and Y. Matsushima, *J. Biochem. (Tokyo)*, 1979, **85**, 335.
[50] H. C. Hoch, G. Hänssler, and H. J. Reisner, *Exp. Mycol.*, 1979, **3**, 164.
[51] F. Reyes, R. Lahoz, and A. V. Moreno, *Trans. Brit. Mycol. Soc.*, 1979, **72**, 447.
[52] D. M. Ferber and R. R. Brubaker, *J. Bacteriol.*, 1979, **139**, 495.

that [^{3}H]2-acetamido-2-deoxy-D-glucose comigrated either with [^{14}C]diaminopimelic acid in the murein peptide or with [^{14}C]isoleucine of the Braun lipoprotein. As judged by these findings and pesticin-dependent release of reducing equivalents but not 4-hydroxy-2-acetamido sugars, the bacteriocin possesses β-D-2-acetamido-2-deoxyglucosidase activity. Hydrolysis of mureinlipoprotein occurred over a broad pH, with an optimum of 4.7. Mureinlipoproteins from a variety of pesticin-sensitive and -resistant organisms were hydrolysed by the bacteriocin, indicating that its antibacterial specificity resides at the level of absorption.

β-D-2-Acetamido-2-deoxyhexosidase has been immobilized with retention of enzyme activity by being co-immobilized with bovine serum albumin by glutaraldehyde coupling, and then being cast into a membrane.[53]

3 α-Arabinofuranosidases and α-L-Arabinopyranosidases

The common identity of human 'acid' β-D-galactosidase, β-D-fucosidase, and α-L-arabinofuranosidase is indicated by similar binding patterns of the glycoside hydrolase activities of each enzyme to various lectins, by similar ratios between their intra- and extracellular levels in normal and I-cell fibroblasts, and by their deficiencies in liver tissues from patients with G_{M_1} gangliosidosis.[54] A 'neutral' β-D-galactosidase, purified to homogeneity from human liver, has been shown to possess all these glycoside hydrolase activities together with β-D-glucosidase and β-D-xylosidase activities at neutral pH. These neutral enzymic activities were not bound by any of the lectins examined and were found to be reduced in liver and spleen of a patient with 'neutral' β-D-galactosidase deficiency.

Whereas the α-L-arabinopyranosidase of almond emulsion and barley are not inhibited by L-arabinono-1,4-lactone, the same compound was found to inhibit the α-L-arabinofuranosidase of *Aspergillus niger*.[55] The opposite effects were found with/assumed for D-glucono-1,5-lactone. Since the inhibiting effect of an aldono-1,4-lactone on an aldofuranosidase with simultaneous inhibition of the corresponding aldopyranosidase had not been previously reported, it was suggested that other enzyme pairs may be influenced in the same sense by 1,4- and 1,5-lactones.

Wild-type *Bacillus subtilis*, when grown on beet arabinan, secretes into its culture medium an *endo*-arabinanase and two arabinosidases.[56] A procedure has been developed for the purification of the *endo*-arabinanase. One of the arabinosidases (mol. wt. $\simeq 6.5 \times 10^4$, pI = 5.3) has been purified to the point that it contains only one quantitatively minor contaminant, as shown by sodium dodecyl sulphate–urea disc gel electrophoresis and isoelectric focusing. The purified arabinosidase, acting on 4-nitrophenyl α-L-arabinofuranoside, has maximal activity at pH 6.5, and, when acting on a branched arabinan substrate,

[53] K. K. Yeung, A. J. Owen, and J. A. Dain, *Carbohyr. Res.*, 1979, **75**, 295.
[54] Y. Ben-Yoseph, M. B. Fiddler, R. Rousson, and H. L. Nadler, *Biochim. Biophys. Acta*, 1979, **568**, 386.
[55] K. Schwabe, A. Grossmann, B. Fehrmann, and B. Tschiersch, *Carbohydr. Res.*, 1978, **67**, 541.
[56] L. Weinstein and P. Albersheim, *Plant Physiol.*, 1979, **63**, 425.

preferentially attacks non-reducing terminal arabinosyl residues linked to the 2 or 3 position of other arabinosyl residues. Neither of the two purified enzymes is capable of hydrolysing a variety of carbohydrate substrates which lack arabinosidic linkages. The purified *endo*-arabinanase is shown to be capable of releasing arabinosyl oligomers from the walls of suspension-cultured sycamore cells, thereby suggesting its usefulness as a probe in studying the structure of the arabinan component of primary cell walls.

4 β-D-Fructofuranosidases

Fructosan is the primary storage polysaccharide in grass species of the Aveneae, Festuceae, and Hordeae tribes.[57] The majority of the research on fructosan metabolism has been accomplished on the (2 → 1) linked inulin class of fructosan found in the Compositeae. The current research was done on the (2 → 6) linked levan class of fructosan stored by the Gramineae. The objective was to investigate the mode of action of β-D-fructofuranosidases isolated from fescue culm base tissue. The results indicate that β-D-fructofuranosidase, isolated from fescue culm base tissue, contained *exo*-action activity since polymer hydrolysis began at the D-fructose end of the fructosan molecule and released only D-fructose residues. The maximum specific activity of this enzyme, at pH 4.5 and 30 min of reaction time, was determined to be 1.23 μmol of D-fructose mg of protein^{-1} h^{-1}. 'Invertase' activity, isolated from the same tissue, was determined to be 0.88 μmol of hexose mg of protein^{-1} h^{-1}.

The cell wall bound β-D-fructofuranosidases of persimmon and soy bean callus cells,[58] and of morning glory callus[59] have been examined.

The induction of β-D-fructofuranosidase was investigated in beet root crops and wheat seedlings by the 'ageing' tissue technique.[60] It was established that ammonium sulphate in a concentration of 0.001 M intensifies β-D-fructofuranosidase induction. This effect of ammonium sulphate was not manifested in the presence of actidione, puromycin, or chloramphenicol. The theory was advanced that the induction of β-D-fructofuranosidase is activated by ammonium sulphate.

The solubilization of β-D-fructofuranosidase bound to cell walls of sugar beet (*Beta vulgaris*) seedlings has been studied.[61] Gel filtration *etc.* studies confirmed that substances which were released from the cell wall together with bound β-D-fructofuranosidase acted on the enzyme in dilute salt solutions and caused insolubilization of the enzyme. The characteristics of two different fractions of the substances were analogous to those of nucleic acids and nucleoproteins.

β-D-Fructofuranosidase in the cell wall preparation of hypocotyls and roots of germinating sugar beets was investigated with reference to β-D-fructofuranosidase isolated from the aged tissue slices of the mature roots.[62] Effect of sodium chloride concentration on release of the enzymes, insolubilization by decreasing

[57] A. E. Smith, *J. Agric. Food Chem.*, 1976, **24**, 476.
[58] S. Hisajima, Y. Arai, and T. Ito, *J. Jpn. Soc. Starch Sci.*, 1978, **25**, 198.
[59] S. Hisajima and Y. Arai, *J. Jpn. Soc. Starch Sci.*, 1978, **25**, 163.
[60] A. A. Anismov and L. V. El'Tsova, *Biochem. USSR*, 1978, **43**, 503.
[61] H. Masuda and S. Sugawara, *Agric. Biol. Chem.*, 1978, **42**, 2151.
[62] H. Masuda and S. Sugawara, *J. Agric. Chem. Soc., Jpn.*, 1977, **51**, 269.

the salt concentration, pH or temperature dependence on the activity and the stability, and pattern of gel filtration on Sepharose® 6B were quite identical with β-D-fructofuranosidase from the mature root tissue. Changes in β-D-fructofuranosidase activity and in sugar content during germination period were also studied. The enzyme activity decreased to a certain extent after four days of germination, and later the activity remained approximately at a constant level. But no appreciable changes in specific activity or activity per mg dry weight of cell wall preparation were observed throughout the germination period tested.

The effect of Blue Dextran® on protection from insolubilization of NaCl-released β-D-fructofuranosidase from sugar beet cell-wall was studied and discussed.[63] The enzyme was insoluble in water or salt solutions of low concentrations but was solubilized by the addition of Blue Dextran. Upon chromatography of the enzyme on a Sepharose® 6B column in the presence of Blue Dextran, the enzyme and Blue Dextran were eluted with the same elution volume. However, they were eluted separately by using 0.5 M-NaCl as an eluting solvent. Solubility of enzyme increased with concentration of Blue Dextran added. It was found that the enzyme was absorbed to Sephadex® G-100, but not to dextran T40 or T500. Some additional experiments revealed that the dependence of pH on solubility of the released enzyme disappeared in the presence of Blue Dextran.

Direct evidence of localization *in vivo* of β-D-fructofuranosidase in tomato fruit cell-wall using an immunofluorescent antibody technique has been reported.[64]

Cytoplasmic and wall-bound β-D-fructofuranosidase were prepared from the seedlings of sugar beet, and absorption-release characteristics of the enzymes were compared by the use of a partially purified wall. Adsorption of the bound enzyme occurred below 0.25 M-NaCl and was unaffected by changes in pH, while the cytoplasmic enzyme was adsorbed only in acidic media of pH 3–4. Release of re-adsorbed enzymes with NaCl depended on its concentration, and the cytoplasmic enzyme was liberated more readily than the bound type, giving their maximum values at 0.2 M and 0.8 M-NaCl, respectively. Thus, the two β-D-fructofuranosidases showed marked differences in adsorption to and release from the wall. Adsorption of the cytoplasmic enzyme to the wall was reversible with changes in pH of the medium. The results suggested that the wall-bound β-D-fructofuranosidase was essentially different from the cytoplasmic enzyme and consequently not an artifact.

Association of the cell wall-bound β-D-fructofuranosidase of sugar beet seedlings with structural polysaccharides of the wall was investigated.[65] After sequential extraction with boiling water, 0.5% H_4edta and 24% KOH from the wall of sugar beet, the polysaccharides were prepared as pectic substances I and II, and hemicellulose. Solubilization of the enzyme in the presence of the polysaccharides and the profiles on gel filtration of the enzyme with the poly-

[63] H. Masuda and S. Sugawara, *J. Agric. Chem. Soc., Jpn.*, 1977, **57**, 335.
[64] K. Iki, H. Nakagawa, N. Ogura, and H. Takehana, *Agric. Biol. Chem.*, 1977, **41**, 1311.
[65] H. Masuda and S. Sugawara, *Agric. Biol. Chem. (Tokyo)*, 1978, **42**, 1485.

saccharide suggested the formation of an enzyme–polysaccharide complex. The complex formation occurred completely at a NaCl concentration below 0.2 M, partly at 0.3 M, and only little at 0.4 M. Among three types of wall polysaccharides used, hemicellulose had the highest affinity with the enzyme, while hexuronic acid content was the lowest. The reverse relation was observed with pectic substance I. It was also observed by gel filtration that the polysaccharides used were associated in aqueous solution and dissociated in salt solution depending on its concentration. The enzyme was considered to form a complex with the associated molecule of the polysaccharide.

The use of Sephadex® columns equilibrated with sodium chloride to purify winter wheat leaf β-D-fructofuranosidase has been described.[66]

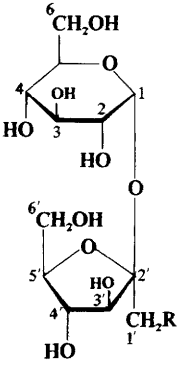
The carbohydrate moiety of β-D-fructofuranosidase of *Candida utilis* was suggested to be localized at certain sites of the enzyme protein.[67] The oxidation of the sugar moeity with periodate to a certain extent had no effect on the enzyme activity, but the stability of the enzyme was decreased. This partially oxidized enzyme bound hide powder or nylon powder and the enzyme activity was retained. The enzyme was shown to be divided into glycoprotein sub-units of a similar molecular size when heated in the presence of sodium dodecyl sulphate. The enzyme did not dissociate upon treatment with reducing agent such as mercaptoethanol.

β-D-Fructofuranosidase of *Candida utilis* purified to an electrophoretically and ultracentrifugally pure state (mol. wt. 3×10^5; pI 3.8) was analysed.[68] Nitrogen content of the enzyme preparation was 8.45%. The preparation contained a great deal of sugar moiety which consisted of D-mannose, D-glucose, and 2-amino-2-deoxy-D-glucose in amounts of about 40%, 4%, and 3%, respectively. Results of amino-acid analysis and the molecular weight, determined by some physicochemical methods reported previously, led to the conclusion that the enzyme consisted of about 1380 moles of amino-acid residues per mole. The β-D-fructofuranosidase was rich in L-aspartic acid, L-glutamic acid, L-serine, and L-threonine; decreasing in that order. The *N*-terminal amino-acid of the enzyme was determined as L-serine.

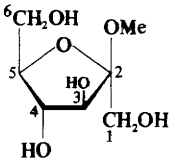
The action of β-D-fructofuranosidase from *Candida utilis* on various derivatives of sucrose (1) and methyl β-D-fructofuranoside (2), as well as on compounds with related structures has been examined.[69] None of the compounds in Table 1 was hydrolysed by the enzyme, even though many of them are only slightly different structurally from sucrose or methyl β-D-fructofuranoside. It is clear that minor changes at the 1′-, 4′-, or 6′-position cause loss of substrate capability, and it was concluded that β-D-fructofuranosidase from *Candida utilis* is a highly specific enzyme.

A mutant strain of *Saccharomyces cerevisiae* (D10-ER1) has been isolated after a two-step mutagenesis of strain 4059-358D (*SUC1*) using ethyl methane

[66] D. W. A. Roberts, *J. Chromatogr.*, 1978, **153**, 532.
[67] M. Iizuka and T. Yamamoto, *Agric. Biol. Chem.*, 1979, **43**, 217.
[68] M. Iizuka, H. Chiura, and T. Yamamoto, *Agric. Biol. Chem. (Tokyo)*, 1978, **42**, 1207.
[69] R. D. Guthrie, I. D. Jenkins, P. J. Rogers, W. F. Som, J. J. Watters, and R. Yamasaki, *Carbohydr. Res.*, 1979, **75**, C1.



(1)



(2)

Table 1 *Compounds not hydrolysed by β-D-fructofuranosidase (from* Candida utilis*)*

Derivatives of methyl β-D-fructofuranoside (2)

1-Azido-1-deoxy
1-Chloro-1-deoxy
1-Deoxy-1-fluoro
1-Deoxy
6-Chloro-6-deoxy
6-Deoxy
1,6-Dichloro-1,6-dideoxy
1,6-Dideoxy-1,6-difluoro

Derivatives of sucrose (1)

1′-Deoxy
1′-Chloro-1′-deoxy
4′-Chloro-4′-deoxy
6′-*O*-Methyl
1′,6′-Di-*O*-methyl
4,6′-Di-*O*-methyl
6,6′-Di-*O*-methyl
6,6′-Diazido-6,6′-dideoxy
6,6′-Diamino-6,6′-dideoxy
6,1′,6′-Trideoxy
6,1′,6′-Trichloro-6,1′-6′-trideoxy

Other compounds

6′-Chloro-6′-deoxyraffinose
6′-Bromo-6′-deoxyraffinose
6′-Deoxy-6′-iodoraffinose
Methyl α-L-sorbofuranoside
Methyl β-D-xylulofuranoside
Methyl β-D-arabinofuranoside
2,6-Anhydro-β-D-fructofuranose
1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside
1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside

sulphonate.[70] Cells of this new strain produced a level of total β-D-fructofuranosidase equalling that of 4059 but contained only trace amounts of the small, internal, aglycan form of the enzyme (<0.1% of total in D10-ER1 compared

[70] B. B. Abrams, R. Hackel, T. Mizunaga, and J. O. Lampen, *J. Bacteriol.*, 1978, **135**, 809.

with 6% in 4059). When D10-ER1 was crossed with a β-D-fructofuranosidase hyperproducing strain dgr3 (*SUC3*), progeny were isolated (HZ400-5A and HZ400-2C) in which levels of total β-D-fructofuranosidase had at least quadrupled. The percentage of small β-D-fructofuranosidase, however, remained insignificant. Levels of small β-D-fructofuranosidase in strain HZ500-5A were determined by affinity chromatography on concanavalin A-Sepharose®, gel permeation chromatography, and isopycnic centrifugation in CsCl. The β-D-fructofuranosidase of the *SUC1* yeasts was found to contain a form apparently greater in size than the large β-D-fructofuranosidase of the *SUC2* strain FH4C; this probably reflects a higher content of carbohydrate. The overall results of this study do not support a direct structural relationship between the large and small β-D-fructofuranosidases. The implications on β-D-fructofuranosidase biosynthesis and structure were discussed.

The purification and characterization of β-D-fructofuranosidase from an oral strain of *Streptococcus mitis* has been described.[71]

5 β-D- and α-L-Fucosidases

Human 'acid' β-D-galactosidase, β-D-fucosidase, and α-L-arabinofuranosidase appear to have a common identity[54] (see p. 429).

A novel technique has been employed to demonstrate that α-L-fucosidase purified from cystic fibrosis and control liver exhibits differential binding to the lectin concanavalin A.[72] The concentration of methyl α-D-mannopyranoside necessary to prevent 50% binding of α-L-fucosidase to concanavalin A is considerably lower for the cystic fibrosis enzyme (13.5 *vs.* 33.3 mM). Comparable results were found when binding studies were done on crude supernatant α-L-fucosidase from eight cystic fibrosis and eight control livers (5.6 ± 0.4 mM and 13.2 ± 3.4 mM, respectively), without any overlap of values between cystic fibrosis and control livers. These results suggest that comparative lectin binding studies on cystic fibrosis and normal glycoproteins from readily available tissues might result in an assay for detecting the cystic fibrosis genotype.

The sub-unit composition of human liver α-L-fucosidase has been studied by using three different procedures for treating and electrophoresing the purified enzyme.[73] Although the presence of only one sub-unit was found by conventional procedures using sodium dodecyl sulphate and urea, two non-identical sub-units with mol. wts. of 5.90×10^4 and 5.40×10^4 were found when the α-L-fucosidase was reduced and *S*-carboxymethylated with iodoacetate, followed by electrophoresis on polyacrylamide gels containing 0.1% sodium dodecyl sulphate and 8.0 M-urea.

α-L-Fucosidase has been purified 12 000-fold from human placenta. The enzyme is a glycoprotein containing: D-galactose, D-mannose, 2-acetamido-2-deoxy-D-glucose, and *N*-acetylneuraminic acid (by weight, 0.9, 1.9, 1.9, and

[71] M. L. Sund and L. Linder, *Arch. Oral Biol.*, 1979, **24**, 439.
[72] J. A. Alhadeff and P. Watkins, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 787.
[73] J. A. Alhadeff and G. L. Andrews-Smith, *Biochem. J.*, 1979, **117**, 753.

1.9%, respectively).[74] Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate separated proteins with molecular weights of 5.50×10^4, 5.14×10^4, and 2.50×10^4. Resolution of the two larger protein bands varied with the gel system and these proteins may differ only in carbohydrate content. Gel filtration of the purified enzyme failed to separate the three proteins. Treatment with the cross-linking reagent dimethyl suberimidate, prior to electrophoresis, resulted in a diminution of the original protein bands and the formation of oligomers with molecular weights of 0.80×10^5, 1.00×10^5, 1.30×10^5, and 1.44×10^5. These results suggest that the heavy 5.50×10^4 and 5.14×10^4 and light 2.50×10^4 proteins are structurally associated. The molecular weight of the native enzyme, measured by gel filtration, was dependent on the pH of the eluting buffer. At pH 5.0 or 6.0 a catalytically active peak was observed, with a molecular weight of 3.05×10^5. At pH 7.5 this peak was completely absent and the enzyme eluted as an asymmetrical peak with an apparent molecular weight of about 6.0×10^4. The reduction in apparent molecular weight at pH 7.5 was reversible by dialysis of isolated fractions at pH 6.0. In agreement with these findings the sedimentation coefficient was 8.5 S at pH 5.0 but only 3.6 S at pH 7.5. The results can be accounted for by the existence of a pH-dependent equilibrium between aggregated and dissociated forms of the enzyme or by pH-dependent conformational changes.

The temperature dependence for the hydrolysis of both 4-methylumbelliferyl α-L-fucopyranoside and 4-nitrophenyl α-L-fucopyranoside has been determined for purified α-L-fucosidase from human placenta.[75] The inhibition of the enzymatic reaction by L-fucose was also studied using the first of these two substrates at different temperatures. The thermodynamic parameters calculated from the pK_m were for the 4-methylumbelliferyl glycoside $\Delta F = -6.6$ kcal mol^{-1}, $\Delta H = -8.5$ kcal mol^{-1}, and $\Delta S = -6.3$ e.u., and for the 4-nitrophenyl glycoside $\Delta F = -5.6$ kcal mol^{-1}, $\Delta H = -12.2$ kcal mol^{-1}, and $\Delta S = -21.1$ e.u. The thermodynamic parameters for L-fucose were $\Delta H = -12.4$ kcal mol^{-1} and $\Delta S = -20.1$ e.u. The lower exothermicity and negative entropy calculated for the 4-methylumbelliferyl substrate compared to the thermodynamic parameters calculated for the 4-nitrophenyl substrate and L-fucose suggest the existence of a secondary hydrophobic binding site for the 4-methylumbelliferyl moiety on the enzyme. The difference in the enthalpy for both substrates is also reflected in a difference in activation energy, being 15.8 kcal mol^{-1} for the 4-methylumbelliferyl substrate and 20.7 kcal mol^{-1} for the 4-nitrophenyl substrate. From these results it may be concluded that altered kinetic properties of the enzyme could be the result of the binding of the aglycone moiety of the fluorogenic substrate to the enzyme.

Decreased binding by the lectins concanavalin A and wheat-germ agglutinin has been found for a number of acidic hydrolases (β-D-2-acetamido-2-deoxyhexosidase, α-L-fucosidase, α- and β-D-glucosidase, β-D-galactosidase, and α-D-mannosidase) from skin fibroblasts of three unrelated patients with mucolipidosis II[29] (see p. 423).

[74] B. M. Turner, *Biochim. Biophys. Acta*, 1979, **578**, 325.

[75] J. Hoebeke, J. Groswasser, N. Bousard, E. Vamos, and A. D. Strosberg, *Arch. Biochem. Biophys.*, 1979, **198**, 556.

The activity of α-L-fucosidase is decreased in the serum of cystic fibrosis patients compared to age-matched controls.[76] This result, combined with the elevated activity in skin fibroblasts, supports the concept of an intracellular and extracellular distribution of α-L-fucosidase in cystic fibrosis.

Investigation of the binding characteristics of urinary 'acid' β-D-galactosidase, β-D-2-acetamido-2-deoxyglucosidase, α-D-galactosidase, and α-L-fucosidase from patients with mucolipidosis II and mucolipidosis III to agarose-immobilized concanavalin A revealed a two- to ten-fold decrease in the proportion of enzyme (that adsorbed on the lectin) activities from patients with mucolipidoses II and III[35] (see p. 424).

Using a simple affinity system which required coupling of α-L-fucopyranose to macroporous agarose by epichlorohydrin treatment of the agarose in the presence of α-L-fucopyranose under alkaline conditions has been used to resolve a partially purified preparation of monkey brain α-L-fucosidase at pH 5.0 into two major fractions; one bound and one retarded.[77] The enzyme that was bound to the affinity column and specifically eluted by 2 mM α-L-fucose at pH 5.0 appeared to be homogeneous by polyacrylamide gel electrophoresis and was constituted mainly by the tetrameric form of the enzyme. The enzyme fraction retarded by the affinity column was found to contain mainly the monomeric form of the enzyme. Additional evidence for the different molecular forms of enzyme in the bound and retarded fractions came from pH–activity profiles and heat inactivation studies. The L-fucose-affinity matrix appeared to bind the tetrameric form of the enzyme specifically and, further, α-L-fucopyranose helped to retain the molecular integrity of the tetrameric enzyme.

Seven renal glycoside hydrolases have been measured in four sub-cellular fractions prepared from highly inbred aglycosuric (*AV*-line) and glycosuric (*XA*-line) Chinese hamsters (*Cricetulus griseus*).[39] Although total α-L- and β-D-fucosidases levels were similar in *AV* and *XA* kidneys, a difference was observed in the supernatant fraction.

In connection with the structural study of D-galacto-L-fucan sulphate (a sulphated polysaccharide of *Undaria pinnatifida*), galactofucanase, and α-L-fucosidase and β-D-galactosidase existing in hepato-pancreas of abalone (*Haliotis* species) were investigated.[78] From the crude mixture of enzymes, only one fraction containing α-L-fucosidase was obtained by ion-exchange chromatography followed by gel filtration. This fraction contained two types (pH optima 3 and 5) of α-L-fucosidase; only the one with pH optimum 3 was inhibited by D-galacto-L-fucan sulphate.

An affinity chromatographic method developed for the purification of α-L-fucosidase I from almond emulsin was based on modified agarose derivatized with 6-aminohexanoyl-lacto-*N*-fucopentaosylamine.[79] The final enzyme preparation was free from other glycoside hydrolases.

[76] T. F. Scanlin, S. S. Matacic, and M. C. Glick, *Clin. Chim. Acta*, 1979, **91**, 197.
[77] T. Alam and A. S. Balasubramanian, *Biochem. Biophys. Acta*, 1979, **566**, 327.
[78] T. Fujikawa, T. Anno, and M. Wada, *J. Agric. Chem. Soc. (Jpn.)*, 1976, **50**, 577.
[79] H. Yoshima, S. Takasaki, S. Ito-Mega, and A. Kobata, *Arch. Biochem. Biophys.*, 1979, **194**, 394.

6 α- and β-D-Galactosidases and D-Galactolipid-oriented Galactosidases

Spontaneous reactions of the irreversible β-D-galactosidase inhibitor 2,6-anhydro-1-deoxy-1-diazo-D-*glycero*-L-*manno*-heptitol have been investigated.[80]

The common identity of human 'acid' β-D-galactosidase, β-D-fucosidase, and α-L-arabinofuranosidase has been recognized (see p. 429).[54]

'Acid' β-D-galactosidase from human liver consists, after gel filtration at pH 7.0, of a monomeric isoenzyme, β-D-galactosidase A and small amounts of an oligomer, β-galactosidase B.[81] New studies show identical gel-filtration patterns for β-D-galactosidase from human liver and cultured skin fibroblasts. Gel filtration in the buffer used for enzyme assays (pH 4.5), however, revealed primarily dimeric β-D-galactosidase, minor amounts of β-D-galactosidase A, but no oligomers. As the transitions between monomeric and dimeric β-D-galactosidase were reversible, dimeric β-D-galactosidase is apparently the enzymatically active isoenzyme. The isoenzymes from patients with four different clinical variants of β-D-galactosidase deficiency showed altered aggregation patterns, although their molecular weights corresponded to the normal molecular weights. It could be demonstrated that the restoration of β-D-galactosidase activity, which occurred after cell fusion of certain combinations of different β-D-galactosidase-deficient fibroblasts, is not the result of intragenic complementation. Mixing experiments with cell-free extracts ruled out the possibility that the formation of dimeric β-D-galactosidase is required for complementation. To explain the complementation, a model was suggested involving two different genes: a structural gene responsible for the synthesis of the polypeptide chain, and a second gene which is involved in a modification process.

D-Gluconamide, D-gluconyl hydrazide, and *N*-(6-aminohexyl)-D-gluconamide have been tested for their inhibitory action on human liver lysosomal glucocerebrosidase and human spleen 'neutral' aryl β-D-glucosidase.[82] Analogous D-galactonamide derivatives were evaluated for their inhibition of human spleen D-galactocerebrosidase and G_{M_1}-ganglioside β-D-galactosidase. D-Gluconyl-hydrazide and D-gluconamide were effective inhibitors of the lysosomal D-glucocerebrosidase, attaining 50% inhibition at 5 and 12 mM, respectively. In contrast, *N*-(6-aminohexyl)-D-gluconamide did not inhibit the D-glucocerebrosidase. D-Gluconyl hydrazide was also the most effective inhibitor of human liver and spleen aryl β-D-glucosidase, 50% inhibition being achieved at 4 mM concentration (competitive inhibition, K_I = 0.4–0.9 mM). D-Galactonamide was the most effective inhibitor of spleen galactocerebrosidase; 4 mM D-galactonamide caused 50% inhibition of the enzyme activity (non-competitive inhibition). *N*-(6-Aminohexyl)-D-galactonamide is a potent inhibitor (90% inhibition, 5 mM) of G_{M_1}-ganglioside β-D-galactosidase but is without effect on D-galactocerebrosidase. It has, therefore, potential usefulness in distinguishing between two of the galactosphingolipid β-D-galactosidases.

[80] M. Brockhaus, H. Fritz, and J. Lehmann, *Carbohydr. Res.*, 1979, **68**, 225.
[81] H. L. Hoeksema, O. P. van Diffelen, and H. Galjaard, *Biochim. Biophys. Acta.*, 1979, **566**, 72.
[82] Y.-B. Chiao, K. Moffitt, Y. Smallwood, R. H. Glew, M. Naoi, and Y. C. Lee, *Arch. Biochem. Biophys.*, 1979, **192**, 1.

A pilot trial of enzyme replacement with human splenic and plasma α-D-galactosidase A isoenzymes was undertaken in two brothers with Fabry disease, an X-linked glycosphingolipid storage disease.[83] Six unentrapped doses (2000 units kg^{-1}) of each isoenzyme were administered intravenously to the respective recipient during a 117-day period. The circulating half-life of the splenic isoenzyme was about 10 min, whereas that for the plasma isoenzyme was approximately 70 min. No immune response was detected by skin and immunodiffusion tests or by alterations in the maximal activity or clearance kinetics for either isoenzyme after successive administrations. After each dose of the splenic isoenzyme, the concentration of the accumulated circulating substrate, trihexosylceramide (globotriaosylceramide), decreased maximally (≈50% of initial values) in 15 min and returned to pre-infusion levels by 2–3 hr. In marked contrast, injection of the plasma isoenzyme decreased the circulating substrate levels by 50–70% in 2–6 hr; the concentrations gradually returned to pre-infusion values by 36–72 hr.

β-D-Galactosidases from human placentae have been purified to homogeneity *via* fractional precipitation, affinity chromatography on a 4-aminophenyl 1-thio-β-D-galactopyranoside derivative of macroporous agarose, and gel filtration.[84] The last step separated the activity into two peaks (I and II), which were both devoid of D-galactosylceramide and lactosylceramide β-D-galactosidase activities. Enzymes I and II (mol. wts. $4.2–4.8 \times 10^5$ and 2.2×10^5 respectively) were inhibited by β-D-galactopyranosylamine, D-galactose, and D-galactono-1,4-lactone. During guanidinium chloride–sodium dodecyl sulphate polyacrylamide gel electrophoresis, in the presence of ethanethiol, II dissociated into a single protein (mol. wt. 7.7×10^4) and I into three bands (mol. wts. 7.7×10^4, 3.1×10^4, and 2.2×10^4). The presence of a common sub-unit between I and II was confirmed immunologically.

Investigation of the binding characteristics of urinary 'acid' β-D-2-acetamido-2-deoxyglucosidase, α-D-galactosidase, and α-L-fucosidase from patients with mucolipidosis II and mucolipidosis III to agarose-immobilized concanavalin A revealed a two- to ten-fold decrease in the proportion of enzyme (that absorbed on the lectin) activities from patients with mucolipidosis II and III[35] (see p. 424).

By cell fusion and a new single cell hydrolase assay technique, the complementation was observed between mucolipidosis II and two other hereditary lysosomal β-D-galactosidase-deficient disorders in humans: G_{M_1}-gangliosidosis type II and β-D-galactosidase deficient-type mucolipidosis.[85] The possible mechanisms with which abnormal ML-II β-D-galactosidase was modified and normalized by the other two different cell strains were discussed.

Decreased binding by the lectins concanavalin A and wheat-germ agglutinin was found for a number of 'acid' hydrolases (β-D-2-acetamido-2-deoxyhexo-

[83] R. J. Desnick, K. J. Dean, G. Grabowski, D. F. Bishop, and C. C. Sweeley, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5326.

[84] J. T. Lo, K. Mukerji, Y. C. Awathi, E. Hanada, K. Suzuki, and S. K. Srivastava, *J. Biol. Chem.*, 1979, **254**, 6710.

[85] S. Okada, T. Kato, H. Yabucchi, and Y. Okada, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 559.

sidase, α-L-fucosidase, α- and β-D-gluconidases, β-D-galactosidase and α-D-mannosidase) from skin fibroblasts of three unrelated patients with mucolipidosis II[29] (see p. 423).

Normal human leucocytes present three molecular forms of α-D-galactosidase, but using isoelectric focusing a new major form (pI = 4.0) characteristic of leucocytes, and two forms that exist in other tissues, forms (pI = 4.5) and II (pI = 4.0), have been found.[31] In normal human leucocytes form II corresponded to the only molecular form of β-D-2-acetamido-2-deoxygalactosidase. In leucocytes from patients with Fabry's disease, electrofocusing shows that the deficient (α-D-galactosidase) activity corresponds to forms I and IV, while the residual activity corresponds to form II.

Three forms of α-D-galactosidase from normal human leucocytes, active against 4-methylumbelliferyl α-D-galactopyranoside have been separated using a preparative electrofocusing technique: form I (pI = 5.00) and form II (pI = 4.5), which are known in other tissues as α-D-galactosidase A and α-D-galactosidase B, respectively, and a new major form, named form IV (pI = 3.95), which is characteristic of leucocytes.[32] In leucocytes from patients with Fabry's disease the deficient activity corresponds to forms I and IV, whereas the residual activity corresponds to form II. This single residual form seems to be identical to the normal α-D-acetamido-2-deoxy-D-galactosidase. In leucocytes the molecular forms of α-D-galactosidase from normal subjects and patients with Fabry's disease can be divided into two groups on the basis of their enzymatic and genetic properties. One group is made of form I (isoenzyme A) and of the new major form IV: heat-labile, with close K_m values for 4-methylumbelliferyl α-D-galactopyranoside (K_m I = 2.1×10^{-3} M and K_m IV = 1.9×10^{-3} M), inhibited by α-D-galactosides and *meso*-inositol, and inactive in Fabry's disease (coded by the same X-linked gene). The other group only concerns α-D-galactosidase form II (isoenzyme B) (or α-D-2-acetamido-2-deoxygalactosidase). This form II from normal subjects has the same properties as the residual one from Fabry's disease: heat-stable, close K_m values for 4-methylumbelliferyl α-D-galactopyranoside (K_m II = 5.1×10^{-3} M and K_m Fabry = 5.5×10^{-3} M) and for 4-nitrophenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (K_m normal = 1.7×10^{-3} M and K_m Fabry = 2.0×10^{-3} M), inhibited by α-D-galactosides and by 2-acetamido-2-deoxy-D-galactosides. This form is coded by a gene different from the X-linked gene.

The possibility of lowering the level of ceramide-3 [α-D-galactosyl-(1 → 4)-β-D-galactosyl-(1 → 4)-β-D-glucosyl-(1 → 1)-ceramide] in the plasma of patients with Fabry's disease was investigated, using an immobilized α-D-galactosidase prepared by coupling purified fig α-D-galactosidase to macroporous agarose.[86] The results suggest that lowering of the level of ceramide-3 in plasma from patients with Fabry's disease by enzymic means is not feasible.

Experiments showing that the presence of some monohydric alcohols in the solution medium perturbs the kinetics of bovine liver β-D-galactosidase have been reported.[87] The effect increases with increasing alcohol concentration and alkyl-

[86] A. W. Schram, M. N. Hamers, E. Oldenbroek-Haverkamp, A. Strijland, A. de Jonge, F. A. J. T. M. van den Bergh, and J. M. Tager, *Biochim. Biophys. Acta*, 1978, **527**, 456.
[87] L. Cordone, V. Izzo, G. Sgroi, and S. L. Fornili, *Biopolymers*, 1979, **18**, 1965.

group size. To explain this effect a model that considers the existence of functional and non-functional enzyme conformations in thermodynamical equilibrium was suggested. This equilibrium is dependent both on alcohol concentration and on alkyl-group size.

'Acid' β-D-galactosidase has been purified 19 000-fold from feline liver with a 13% recovery using a four-step procedure that involved (i) lectin chromatography on agarose-immobilized concanavalin A, (ii) ion-exchange chromatography, (iii) affinity chromatography on a 6-aminohexyl 1-thio-β-D-galactopyranoside derivative of agarose, and (iv) gel filtration.[88] The purified protein existed in two forms (mol. wts. 7.0×10^5 and 1.3×10^5). Molecular weight estimation by sucrose gradient centrifugation revealed a single 1.15×10^5 molecular weight form. Reduced and denatured β-D-galactosidase migrated as a single major band with an apparent molecular weight of 6.2×10^4 during polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. The purified protein demonstrated a single protein precipitin arc that coincided with a single arc of enzyme activity when examined by immunoelectrophoresis, using an antiserum to partially purified feline liver 'acid' β-D-galactosidase. Purified β-D-galactosidase hydrolysed synthetic β-D-galactosidases, α-L-arabinosides, and β-D-fucosides, as well as the β-linked D-galactose moieties of G_{M_1} ganglioside and asialofetuin. The enzyme also catalysed the transfer of galactose from 4-nitrophenyl β-D-galactopyranoside and G_{M_1} ganglioside to various carbohydrate acceptors.

Rabbit liver β-D-galactosidase has been fractioned into peaks of mol. wts. 12.0×10^4 and 2.7×10^4 by gel filtration, three peaks by ion-exchange chromatography, or into two peaks by isoelectric focusing.[89] Four electrophoretic bands could be resolved in the original liver supernatant and in all of the peaks obtained by chromatography or isoelectric focusing.

Seven renal glycoside hydrolases have been measured in four sub-cellular fractions prepared from highly inbred aglycosuric (*AV*-line) and glycosuric (*XA*-line) Chinese hamsters (*Cricetulus griseus*).[39] α- and β-D-Galactosidases were highest in the nuclear and supernatant fractions and both showed reduced activities in the *XA* animals.

Lactose 6′-*O*-sulphate neither behaved as a substrate nor acted as an inhibitor with respect to unsubstituted lactose and 4-nitrophenyl β-D-galactopyranoside when tested with β-D-galactosidase ('lactase') of suckling rat intestine and *Escherichia coli* β-D-galactosidase.[90]

α-D-Glucosidase ('maltase') and β-D-galactosidase ('lactase'), the predominant glycoside hydrolases associated with the intestinal brush-border membrane of the suckling rat, have been purified, essentially free from each other to near homogeneity and their specific physicochemical properties determined.[91] Antisera prepared to each showed, by immunodiffusion, a single common precipitin line with pure enzyme and solubilized proteins of the brush-border membrane. Brush-border membranes were purified from infant rat intestine, and 'lactase'

[88] E. W. Holmes and J. S. O'Brien, *Biochemistry*, 1979, **18**, 952.
[89] P. S. J. Cheetham, *Int. J. Biochem.*, 1977, **8**, 857.
[90] N. Mian, C. E. Anderson, and P. W. Kent, *Biochem. J.*, 1979, **181**, 387.
[91] K. K. Tsuboi, S. M. Schwartz, P. H. Burrill, L. K. Kwong, and P. Sunshine, *Biochim. Biophys. Acta*, 1979, **554**, 234.

and 'maltase' were estimated to comprise in excess of 10% and 2%, respectively, of the membrane protein. Immunotitration curves of 'lactase' and 'maltase' showed equivalent antibody binding by the membrane-bound and free enzyme forms. Furthermore, antibody binding to one enzyme did not affect the immunotitration curve or the extractability (papain or Triton X-100) of the other membrane-bound enzyme. It was concluded that the 'lactase' and 'maltase' molecules are attached singly on the external membrane surface in a spatially independent manner with their antigenic sites as freely available to antibody binding as exhibited by their papain-solubilized counterparts.

The rate of synthesis of β-D-galactosidase in mouse tissues is under control of a regulatory locus, *Bgl-s*, that is tightly linked to the enzyme structural gene.[92] The *cis–trans* nature of the *Bgl-s* action has been tested by analysis of the electrophoretic mobility of the enzyme from animals heterozygous for the appointment regulatory and structural alleles. Results indicated that *Bgl-s* acts *cis*, controlling the expression of the structural gene located on the same chromosome.

In connection with the structural study of D-galacto-L-fucan sulphate (sulphated polysaccharide of *Undaria pinnatifida*), D-galacto-L-fucanase and α-L-fucosidase and β-D-galactosidase existing in hepato-pancreas of abalone (*Haliotis* sp.) were investigated.[78] Fractionations were conducted using ion-exchange chromatography and gel filtration.

Sweet almond α-D-galactosidase has been shown to catalyse hydrolytic, synthetic (*de novo*), and transfer reactions.[93] Transfer products were formed using 4-nitrophenyl α-D-galactopyranoside as the D-galactosyl donor and D-glucose, D-galactose, sucrose, maltose, and lactose as acceptors; several of the products were identified. The enzyme also caused elongation of the oligosaccharide chain of two substrates (melibiose and raffinose). In addition, the enzyme catalysed condensation of free D-galactose, yielding oligosaccharides.

An α-D-galactosidase from alfalfa (common lucerne, *Medicago sativa*) seeds was purified 140-fold by ammonium sulphate fractionation and ion-exchange chromatography.[94] Polyacrylamide gel electrophoresis of the purified enzyme showed a single protein band (mol. wt. 5.7×10^4 by gel filtration). The purified enzyme hydrolysed 4-nitrophenyl α-D-galactopyranoside more rapidly than raffinose. The maximal enzyme activities were obtained at pH 4.0 and 5.5 for 4-nitrophenyl α-D-galactopyranoside, and at 4.5 for raffinose. The enzyme was shown to be inhibited by Hg^{2+} and Ag^{+} ions, and D-galactose.

The major phytohaemagglutinin from mung bean seeds after purification found to possess a strong enzymic activity (α-D-galactosidase).[95] The lectin and enzymic activities were co-purified by methods that would resolve components with significantly different size or charge properties. The purified extract contained a single tetrameric glycoprotein (mol. wt. 1.6×10^5) composed of identical sub-units (mol. wt. 4.5×10^4). The lectin and enzyme possessed

[92] F. G. Berger and K. Paigen, *Nature*, 1979, **282**, 314.
[93] P. M. Dey, *Phytochemistry*, 1979, **18**, 35.
[94] T. Itoh, S. Shimura, and S. Adachi, *Agric. Biol. Chem.*, 1979, **43**, 1499.
[95] C. N. Hankins and L. M. Shannon, *J. Biol. Chem.*, 1978, **253**, 7791.

indistinguishable carbohydrate specificities, were equally heat-labile in the presence and absence of sugars, and were equally sensitive to a thiol-directed agent, providing compelling evidence that the enzymic and lectin activities reside in a single species. Possible reasons as to why the enzymic nature of lectins has remained obscure are discussed.

The distribution of α-D-galactosidase in squash plants (*Cucurbita pepo*) has been determined in an attempt to assess its involvement in hydrolysis of transport sugars of the raffinose oligosaccharide series ([6-*O*-α-D-galactopyranosyl]$_n$-sucrose).[96] Extracts prepared from leaves and petioles at different stages of development, roots, flowers, dry and germinating seeds, all contained appreciable levels of α-D-galactosidase activity. Chromatography of these extracts on DEAE-Sephadex® resolved the enzyme into three active isoenzymic forms. These isoenzymes were present in all regions of the plant analysed but their relative proportions varied between tissues and changed within leaf and petiole tissues during development and in seeds during their germination. The level of total α-D-galactosidase activity in the leaf blade measured on a fresh weight or total protein basis remained constant at all developmental stages analysed. The occurrence of these isoenzymes in mature exporting leaves indicates an effective intracellular compartmentation between their location and the sites of D-galactosyl oligosaccharide biosynthesis, accumulation and movement in the tissue. These results were used to comment on the transport pathway of D-galactosyl oligosaccharides between the phloem and surrounding tissues in this plant.

Five α-D-galactosidases have been identified, by chromatography and by their different electrophoretic mobilities, in the germinated seeds of white clover (*Trifolium repens*).[97] α-D-Galactosidases II, III, and IV were purified to homogeneity, with increases in specific activity of approximately 4600-, 4900-, and 2800-fold, respectively. The enzymes were purified by a procedure that included ammonium sulphate precipitation, hydroxyapatite, Sephadex® G-75 and DEAE-cellulose chromatography, and preparative polyacrylamide gel disc electrophoresis. The purified enzymes showed a single protein band, corresponding to the α-D-galactosidase activity, when examined by polyacrylamide gel electrophoresis. The pH optimum was determined with 2-nitrophenyl α-D-galactopyranoside and the D-galacto-D-mannan of *T. repens* T_0 as substrates. All three enzymes are highly thermolabile. Hydrolysis of oligosaccharides and D-galacto-D-mannans was examined, including two D-galacto-D-mannans from the germinated seeds of *T. repens* (T24 and T36). By sodium dodecyl sulphate–polyacrylamide gel electrophoresis the molecular weights of the multiple forms of enzyme were found to be identical (4.1×10^4).

The use of Sephadex® columns equilibrated with sodium chloride to purify winter wheat leaf β-D-galactosidase has been described.[66]

The immunological relationship between β-D-galactosidases of different bacterial origins has been investigated.[98]

[96] B. Thomas and J. A. Webb, *Plant Physiol.*, 1978, **42**, 713.
[97] J. Williams, H. Villarroya, and F. Peter, *Biochem. J.*, 1978, **175**, 1069.
[98] N. Guiso, J. C. Hubert, and A. Ullmann, *Mol. Immunol.*, 1979, **16**, 409.

β-D-Galactosidase from *Aspergillus niger* has been purified and resolved into three multiple forms, using molecular sieving, ion-exchange, and hydrophobic chromatography.[99] The isolated enzyme forms accounted for 83%, 8%, and 9% of the total β-D-galactosidase activity, respectively. They were glycoproteins (mol. wts. 1.24×10^5, 1.5×10^5, 1.73×10^5, pI's 4.6, and pH optima 2.5–4.0). Amino-acid and carbohydrate analyses showed that multiplicity was mainly due to dissimilar carbohydrate contents (about 12.5%, 20.5%, and 29% neutral carbohydrates, respectively). The multiple form pattern might depend on the culture conditions. The β-D-galactosidase forms were heat-stable up to about 60 °C. The K_m values for lactose ranged from 85 mM to 125 mM, whereas for the synthetic substrate 2-nitrophenyl β-D-galactopyranoside were 2.4 mM. The slight reactional dissimilarities between the three enzyme forms are unlikely to be physiologically relevant. The biological significance of *A. niger* β-galactosidase multiplicity might be related to the observed differences in carbohydrate content, as suggested by recent reports on other microbial glycoprotein enzymes.

β-D-Galactosidase of Takadiastase (*ex Aspergillus oryzae*) has been purified by acetone fractionation and ion-exchange chromatographies.[49] Purity was confirmed by disc electrophoresis, ultracentrifugation, and measurement of other glycoside hydrolase activities which co-existed in Takadiastase. The molecular weight of the β-D-galactosidase was 1.12×10^5 by sedimentation and 5.6×10^4–5.9×10^4 by sodium dodecyl sulphate–disc electrophoresis, showing that the enzyme consisted of two sub-units. The enzyme was a glycoprotein containing 2-amino-2-deoxy-D-glucose and neutral sugar. Stability, pH optima, isoelectric point, and some specificities of the enzyme were determined.

A strain of *Bacillus* species was found to produce a β-D-galactosidase inducibly, and the induction of the enzyme was faster at pH 10.2 than at pH 7.2.[100] At pH 8.5, intact cells exhibited higher enzyme activity than toluenized cells. At 60 °C, pH 10.2 grown cells exhibited relatively higher enzyme activity than pH 7.6 grown cells. This difference in stabilization of the enzyme was explained by the difference in the outer surface under different culture conditions.

Further to the isolation and identification of an alkalophilic strain of a bacterium, which produced β-D-galactosidase inducibly, this bacterium, *Bacillus* No. C-125, was found to produce the enzyme not only in alkaline media but also in neutral media.[101] Comparative studies on the β-D-galactosidases (pH optima 6.5, temperature optima 44 °C, mol. wts. 1.85×10^5) produced in alkaline and in neutral media were investigated from a view point of the effects of pH value of the media. D-Galactose and melibiose at 10 mM inhibited the activity and heavy-metal ions such as Hg^{2+}, Ag^{+}, Pb^{2+}, Zn^{2+}, and Cu^{2+} almost completely inhibited the enzyme activity.

The properties of a β-D-galactosidase from thermophilic *Bacillus* have been studied and compared with the properties of the enzyme contained in whole

[99] F. Widmer and J.-L. Leuba, *Eur. J. Biochem.*, 1979, **100**, 559.
[100] Y. Ikura and K. Horikoshi, *Agric. Biol. Chem.*, 1979, **43**, 85.
[101] Y. Ikura and K. Horikoshi, *Agric. Biol. Chem.*, 1979, **43**, 1359.

cells, and in cells entrapped in a polyacrylamide gel matrix.[102] The partially purified enzyme appeared to be optimally active at a temperature of 60 °C and pH 6.0. The enzyme activities from whole cells and immobilized whole cells had slightly different temperature optima (75 and 65 °C respectively) and pH optima (6.2 and 6.6 respectively). The enzyme activity from whole cells was significantly more thermostable than that of the partially purified enzyme (half-lives 125 and 2 min respectively at 70 °C). In other properties studied, the enzyme activities of the three sources were similar. The thermostability of the enzyme and the broad pH range over which it is active makes this enzyme suitable for use in the dairy industry.

A 1000-litre fermenter has been used to produce a continuous feed of *Escherichia coli* containing a high level of β-D-galactosidase.[103] Investigation of the individual unit operations for the isolation of the enzyme, cell disruption, nucleic acid removal, protein precipitation and solid–liquid separation after each stage, permitted operation of a semi-continuous process which would yield 100 g protein hr^{-1} comprising 23% β-D-galactosidase.

Sedimentation analyses of an activatable mutant β-D-galactosidase from *E. coli* and of the products of its reaction with Fab fragments of activating antibody showed that the enzyme exists mainly as 10 S dimers.[104] Activations by antibody resulted in formation of 16 S tetramers. A hypothesis postulating a dimer–tetramer equilibrium accounts for this observation as the counterpart of inactivation.

α-Complementation in *E. coli* β-D-galactosidase is the restoration of enzyme activity by addition of the α-donor 'CNBr2' polypeptide, from amino-acid residues 3–92 of the polypeptide, to inactive M15 protein from the *lacZ* deletion mutant strain M15.[105] M15 protein lacks residues 11–41 and is a dimer; the active complex, like native β-D-galactosidase, is tetrameric. A dimer–dimer binding region in β-D-galactosidase has been identified by proteolytic and immunologic studies of α-complementation. Proteolytic experiments were carried out with trypsin. Treatment of native β-D-galactosidase with trypsin, followed by treatment of the mixture with cyanogen bromide, yields intact CNBr2 as measured by its ability to complement M15 protein. Active CNBr2 is not obtained when urea-denatured β-D-galactosidase is treated in the same way. Therefore the segment corresponding to CNBr2 is apparently buried within the folded protein. Immunologic experiments were carried out with antibodies against CNBr2, tryptic peptide T8 (residues 60–140), and CNBr2 (residues 93–187). Anti-CNBr2 and anti-T8 bind to M15 protein but not to β-D-galactosidase, indicating that this area is exposed in the dimer. Anti-CNBr2, but not anti-T8 or anti-CNBr3, inhibits the formation of α-complemented enzyme. These results indicate that an early part of the sequence, within the segment corresponding to CNBr2, is involved in dimer–dimer interaction.

[102] M. W. Griffiths and D. D. Muir, *J. Sci. Food Agric.*, 1978, **29**, 753.
[103] J. J. Higgins, D. J. Lewis, W. H. Daly, F. G. Mosqueira, P. Dunnill, and M. D. Lilly, *Biotechnol. Bioeng.*, 1978, **20**, 159.
[104] E. Conwaydemacario, J. Ellis, R. Guzman, and B. Rotman, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 720.
[105] F. Celada and I. Zabin, *Biochemistry*, 1979, **18**, 404.

A protein processing both *lac* repressor and β-D-galactosidase activities in a single polypeptide (mol. wt. 1.55×10^5) has been purified from a deletion mutant of *E. coli* in which the *lacI* and *Z* genes are fused.[106] A 77-residue cyanogen bromide-produced peptide containing a fusion joint was isolated. The site of joining is from residue 355 of *lac* repressor to residue 24 of β-D-galactosidase (or 356 to 25), indicating that the last four residues at the *C*-terminus of *lac* repressor and the first 23 residues of the *N*-terminus of β-D-galactosidase are not essential for the activities of these two proteins. The exact site of the fusion was not known because *lac* repressor residue 356 and β-D-galactosidase residue 24 are both L-leucine residues.

Carboxymethylation with [^{14}C]iodoacetate of L-cysteine residues in wild-type β-D-galactosidase from *E. coli*, and in a defective β-D-galactosidase from deletion mutant strain M15 has been investigated in order to determine accessible positions in the tetrameric wild-type form and the dimeric mutant M15 protein.[107] The extent of carboxymethylation, the effects on biological activity, antibody activation, physical stability, and the labelling of particular residues were studied. The results distinguish three groups of spatial relationships for L-cysteine residues in the protein, define possible regions for sub-unit interactions, and confirm that no L-cysteine residue is specifically involved in catalysis. Residue 1019, and to a lesser extent 498, are accessible in the tetrameric protein and probably represent exposed areas. In the M15 protein these two, and three additional residues, at 76, 387, and 600, were found to react significantly with reagent. One or more of the latter are suggested to be in the dimer–dimer interface. Complementation and activation by antibody are inhibited by carboxymethylation of M15 protein.

Antibodies were prepared against 18 tryptic and cyanogen bromide peptides from *E. coli* β-D-galactosidase ranging in size from 15 to 96 amino-acid residues representing more than 80% of the polypeptide chain.[108] They were tested for binding capacity and affinity toward their homologous antigens and toward the whole native protein. Nine antisera became bound to β-D-galactosidase; these had been raised against certain peptides from the central and terminal regions of the polypeptide chain. Based on these results a preliminary model of the three-dimensional structure of the folded protein is suggested.

The location of the bivalent metal cation with respect to bound competitive inhibitors in *E. coli* (*lacZ*) β-D-galactosidase has been investigated by proton magnetic resonance.[109] Replacement of Mg^{2+} by Mn^{2+} enhances both longitudinal and transverse relaxation of the methyl groups of the β-D-galactopyranosyltrimethylammonium ion, and of methyl 1-thio-β-D-galactopyranoside; linewidths are narrowed by increasing temperature. The Mn^{2+} ion is located 8–9 Å (0.8–0.9 nm) from the centroid of the trimethylammonium group and 9 Å (0.9 nm) from the average position of the methylthio protons. The

[106] A. J. Brake, A. V. Fowler, I. Zabin, J. Kania, and B. Mullerhill, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 4824.
[107] H. Jornvall, A. V. Fowler, and I. Zabin, *Biochemistry*, 1978, **17**, 5160.
[108] F. Celada, A. V. Fowler, and I. Zabin, *Biochemistry*, 1978, **17**, 5156.
[109] R. S. T. Loeffler, M. L. Sinnoit, B. D. Sykes, and S. G. Withers, *Biochem. J.*, 1979, **177**, 145.

effective charge at the active site was probed by measurement of competitive inhibition constants (K_i^0 and K_i^+ respectively) for the isosteric ligands, β-D-galactopyranosylbenzene and the β-D-galactopyranosylpyridinium ion. The ratio of inhibition constants ($Q = K_i^+/K_i^0$) obtained with 2-(β-D-galactopyranosyl)-naphthalene and the β-D-galactopyranosylisoquinolinium ion at pH 7 with Mg^{2+}-enzyme was identical, within experimental error, with that obtained with the monocyclic compounds. The variation of Q for Mg^{2+}-enzyme can be described by

$$Q = 0.1\left(1 + \frac{[H^+]}{4.17 \times 10^{-10}}\right)\left(1 + \frac{[H^+]}{10^{-8}}\right)^{-1}$$

This, in the theoretical form for a single ionizable group, is ascribed to the ionization of the phenolic hydroxy-group of L-tyrosine-501. The variation of Q for Mg^{2+}-free enzyme is complex, probably because of deprotonation of the groups normally attached to Mg^{2+} as well as L-tyrosine-501.

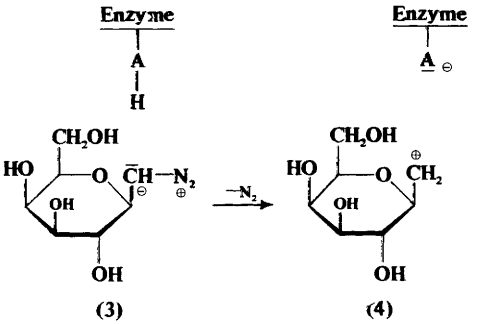
Although the addition of various divalent metals to *E. coli* β-D-galactosidase resulted in apparent activation, only Mg^{2+} and Mn^{2+} actually did activate.[110] The apparent activation by the other divalent metals was shown to be due to Mg^{2+} impurities. Calcium did not activate, but experiments suggested that it did bind. Other divalent metals which were studied failed to bind. The dissociation constants for Mg^{2+} and Mn^{2+} were 2.8×10^{-7} and 1.1×10^{-8} M, respectively, and in each case one ion became bound per monomer. These constants corresponded very closely to apparent values which were obtained from activation studies. The apparent binding constant for Ca^{2+}, obtained from competition studies, was 1.5×10^{-5} M. Data were obtained which showed that Mg^{2+}, Mn^{2+}, and Ca^{2+} all compete for binding at a single site. Of interest and of possible molecular biological importance was the observation that, while Mg^{2+} bound non-co-operatively ($n = 1.0$), Mn^{2+} did so in a highly co-operative manner ($n = 3.4$). The binding of Mn^{2+} (as compared to Mg^{2+}) resulted in a two-fold drop in the V_{max} for the hydrolysis and transgalactosylis reactions of lactose but had little effect on the V_{max} of hydrolysis of allolactose, or 2- or 4-nitrophenyl β-D-galacto-pyranosides; K_m values were not affected differently for any of the substrates by Mn^{2+} as compared to Mg^{2+}. When very low levels of divalent metal ions were present (0.01 M H_4 edta added) or when Ca^{2+} was bound with lactose as the substrate, a greater decrease was observed in the rate of the transgalactosylic reaction than in the rate of the hydrolytic reaction, and the K_m values for lactose and 2-nitrophenyl β-D-galactopyranoside were increased. Of the three divalent-metal ions which bound to β-D-galactosidase, only Mn^{2+} had significant stabilizing effects toward denaturing urea and heat conditions.

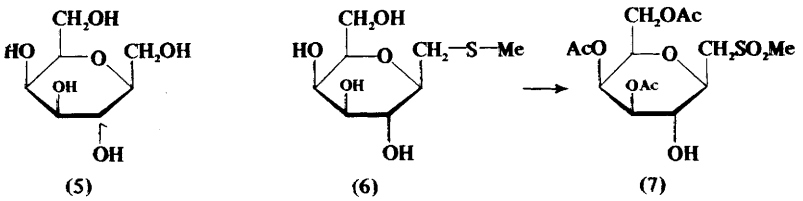
A very detailed study of the involvement of ion and ion-pair phenomena in the catalysis of *lacZ* β-D-galactosidase of *E. coli* considers: the nature of the D-galactosyl–enzyme complex, the number and nature of the molecular events leading to it, and the formation of the D-galactosyl–enzyme complex being faster than the hydrolysis of D-galactosyl derivatives in free solution.[111]

[110] R. E. Huber, C. Parfett, H. Woulfe-Flanagan, and D. J. Thompson, *Biochemistry*, 1979, **18**, 4090.

[111] M. L. Sinnot, *FEBS Lett.*, 1978, **94**, 1.

Ester and sulphonium salt formation in the active-site labelling of β-D-galactosidase from *E. coli* by 2,6-anhydro-1-deoxy-1-diazo-D-*glycero*-L-*manno*-heptitol has been examined.[112] 2,6-Anhydro-1-deoxy-1-diazo-D-*glycero*-L-*manno*-heptitol (3) is an efficient, active-site-directed, irreversible inhibitor of β-D-galactosidase from *E. coli*. It was considered that its effect on a hypothetical proton-donating group 'AH', normally responsible for the protonation of the glycosidic oxygen atom, should be that of a suicide substrate. Compound (3), which bears close steric as well as polar resemblance to a β-D-galactoside, was also expected to be bound to the enzyme in much the same way. Protonation by 'AH' of its C-1 atom creates a highly reactive carbonium ion (4). It is reasonable to assume that (4) will react with the conjugate base of 'AH'. Experiments proved that (3) not only reacted as expected with the acid 'AH', but also with the thioether group of a methionine residue to yield a sulphonium salt. A methionine residue was also labelled in the active-site-directed inactivation of β-D-galactosidase with *N*-bromoacetyl-β-D-galactopyranosylamine, *N*-bromoacetyl-β-D-glucopyranosylamine, and 3-(β-D-galactopyranosylmethyl)-1-(4-nitrophenyl)triazine. It was suggested that methionine is not involved as a reactive group in the catalytic action of β-D-galactosidase. It is either situated close to the actual protonating carboxylic acid 'AH', and is therefore able to compete for the carbonium ion (4) because of its superior nucleophilicity, or it may be part of another binding-site, possibly the aglycon binding-site, where it becomes alkylated independently.





[112] M. Brockhaus and J. Lehmann, *Carbohydr. Res.*, 1978, 63, 301.

The effects of D-glucose and D-glucose 6-phosphate in initiating the repression of β-D-galactosidase biosynthesis have been studied using a mutant of *E. coli* K12 which lacks D-glucose-specific enzyme II of the phosphoenolpyruvate–sugar phosphotransferase system.[113] It was found that D-glucose 6-phosphate caused transient repression of β-D-galactosidase synthesis but D-glucose did not cause transient repression in this mutant. Evidence was obtained that both the presence of an active transport system for D-glucose 6-phosphate in the cells and D-glucose 6-phosphate in the medium are necessary for the initiation of transient repression.

A number of abnormal polypeptides which are products of the *lacZ* gene in *E. coli* have been characterized.[114] A variety of experiments indicated that one class of at least nine different fragments arises from premature termination of protein synthesis. They have been detected as products of protein synthesis *in vitro* and *in vivo*, although the percentage of fragments made *in vitro* is greater than *in vivo*. The percentage of total *Z* gene-encoded protein which is found as fragments is estimated to be roughly 23% *in vivo*; *i.e.* about 31% of the total number of β-D-galactosidase monomers synthesized are prematurely terminated molecules. The average molecular weight of 9.1×10^4 of the polypeptides produced corresponds to a termination event occurring on average one every 3200 codons. Polypeptides synthesized *in vitro* and *in vivo* from templates containing mutations in the *Z* gene have also been compared. Most of the mutationally generated fragments synthesized *in vitro* are stable, unlike their *in vivo* counterparts which are often rapidly degraded.

Internal re-initiation polypeptides which are products of the *lacZ* gene of *E. coli* have been synthesized in a DNA-directed cell-free protein synthesis system.[115] The *de novo* biosynthesized re-initiation proteins, unlike both *in vitro* biosynthesized wild-type β-D-galactosidase and nonsense termination fragments, were insoluble when biosynthesized at 37 °C, but soluble if biosynthesis took place at 25 °C. Both *in vivo* and *in vitro*, the re-initiation proteins are not synthesized from DNA containing a wild-type *Z* gene, but require a specific nonsense mutation in order to be expressed. Furthermore, the location of the mutation within the *Z* gene is very important in determining whether or not re-initiation will occur at a given site. Several nonsense mutations which do not result in the synthesis of detectable amounts of restart protein *in vivo* produce specific re-initiation polypeptides *in vitro*. Experiments showed that when complicating factors, such as polarity and protein degradation, are eliminated, translational re-initiation can be detected at many sites in the *lacZ* gene.

The replication defective transducing phage λ*plac5O29P3* carries a portion of the *E. coli lac* operon in the *b2* region of the lambda phage.[116] This *lac* operon segment contains the *lac* promoter, the *lac* operator, and the β-D-galactosidase *z* gene, but does not contain the *lac* repressor *i* gene. The *z* gene can be expressed from both the inserted *lac* promoter and the phage promoter. When *E. coli*

[113] H. Kanazawa and Y. Anraku, *J. Biochem. (Tokyo)*, 1978, **83**, 1337.
[114] J. L. Manley, *J. Mol. Biol.*, 1978, **125**, 407.
[115] J. L. Manley, *J. Mol. Biol.*, 1978, **125**, 449.
[116] K. C. Luk and K. Mark, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 64.

strain 594 (z^-, i^+) or JC6256 (Δlac) is infected by $\lambda plac5O29P3$ in the absence of additional adenosine 3′,5′-phosphate, β-D-galactosidase synthesis is shown to be expressed from the phage promoter. When 594 (λ^+) or JC6256 (λ^+) is infected by $\lambda plac5O29P3$ in the presence of additional adenosine 3′,5′-phosphate, β-D-galactosidase synthesis is shown to be expressed from the inserted *lac* promoter. The ability to separate the phage promoter from the inserted *lac* promoter for β-D-galactosidase expression will simplify the interpretation whenever $\lambda plac5$ is used.

Localization of α-D-galactosidase in an alkalophilic strain of *Micrococcus* was investigated in relation to the cell membrane as a permeability barrier.[117] The α-D-galactosidase appeared to be mostly intracellular; only about 4% of α-D-galactosidase was released by lysozyme or freeze–thaw treatments of the whole cells. The enzyme activity was not inhibited by treatment of the whole cells with diazo-7-amino-1,3-naphthalene disulphonic acid, which penetrated the cell wall but not the cytoplasmic membrane. The enzyme activity of the whole cells increased about four-fold by toluene–acetone treatment which caused an alteration in the membrane permeability. The enzyme in such cells became to be relatively sensitive to pH. These results showed that cell membrane played a protective role as a permeability barrier against alkaline environment.

'Lactase' (β-D-galactosidase) from the yeast *Saccharomyces lactis* was tested for activity at −9, −13, −19, −23, and −30 °C.[118] After 1, 4, 10, and 30 days storage at each temperature, the percent lactase remaining was determined. There was no lactase activity in samples frozen at −19, −23, and −30 °C; however, there was significant hydrolysis of lactose at −9 and −13 °C.

The distributions of β-D-galactosidase and 6-phospho-β-D-galactosidase activities among 40 strains of lactic streptococci (*Streptococcus cremosis, S. lactis, S. diacetilactis, etc.*) have been examined.[119] In general, the abilities of the organisms to hydrolyse 2-nitrophenyl β-D-galactopyranoside 6-phosphate were far higher than their abilities to hydrolyse 2-nitrophenyl β-D-galactopyranoside and it was concluded that the main enzyme involved in lactose utilization by lactic streptococci is β-phospho-D-galactosidase, the lactose being pre-phosphorylated in the transport system.

α-D-Galactosidase has been immobilized with retention of enzymic activity by treatment with derivatized macroporous agarose.[86] β-D-Galatosidases have been immobilized with retention of enzymic activity by treatment with glucagon and its fragment peptides using *meta*-maleimidobenzoyl *N*-hydroxysuccinimide ester as a coupling reagent,[120] and by entrapment in crosslinked polyacrylamide gel beads.[121] Immobilization but with added retention of solubility of the β-D-galactosidase was achieved by conjugation with rabbit (anti-human immunoglobulin G) immunoglobulin G and Fab′ antibodies.[122]

[117] T. Akiba and K. Horikoshi, *Agric. Biol. Chem.*, 1978, **42**, 2091.
[118] R. J. Baer and M. Lowenstein, *J. Dairy Sci.*, 1979, **62**, 1041.
[119] T. Okamoto and T. Morichi, *Agric. Biol. Chem.*, 1979, **43**, 2389.
[120] S. Iwasa, H. Ueno, T. Miya, M. Wakimasu, K. Kondo, and A. Ohneda, *J. Biochem. (Tokyo)*, 1979, **86**, 943.
[121] K. Ohmiya, C. Terao, S. Shimizu, and T. Kobayashi, *Agric. Biol. Chem.*, 1975, **39**, 491.
[122] Y. Hamoguchi, S. Yoshitake, E. Ishikawa, Y. Endo, and S. Ohtaki, *J. Biochem. (Tokyo)*, 1979, **85**, 1289.

7 α- and β-D-Glucosidases

The common identity of human 'acid' β-D-glucosidase and β-D-xylosidase is indicated by similar binding patterns of the glycoside hydrolase activities of each enzyme to various lectins, by similar ratios between their intra- and extracellular levels in normal and I-cell fibroblasts, and by their deficiencies in liver tissues from patients with Gaucher disease.[54]

D-Gluconamide, D-gluconyl hydrazide, and *N*-(6-aminohexyl)-D-gluconamide have been tested for their inhibitory action on human liver lysosomal glucocerebrosidase and human spleen 'neutral' aryl β-D-glucosidase[82] (see p. 437).

Anomeric forms of D-glucose produced from phenyl α-maltoside, maltose, or phenyl α-D-glucopyranoside have been determined quantitatively by simultaneous measurements of optical rotation and reducing power, for eight kinds of D-glucose-producing (1 → 4)-α-D-glucosyl hydrolases [*exo*-(1 → 4)-α-D-glucanases] including D-glucose-forming amylase from human urine, and α-D-glucosidases from pig serum, honey bee, buckwheat seed, rice seed, sugar-beet seed, flint-corn seed, and brewer's yeast. All the eight enzymes studied were found to produce α-D-glucopyranose exclusively.[123]

Decreased binding by the lectins concanavalin A and wheat-germ agglutinin was found for a number of acidic hydrolases (β-D-2-acetamido-2-deoxyhexosidase, α-L-fucosidase, α- and β-D-glucosidases, β-D-galactosidase and α-D-mannosidase) from skin fibroblasts of three unrelated patients with mucolipidosis II[29] (see p. 423).

A search for lysosomal hydrolases and related enzymes has been made in haemolysates from human and rabbit red cells.[33] Apart from acid phosphatases, significant activities were found only for α-D-mannosidase, 'neutral' α-D-glucosidase and β-D-2-acetamido-2-deoxyhexosidase.

The isoenzymes of α-D-glucosidases have been studied in human white blood cells by immunological and electrophoretic techniques.[124] Three isoenzymes have been found in leucocyte extracts: lysosomal α-D-glucosidase ('acid' maltase, glucoamylase), cytoplasmic 'neutral' α-D-glucosidase, and an enzyme immunologically identical to renal 'maltase' (α-D-glucosidase). In Pompe's disease and other types of 'acid maltase' deficiency, the deficiency is as complete in leucocytes as in other tissues, and the residual activity is due to 'renal' 'maltase'.

In a study of the processing of carbohydrate moieties of glycoproteins in calf thymus, the occurrence of a microsomal enzyme which can release D-glucose selectively from the dolichol-linked oligosaccharide, or from the oligosaccharide after transfer to endogenous protein, was demonstrated.[125] The enzyme (pH optimum 6.0–7.0) was unaffected by H_4edta and uninhibited by any of a large range of α- and β-D-glucosides but marked stimulation of D-glucose release was observed in the presence of antichaotropic ions, and the specificity, pH optimum and low K_m value of the enzyme distinguished it from other thyroid D-glucosidases. Substrate structural studies indicated the enzyme to be an α-D-

[123] S. Chiba, K. Hiromi, N. Minamiura, M. Ohnishi, T. Shimomura, K. Suga, T. Suganuma, A. Tanaka, S. Tomioka, and T. Yamamoto, *J. Biochem. (Tokyo)*, 1979, **85**, 1135.
[124] J. Dreyfus and L. Poenaru, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 615.
[125] R. G. Spiro, M. J. Spiro, and V. D. Bhoyrod, *J. Biol. Chem.*, 1979, **254**, 7659.

glucosidase, and it was suggested that, in addition to initiating processing of the oligosaccharide after its transfer to protein, the enzyme may also control the level of D-glucose-containing oligosaccharide-lipids available for protein glycosylation. Further studies on the glycosylation step and the involvement of the α-D-glucosidase have been reported.

The anomeric configuration of the products of reactions catalysed by glucosidases from bovine liver and the mold fungus *Aspergillus awamori* was determined.[126] It was found that the enzyme from bovine liver is an α-D-glucosidase and that from *A. awamori* an *exo*-D-glucanase. It was shown that the α-D-glucosidase hydrolyses the C_1—O bond in the course of the reaction; D-glucono-1,4-lactone is a competitive inhibitor of both enzymes. The secondary kinetic isotope effects were measured and the isotope effect for α-D-glucosidase was found to be equal to 1.00 and that for *exo*-(1 → 4)-α-D-glucanase 1.10 relative to glycogen and 1.18 relative to maltose. Some aspects of the mechanism of the reactions catalysed by the two enzymes are discussed on the basis of these data.

Kinetic studies on the active site of pig serum α-D-glucosidase and buckwheat α-D-glucosidase catalysing the hydrolyses of maltose and soluble starch were made.[127] The kinetic features obtained by the experiments with the mixed substrates, the linearity of Lineweaver–Burk plots, and the dependence of the apparent Michaelis constants and the apparent maximal velocities on the fraction f of maltose in the mixed substrate solutions, that is f = maltose/(maltose + soluble starch), were in good agreement with those expected for the single active-site catalysing the hydrolyses of both substrates. From the results it was concluded that these enzymes attacked maltose and soluble starch by the single active-site mechanism.

Pure rat intestinal α-D-glucosidase–glucoamylase ('maltase'–glucoamylase) was partially inactivated in 1% sodium dodecyl sulphate by heating at 40–70 °C for 5 min at pH 7.5 or by lowering the pH to 5.4–6.6 at 24 °C.[128] When these partially active preparations were electrophoresed in the presence of sodium dodecyl sulphate, a complicated protein band pattern of incompletely dissociated fragments of the enzyme was observed. Complete dissociation of the enzyme in sodium dodecyl sulphate, induced by boiling or by pH values below 5.4, was accompanied by total loss of enzyme activity and simplification of the protein pattern to five major species. Although the original enzyme band was absent from some partially dissociated preparations, enzyme activity was present and was associated with several transient protein bands on the gels. 'Maltase' and α-D-glucosidase activities were detected in these bands, but glucoamylase activity was absent.

Oligo-1,6-D-glucosidase–sucrose α-D-glucohydrolase ('sucrase–isomaltase') is an intestinal membrane enzyme consisting of two active moieties, each with its hydrolytic site available for nutrient digestion at the luminal-cell interface.[129]

[126] L. M. Firsov, *Biochemistry*, 1978, **43**, 1757.
[127] S. Chiba, N. Hibi, K. Kanaya, and T. Shimomura, *Agric. Biol. Chem.*, 1977, **41**, 1245.
[128] P. R. Flanagan and G. G. Forstner, *Biochem. J.*, 1979, **177**, 487.
[129] J. P. Cezard, K. A. Conklin, B. C. Das, and G. M. Gray, *J. Biol. Chem.*, 1979, **254**, 8969.

At least 90% of hydrolytic activity can be localized to the brush-border membrane and the remainder in the cytoplasm has been considered to originate from brush border contamination. The intracellular cytosol from rat jejunum was examined for the presence of a precursor of the fully formed brush border membrane enzyme. The cytosol contained 1–4% of the total cellular sucrose α-D-glucohydrolase activity. Whereas brush-border sucrose α-D-glucohydrolase is essentially all (99%) in the form of oligo-1,6-D-glucosidase–sucrose α-D-glucohydrolase hybrid, the predominant form in the cytosol was free sucrose α-D-glucohydrolase (60–85%). Smaller amounts of free oligo-1,6-D-glucosidase were also identified. As estimated from gel filtration chromatography and density gradient centrifugation, sucrose α-D-glucohydrolase, free sucrose α-D-glucohydrolase, and free oligo-1,6-D-glucosidase in the cytosol were distinctly smaller in molecular weight (-2.0×10^4/enzyme moiety) than the corresponding membrane enzymes. Like their brush-border counterparts, the cytosol sucrose α-D-glucohydrolases bound nearly completely to agarose concanavalin A immobilized. The intracellular cytosol sucrose α-D-glucohydrolases therefore appear to be closely related to brush-border membrane oligo-1,6-D-glucosidase–sucrose α-D-glucohydrolase and have the appropriate characteristics to serve as intracellular precursors for the fully formed brush-border membrane enzyme at some stage of the intracellular assembly process.

α-D-Glucosidase and β-D-galactosidase ('maltase' and 'lactase'), the predominant glycoside hydrolases associated with the intestinal brush-border membrane of the suckling rat, have been purified essentially free from each other to near homogeneity and their specific physicochemical properties determined[91] (see p. 440).

Evidence has been presented for a similar distribution of α-D-glucosidase activities in subcellular fractions of rat and trout livers.[130]

L-Asparagine-linked oligosaccharides of glycoproteins undergo extensive modification or 'processing' following their attachment to protein.[131] A key step in post-glycosylation processing is the sequential removal of D-glucose residues from the protein-linked oligosaccharide. Rat liver preparations which catalyse removal of D-glucose from (8)–(10) were studied.

$$\text{D-Glc}_3\text{-D-Man}_9\text{-D-GlcNAc}$$

(8)

$$\text{D-Glc}_2\text{-D-Man}_9\text{-D-GlcNAc}$$

(9)

$$\text{D-Glc}_1\text{-D-Man}_9\text{-D-GlcNAc}$$

(10

Detergent solubilization studies, inhibitor studies, and temperature-activity profiles indicate that at least two distinct D-glucosidases are present in the membranes. One of these D-glucosidases removes the distal D-glucose from (8), and the other D-glucosidase sequentially removes D-glucose from (9) and (10).

[130] C. Castilla, H. Paris, and J. C. Murat, *C. R. Seances Soc. Biol. Ses Fil.*, 1978, **172**, 968.
[131] L. S. Grinna and P. W. Robbins, *J. Biol. Chem.*, 1979, **254**, 8814.

The latter D-glucosidase has been solubilized from the microsomal membranes and purified 12-fold. The D-glucosidases, which are integral membrane proteins, are localized in the rough and smooth microsomes and appear to be located on the cisternal surface of the microsomal vesicles. These D-glucosidases are suggested to be of biological importance in catalysing the initial events in the post-glycosylation processing of cellular glycoprotein.

Levels of D-glucosyl ceramidase in mice dropped very rapidly after conduritol B epoxide injection, reaching a minimum after 1 hr in liver.[132] There was a noticeable restoration of activity within the next 2–3 hr. The level of aryl β-D-glucosidase decreased somewhat less than that of cerebrosidase, reaching a minimum within 2 h; it too showed some recovery of activity within 3 hr.

Injection of a single dose of conduritol B epoxide into mice produced almost complete destruction of D-glucosylceramidase in liver, spleen, brain, and kidney within 5 hr.[133] Restoration of activity became noticeable within 1 day (2 days in the case of brain) and was about 80% of normal within 16 days. The same injection produced less destruction of aryl β-D-glucosidase, measured at pH 5.4 with 4-methylumbelliferyl β-D-glucopyranoside in the absence of taurocholate. Brain showed the least amount of destruction, about 50%, but measurements of activity at lower pH values revealed complete loss of activity. This suggests that brain contains two different aryl β-D-glucosidases with different sensitivity to the inhibitor. Liver, on the other hand, did not show differential destruction when assayed at different pH values. Re-biosynthesis of the enzyme activities was almost complete by 16 days. Injection of phenylhydrazine produced haemolysis and spleen enlargement, with concomitant increases in specific activities of D-glucocerebrosidase and aryl β-D-glucosidase in liver and spleen (but not in kidney). When this experiment was done in mice previously treated with conduritol B epoxide, the reappearance of cerebrosidase was found to be accelerated. This is interpreted to mean that the increased load of glucolipids from the erythrocytes had induced an enhanced synthesis of the D-glucohydrolase. A similar explanation may apply to aryl β-D-glucosidase and D-glucopeptides in the cells.

Two kinds of α-D-glucosidase have been found in chick embryos.[134] One of them is an enzyme that hydrolyses maltose, and the other is one that releases D-glucose from 2-*O*-α-D-glucopyranosyl-D-galactose linked to L-hydroxylysine. The latter was purified from an ultracentrifuged supernatant fraction of chick-embryo homogenate by affinity chromatography using 2-amino-2-deoxy-D-glucose-agarose as an adsorbent. The enzyme was eluted from the adsorbent specifically with 0.1 M 2-amino-2-deoxy-D-glucose. A highly purified α-D-glucosidase was obtained with a specific activity 30 000 times that in the ultracentrifuged supernatant fraction. The enzyme preparation appeared to be homogeneous in gel filtration and sodium dodecyl sulphate polyacrylamide gel electrophoresis. The molecular weight of this enzyme was estimated at 6.5×10^4 and 7.5×10^4 using gel filtration and sodium dodecyl sulphate polyacrylamide gel electrophoresis, respectively. The enzyme may have no isoenzyme forms, as

[132] A. Hara and N. S. Radin, *Biochim. Biophys. Acta*, 1979, **582**, 423.
[133] A. Hara and N. S. Radin, *Biochim. Biophys. Acta*, 1979, **582**, 412.
[134] K. Kanaya, S. Chiba, and T. Shimomura, *Agric. Biol. Chem.*, 1979, **43**, 1841.

judged by isoelectric focusing. The enzyme did not catalyse the hydrolysis of maltose, methyl α-D-glucopyranoside, or 4-nitrophenyl α-D-glucopyranoside. The enzyme had a pH optimum around 5.3 and K_m value of 2.2 mM with 2-*O*-α-D-glucopyransyl-*O*-β-D-galactopyranosyl-L-hydroxylysine (Glc-Gal-Hyl) as a substrate. The ε-amino-group of L-hydroxylysine was assumed to be essential to this substrate since the hydrolysis rate was reduced greatly when the amino-groups of Glc-Gal-Hyl were acetylated, and since the enzyme liberated D-glucose from basement membrane. The enzyme was inhibited competitively by 2-amino-2-deoxy-D-glucose and -D-mannose, but not by 2-amino-2-deoxy-D-galactose, 2-acetamido-2-deoxyhexose, or 2-amino-2-deoxy-D-glucitol. K_i value of 2-amino-2-deoxy-D-glucose and -D-mannose at pH 5.3 were 160 and 5.6 μM, respectively. Cu^{2+} and Zn^{2+} at a concentration of 1 mM inhibited the enzyme, whereas Mg^{2+}, Ca^{2+}, Mn^{2+}, and H_4edta had no effect. It was concluded that this enzyme probably functions in degradation of the L-hydroxylysine-linked disaccharide derived from collagen.

The crystalline style of the gastropod *Telescopium telescopium* has been shown to contain a β-D-glucosidase and two (1 → 3)-β-D-glucanases.[135]

The amino-acid residues(s) involved in the activity of buckwheat α-D-glucosidase was modified by 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide in the presence of glycine ethyl ester.[136] The modification resulted in the decrease in the hydrolytic activity of the enzyme following pseudo-first order kinetics. Competitive inhibitors, such as tris and turanose, protected the enzyme against the inactivation. Protection was provided also by alkali metal, alkaline-earth metal, and ammonium ions, though these cations are non-essential for the activity of the enzyme. Turanose or K^+ protected one carboxy-group per enzyme from the modification with carbodi-imide and glycine ethyl ester. The free third group of the enzyme was also partially modified with carbodi-imide, but the inactivation was considered to be mainly attributed to the modification of essential carboxy-group, rather than to that of the free third group.

Buckwheat α-D-glucosidase showed especially high activity toward malto-oligosaccharides, phenyl α-maltoside and nigerose.[137] Its activities towards isomaltose and phenyl α-D-glucopyranoside were very weak. The ratios of the maximum velocities for maltose, nigerose, kojibiose, isomaltose, phenyl α-D-glucopyranoside and phenyl α-maltoside were estimated to be 100: 101: 17: 2: 4: 106 in this order; those for maltose, malto-triose, -tetraose, -pentaose, -hexaose, -heptaose, -octaose and maltodextrin ($DP_n = 13$), 100: 110: 106: 121: 131: 109: 111: 86. The K_m values, the molecular activities k_0 and the ratios k_0/K_m for a series of malto-oligosaccharides were determined. From the experimental data, the sub-site affinity of each subside in the enzyme was evaluated according to the sub-site theory. The difference in the substrate specificities between glucoamylase and α-D-glucosidase was interpreted by the relative value of sub-site affinities of the first and the third sub-sites counting from the terminal (the first sub-site) to which the non-reducing end of substrate is bound.

[135] S. Chiba, K. Kanaya, K. Hiromi, and T. Shimomura, *Agric. Biol. Chem.*, 1979, **43**, 237.
[136] S. Chiba and T. Shimomura, *Agric. Biol. Chem.*, 1975, **39**, 1041.
[137] S. Murata, H. Matsui, S. Chiba, and T. Shimomura, *Agric. Biol. Chem.*, 1979, **43**, 2131.

The substrate specificity of flint corn of α-D-glucosidase has been investigated.[138] The enzyme was active, especially on nigerose, phenyl α-maltoside, and malto-oligosaccharides. The action on isomalto-oligosaccharides was very weak. The α-D-glucans, such as amylopectin, β-limit dextrin, glycogen, and amylose besides soluble starch, also were hydrolysed, though no activity on dextran was observed. The ratio of hydrolysis rate for maltose, nigerose, phenyl α-maltoside, maltotriose, maltotetraose, and maltopentaose was estimated to be 100: 105: 110: 97: 100: 90 in this order. The extrapolated K_m values for maltose, nigerose, phenyl α-maltoside, maltotriose, maltotetraose, and maltopentaose were 1.25 mM, 1.43 mM, 1.00 mM, 0.71 mM, 0.60 mM, and 1.25 mM, respectively. The enzyme is considered to be essentially an 'acid' α-glucosidase with a preferential activity on malto-oligosaccharides to α-D-glucans.

The substrate specificity of rice α-D-glucosidase II has been studied.[139] The enzyme was especially active on nigerose, phenyl α-maltoside, and malto-oligosaccharides. The actions on isomaltose and phenyl α-D-glucopyranoside were weak, and on sucrose and methyl α-D-glucopyranoside, negligible. The α-D-glucans, such as soluble starch, amylopectin, β-limit dextrin, glycogen, and amylose, were also hydrolysed. The ratio of the maximum velocities for hydrolyses of maltose (G_2), nigerose (N), kojibiose (K), isomaltose (I), phenyl α-maltoside (φM) and soluble starch (SS) was estimated to be 100: 94.4: 14.2: 7.1: 89.5: 103.1 in this order, and that for hydrolyses of malto-triose (G_3), -tetraose (G_4), -pentaose (G_5), -hexaose (G_6), -heptaose (G_7), -octaose (G_8), and amyloses ($G_{\overline{13}}$) and ($G_{\overline{17}}$), 113: 113: 113: 106: 113: 100: 106: 106. The K_m values for N, K, I, φM, and SS were 2.4 mM, 0.58 mM, 20 mM, 1.6 mM, and 5.0 mg ml^{-1}, respectively; those for G_2, G_3, G_4, G_5, G_6, G_7, G_8, $G_{\overline{13}}$, and $G_{\overline{17}}$, 2.4 mM, 2.2 mM, 2.1 mM, 1.5 mM, 1.0 mM, 1.1 mM, 0.95 mM, 1.5 mM, and 1.1 mM. Rice α-D-glucosidase II was considered to be an enzyme with a preferential activity on malto-oligosaccharides.

Chloroplasts isolated from spinach (*Spinacia oleracea*) plants grown under controlled light/dark and temperature regimes contained the phosphorolytic and amylolytic pathways for starch breakdown. The latter consists at least of α- and β-amylase and β-D-glucosidase ('maltase').[140] Only low amylolytic activity was observed in chloroplasts isolated during the light phase. In chloroplasts prepared during the dark phase, this activity was almost twice as high. These diurnal oscillations of the amylolytic activity were maintained when the plants were kept in prolonged darkness or continuous light. The amylolytic system exhibited a sharp pH optimum between 5.8 and 6.0. Phosphorylase activity, when assayed with saturating concentrations of inorganic phosphate, did not show diurnal fluctuations.

An α-D-glucosidase which was homogeneous in ultracentrifugal and disc electrophoretic analyses was purified from sugar beet (*Beta vulgaris*) seed by fractionation with ammonium sulphate, chromatography on CM-cellulose, and gel filtrations.[141] The sedimentation coefficient ($s_{20,w}$) was calculated to be

[138] P. Pongratz and E. Beck, *Plant Physiol.*, 1978, **62**, 687.
[139] S. Chiba, S. Inomata, H. Matsui, and T. Shimomura, *Agric. Biol. Chem.*, 1978, **42**, 241.
[140] H. Matsui, S. Chiba, and T. Shimomura, *Agric. Biol. Chem.*, 1978, **42**, 1855.
[141] Y. Suzuki, K. Uchida, and A. Tsuboi, *J. Agric. Chem. Soc. Jpn.*, 1979, **53**, 189.

5.9 S. The molecular weight was estimated to be approximately 9.1×10^4 by sodium dodecyl sulphate disc electrophoresis. The α-D-glucosidase ($s_{20,w}$ 5.59; mol. wt. 9.1×10^4 by sodium dodecyl sulphate disc electrophoresis) showed also pronounced glucoamylase activity. The optimal pH was found to be in the range of 4.1 to 4.7 for both maltose and soluble starch. The enzyme exhibited higher substrate-binding affinity and hydrolytic activity towards soluble starch than towards maltose. The ratio of initial velocity of hydrolysis for maltose (K_m, 6.8 mg ml^{-1}) and soluble starch (K_m, 1.9 mg ml^{-1}) was calculated to be 100: 120 in this order.

The substrate specificity of sugar beet (*Beta vulgaris*) α-D-glucosidase has been investigated.[142] The enzyme showed a relatively wide specificity upon various substrates, having (1 → 2), (1 → 3), (1 → 4), and (1 → 6)-α-D-glucosidic linkages. The relative hydrolysis velocity for maltose (G_2), nigerose (N), kojibiose (K), isomaltose (I), panose (P), phenyl α-maltoside (φM) and soluble starch (SS) was estimated to be 100: 130: 10.7: 22.6: 54.6: 55.8: 120 in this order; that for malto-triose (G_3), -tetraose (G_4), -pentaose (G_5), -hexaose (G_6), -heptaose (G_7), -octaose (G_8), -amyloses ($G_{\overline{13}}$) and ($G_{\overline{17}}$), 91: 91: 91: 91: 80: 57: 75: 73. The K_m values for N, K, I, P, φM, and SS were 16.7 mM, 1.25 mM, 10.8 mM, 8.0 mM, 4.12 mM, and 1.90 mg ml^{-1}, respectively; that for G_2, G_3, G_4, G_5, G_6, G_7, G_8, $G_{\overline{13}}$, and $G_{\overline{17}}$ were 20.0 mM, 3.67 mM, 2.34 mM, 0.64 mM, 0.42 mM, 0.32 mM, 0.23 mM, 0.36 mM, and 0.26 mM, respectively. The enzyme, though, showed higher affinity and activity toward soluble starch than toward maltose, was considered essentially to be an α-D-glucosidase.

The enzyme-catalysed formation of pyridoxine β-D-glucopyranosides by the β-D-glucosidase of wheat bran has been studied.[143]

The use of Sephadex® columns equilibrated with sodium chloride to purify winter wheat leaf β-D-glucosidase has been described.[66]

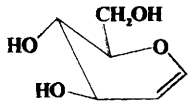
Aspergillus awamori produced multiple forms (isoenzymes) of α-D-glucosidase, three of which could be separated from one another by ion-exchange chromatography, and a glucoamylase.[144] The α-D-glucosidases isolated (I–III) (mol. wts. 1.25×10^5, 1.40×10^5, 1.30×10^5) were homogeneous on gel electrofocusing and ultracentrifugation. They hydrolysed maltose, maltotriose, phenyl α-maltoside, isomaltose, panose, phenyl α-D-glucopyranoside, and amylose liberating D-glucose, but did not act on sucrose. Isomaltose and panose were the main α-D-glucosyltransfer products formed from maltose by the three α-D-glucosidases.

β-D-Glucosidase of *Aspergillus oryzae* 'Takadiastase' has been purified by acetone fractionation and ion-exchange chromatographics.[49] Purity was confirmed by disc electrophoresis, ultracentrifugation, and measurement of other glycoside hydrolase activities which coexisted in Takadiastase. The molecular weight of the β-D-glucosidase was 2.18×10^5 by sedimentation equilibrium and 1.10×10^5–1.16×10^5 by sodium dodecyl soluble disc electrophoresis, suggesting that the enzyme consisted of two subunits. The enzyme was a glycoprotein

[142] H. Hamazaki and K. Hotta, *J. Biol. Chem.*, 1979, **254**, 9682.
[143] R. L. Cutler and D. Yellowlees, *Carbohydr. Res.*, 1979, **75**, 221.
[144] Y. Yamasaki, Y. Suzuki, and J. Ozawa, *Agric. Biol. Chem.*, 1977, **41**, 2149.

containing 2-amino-2-deoxy-D-glucose and neutral sugar. Stability, pH optimum, isoelectric point, and some specificities were observed.

D-Glucal (11) acted as mixed competitive/non-competitive inhibitor against β-D-glucosidase A_3 from *Aspergillus wentii* with slow approach to the steady-state rate of substrate hydrolysis.[145] The same rate is reached whether D-glucal competes directly with the substrate or the substrate displaces D-glucal that has been bound by pre-incubation in the absence of substrate. The K_i for competitive inhibition and the rate constants for the approach to the steady-state are in agreement with the kinetic constants for the hydration of D-glucal to 2-deoxy-D-glucose. The concentration dependence of the inhibition shows that one molecule of D-glucal binds with complete inhibition but the biphasic dissociation kinetics of the enzyme–glucal complex and labelling studies point to an additional binding site. An EI_2 complex can be isolated at pH 6 and 0 °C by rapid ion-exchange chromatography. This complex can be reactivated at pH 4 and room temperature; all the D-glucal is released as 2-deoxy-D-glucose. After denaturation of the EI_2 complex one molecule of D-glucal remains bound to the enzyme. The binding site was identified (by isolation and structure determination of a radioactive peptide) as the same L-aspartate residue that had been labelled with the active-site-directed inhibitor, conduritol B epoxide, in a previous study.



(11)

Bacillus brevis produced extracellular 'maltase' (α-D-glucosidase) only in the presence of short (1 → 4)-α-D-glucosidic polymers, such as maltose and maltotriose.[146] An optimum medium was developed; it contained 2.5% maltose, 0.5% non-fat dry milk, 0.4% yeast extract, and 0.01% $CaCl_2$. The enzyme was produced extracellularly during the logarithmic phase of growth; no cell-bound activity was detected at any time. Partial purification of the α-D-glucosidase was accomplished by using diethylaminoethylcellulose batch adsorption, ammonium sulphate precipitation, and Sephadex® G-200 gel filtration. α-D-Glucosidase, 'isomaltase' (oligo-1,6-D-glucosidase), and D-glucosyltransferase activities were purified 20.0-, 19.1-, and 11.5-fold, respectively. Some properties of the partially purified α-D-glucosidase were determined; optimum pH 6.5; optimum temperature, 48–50 °C; pH stability range, 5.0–7.0; temperature stability range, 0–50 °C; pI, 5.2; and molecular weight, 5.2×10^4. The relative rates of hydrolysis of maltose (G_2), maltotriose (G_3), G_4, methyl α-D-maltoside, G_{40}, dextrin, and isomaltose were 100, 22, 12, 10, 10, 8, and 5% respectively; the K_m for maltose was 5.8 mM; D-glucose, 4-nitrophenyl α-D-glucopyranoside,

[145] G. Legler, K.-R. Roeser, and H.-K. Illig, *Eur. J. Biochem.*, 1979, **101**, 85.
[146] S. J. McWethy and P. A. Hartman, *Appl. Envir. Microbiol.*, 1979, **37**, 1096.

and tris(hydroxymethyl)aminomethane were competitive inhibitors; trans-D-glucosylase activity of the enzyme on maltose resulted in the synthesis of isomaltose, isomaltotriose, and larger oligosaccharides.

A 4-nitrophenyl α-D-glucopyranoside-hydrolysing α-D-glucosidase of a thermophile, *Bacillus thermoglucosidius*, has been purified to an electrophoretically homogeneous state.[147] Its molecular weight was estimated as 6.0×10^4 by gel electrophoresis. The molecular activity (k_0) and the K_m value at 60 °C and pH 6.8 for 4-nitrophenyl α-D-glucopyranoside were 233 s^{-1} and 0.24 mM, respectively. The enzyme cleft the non-reducing terminal (1 → 6)-α-D-glucosidic bonds of isomaltose, panose, isomaltotriose, and isomaltopentaose. The k_0 values were 72.4, 194, 208, 233, and 167 s^{-1}, and K_m values were 3.3, 9.5, 11, 13, and 21 mM, respectively. Each isomalto-oligosaccharide was hydrolysed to D-glucose by the cleavage of single D-glucose units from its non-reducing end. The present study suggests that the enzyme is an oligo-1,6-D-glucosidase and an *exo*-D-glucosidase.

A high-molecular-weight β-D-glucosidase (component B1, mol. wt. 3.5×10^5–3.8×10^5) predominated in filtrates from young cultures of *Botryodiplodia theobromae*, but a low-molecular-weight enzyme (B4, mol. wt. 4.5×10^4–4.7×10^4) predominated in older filtrates.[148] Intermediate molecular species of β-D-glucosidase (B2, mol. wt. 1.7×10^5–1.8×10^5; B3, mol wt. 8.3×10^4–8.7×10^4) were also found. These enzymes acted synergistically in conjunction with the cellulases also present.

The effects of pH and temperature on K_m and V_{max} and of NaCl on the activity of the high-molecular-weight β-D-glucosidase from cultures of *B. theobromae* have been studied.[149] Donor binding and inhibition of activity by D-glucose were dependent on the ionization of a group (pK 6.0) that appeared to be an imidazole group. Catalytic activity and the simulation of activity by glycerol were dependent on the ionization of two groups, which appeared to be a carboxy-group and an imidazole group. The Arrhenius activation energy (E_a) calculated from results obtained at pH 4.0 and 5.0 was about 45–46 kJ mol^{-1}. The enthalpies (ΔH^0) calculated from results obtained at pH 4.0 and 5.0 were similar (about −4 kJ mol^{-1}), whereas at pH 6.5 the value was about −33 kJ mol^{-1}. The entropies (ΔS^0) calculated from these results at 37 °C were −21, −22, and −118 J K^{-1} mol^{-1} at pH 4.0, 5.0, and 6.5, respectively. A low concentration of NaCl (16.6 mM) stimulated enzymic activity and decreased the K_m for the donor, whereas high concentrations (up to 500 mM) inhibited enzymic activity, increased the K_m and had no effect on V_{max}. Plots of initial velocity data obtained in the presence of dioxan as v^{-1} against the ratio of the molar concentration of dioxan to that of water were linear. These results are discussed in terms of the enzyme mechanism.

A β-D-glucosidase has been isolated from *Clostridium thermocellum*; the enzyme was localized in the periplasmic space.[150] It was purified in a five-step procedure including ion-exchange chromatography, gel filtration, and isoelectric

[147] Y. Suzuki, Y. Ueda, N. Nakamura, and S. Abe, *Biochim. Biophys. Acta*, 1979, **566**, 62.
[148] G. M. Umezurike, *Biochem. J.*, 1979, **177**, 9.
[149] G. M. Umezurike, *Biochem. J.*, 1979, **179**, 503.
[150] N. Ait, N. Creuzet, and J. Cattaneo, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 537.

focusing. The final preparation was purified 944-fold with a recovery of about 5% of the initial enzyme activity. Polyacrylamide disc electrophoresis of the purified enzyme gave a single band at pH 8.3. The enzyme is active towards cellobiose and 4-nitrophenyl β-D-glucopyranoside and developed maximum activities at pH 6.0 and 65 °C. A molecular weight of 5.0×10^4 was estimated by gel filtration and the enzyme was isoelectric at pH 4.68.

An α-D-glucosidase has been isolated from fruit bodies of *Lentinus edodes* by a procedure including fractionation with ammonium sulphate, ion-exchange chromatography, and preparative gel electrofocusing.[151] The preparation was homogeneous on gel electrofocusing and ultracentrifugation. The α-D-glucosidase (mol. wt. 5.1×10^4) hydrolysed maltose, maltotriose, phenyl α-maltoside, amylose, and soluble starch, but did not act on sucrose. The enzyme hydrolysed phenyl α-maltoside into D-glucose and phenyl α-glucoside. α-D-Glucose and not β-D-glucose was released by the α-D-glucosidase. Maltotriose was the main α-D-glucosyltransfer product formed from maltose by the α-D-glucosidase.

An α-D-glucosidase has been isolated from the mycelia of *Mucor racemosus*, but a procedure including extraction with urea, fractionation with acetone, treatment with $MnCl_2$, and successive separation by ion-exchange chromatography.[152] The purified enzyme was homogeneous on polyacrylamide gel electrophoresis, gel electrofocusing, and ultracentrifugation. The sedimentation constant ($s^0_{20,w}$) of the enzyme was 6.2 S, and its molecular weight was 0.97×10^5–1.14×10^5. The pH optimum of the enzyme was in the pH range of 4.0–6.0, and the temperature optimum was 50 °C. The purified enzyme was stable in the pH range of 4.0–7.0, and also stable up to 45 °C. The enzyme also catalysed the transfer of an α-D-glucosyl residue from maltose to riboflavin. The substrate and inhibitor specificities, and α-D-glucosyltransfer products of the purified α-D-glucosidase from the mycelia of *M. racemosus* have been investigated.[153] The enzyme hydrolysed maltose, maltotriose, phenyl α-maltoside, isomaltose, soluble starch, and amylose, liberating D-glucose, but did not act on sucrose. The enzyme hydrolysed phenyl α-maltoside into D-glucose and phenyl α-D-glucoside. Maltotriose was the main α-D-glucosyltransfer product formed from maltose, and isomaltose was that from soluble starch. Tris and turanose inhibited the enzyme activity, but 4-chloromercuribenzoate and H_4edta did not. The enzyme hydrolysed amylose liberating α-D-glucopyranose. The enzyme was a glycoprotein containing 4.1% of neutral sugar which was identified as D-mannose.

Penicillium oxalicum was shown to produce two forms of glucoamylase and an α-D-glucosidase.[154] The α-D-glucosidase has been isolated from the mycelia in an electrophoretically homogeneous form, and its properties have been investigated. The enzyme had a pH optimum at 3.5 with maltose as substrate. The enzyme hydrolysed maltose, maltotriose, phenyl α-maltoside, isomaltose, panose, isomaltotriose, amylose, and soluble starch liberating D-glucose, but not

[151] Y. Yamasaki and Y. Suzuki, *Agric. Biol. Chem.*, 1978, **42**, 971.
[152] Y. Yamasaki, Y. Suzuki, and J. Ozawa, *Agric. Biol. Chem.*, 1977, **41**, 1553.
[153] Y. Yamasaki, Y. Suzuki, and J. Ozawa, *Agric. Biol. Chem.*, 1977, **41**, 1559.
[154] Y. Yamasaki, Y. Suzuki, and J. Ozawa, *Agric. Biol. Chem.*, 1977, **41**, 1451.

sucrose. The K_m value for maltose was 1.232 mM. Phenyl α-maltoside was hydrolysed to D-glucose and phenyl α-D-glucopyranoside by the enzyme. Isomaltose was the main trans-D-glucosylation product from maltose and soluble starch. It was found that the enzyme liberated α-D-glucopyranose from amylose.

The β-D-glucosidases which exist in the yeast *Pichia polymorpha* have been examined.[155]

Germinating microcysts of *Polysphondylium pallidum* possess the ability to digest carboxymethylcellulose to its D-glucose units.[156] This activity increases several-fold intracellularly during germination, and is excreted into the extracellular medium. The inhibition of β-D-glucosidase activity with D-glucono-1,4-lactone also inhibits emergence, implicating this enzyme in germination as well.

The different α-D-glucosidases have been purified as part of the re-examination, mainly by isoelectric focusing, of the intracellular enzymes of *Pseudomonas* UQM 733 which act on dextran.[157] The action patterns have been studied for a purified, intracellular dextranase and three intracellular α-D-glucosidases from *Pseudomonas* UQN 733, on pure isomalto-oligosaccharides.[158] The α-D-glucosidases have optimal activity on isomaltotetraose and are therefore classified as 'oligo-D-glucanases'. They have been used to determine the structure of two branched isomalto-oligosaccharides obtained by enzymic degradation of dextran.

'Amylomaltase' and 'disproportionating enzyme' ('D-enzyme') (α-D-glucosidases) have been purified to homogeneity from cell-free extracts of *Pseudomonas stutzeri* using a six-step procedure.[159] The presence of both glycosyltransferases in the same organism has not been reported before. Molecular weight determination by gel chromatography gave a value of 7.4×10^4 for the 'amylomaltase' and 1.15×10^5 for the 'D-enzyme'. Two sub-units of different molecular weight were found in each enzyme, as proved by sodium dodecyl sulphate–gel electrophoresis. The optimum pH of 'amylomaltase' and 'D-enzyme' activity is 7.6–7.7. Action of both D-glycosyltransferases on different maltodextrins showed that amylomaltase is most active with maltotetraose, and the K_m value for this substrate is 7.1 mM. 'D-Enzyme' catalysed D-glucose release from maltose (K_m = 8.3 mM) at a higher rate than from maltotriose and maltotetraose. With maltotriose as initial substrate, 'D-enzyme' forms glucose, maltopentaose, maltoheptaose, maltononaose, and maltoundecaose as major products. 'Amylomaltase' acts on maltotriose, maltotetraose, and maltopentaose to form a series of homologous (1 → 4)-α-D-glucans. No essential chain-lengthening reaction occurred with maltohexaose.

Three components (GA, GB-1, and GB-2) with β-D-glucosidase activity have been detected in the culture filtrate of *Pyricularia oryzae* grown in a cellulose or cellulose-derivative medium.[160] Among them, GB-1 was induced most

[155] T. G. Villa, V. Notario, and J. R. Villanueva, *FEMS Microbiol. Lett.*, 1979, **6**, 91.
[156] D. H. O'Day and G. Paterno, *Arch. Microbiol.*, 1979, **121**, 231.
[157] M. T. Covacevich and G. N. Richards, *Carbohydr. Res.*, 1978, **64**, 169.
[158] M. T. Covacevich and G. N. Richards, *Carbohydr. Res.*, 1970, **70**, 283.
[159] J. Schmidt and M. John, *Biochim. Biophys. Acta*, 1979, **566**, 100.
[160] T. Hirayama, S. Horie, H. Nagayama, and K. Matsuda, *J. Biochem. (Tokyo)*, 1978, **84**, 27.

strongly. Purified GB-1 was homogeneous on polyacrylamide gel electrophoresis and showed an approximately 1400-fold increase of specific activity over the starting material. The molecular weight was determined to be 2.4×10^5 by sodium dodecyl sulphate–gel electrophoresis. A similar value was also obtained by sucrose density gradient centrifugation. The enzyme contained a high proportion of acidic amino-acids and D-mannose, and the isoelectric point of the enzyme was pH 4.15. The enzyme had a pH optimum of 5.5 and a temperature optimum at 55 °C. β-D-Glucosidase activity was inhibited by Mn^{2+}, Cu^{2+}, Hg^{2+}, 4-chloromercuribenzoate, and D-glucono-1,4-lactone. The enzyme split off D-glucose units one by one from the non-reducing ends of not only β-D-gluco-oligosaccharides but also some β-D-glucans, such as carboxymethylcellulose, laminaran, pustulan, and zeagallan. The affinity for cello- and laminari-oligosaccharides tended to increase in parallel with the chain length.

Enzyme preparations active against crystalline cellulose, marble-milled filter paper, carboxymethylcellulose, hemicellulose, and xylan were obtained from cultures of *Ruminococcus flavefaciens*.[161] These preparations also contained swelling factor, pectin methylesterase, pectin lyase and low levels of β-D-glucosidase and β-D-xylosidase. 'Carboxymethylcellulase' and xylanase activities were present in a high molecular weight complex, but substrate competition studies showed that different active sites were probably responsible for each activity. Analysis of products and viscosity changes during enzymic hydrolysis of carboxymethylcellulose and xylan indicated that the most active enzymes were of the *exo*-(1 → 4)-β-D-glycanase type. A variety of reducing sugars was released from cell walls of *Lolium perenne* (perennial ryegrass) by enzyme preparations.

α-D-Glucosidase has been purified to homogeneity from logarithmically growing cells of *Saccharomyces carlsbergensis*.[162] The purification involved ammonium sulphate fractionation, gel filtration, ion-exchange chromatography, and hydroxyapatite chromatography. This procedure gave a preparation judged to be greater than 98% pure by sodium dodecyl sulphate–polyacrylamide gel electrophoresis. The enzyme was shown to be a monomer (6.3×10^4) by gel filtration under native conditions, and by polyacrylamide gel electrophoresis under denaturing conditions. The K_m values of the enzyme for the substrates maltose and 4-nitrophenyl α-D-glucopyranoside were 1.66×10^{-2} and 3.1×10^{-4} M, respectively. The pH optimum for the purified enzyme was found to be between pH 6.7 and 6.8. The enzyme has an absolute anomeric specificity for α-D-glycosidic linkages and appears to recognize a D-glucosyl residue in a linkage on the non-reducing end of its substrate. For the strain used in this study, which carries the MAL 6 locus, only a single form of the enzyme was detected.

The contribution of β-D-glucosidase(s) to the cellulolytic function of *Sporotrichum pulverulentum* has been discussed in a review of cellulose degradation by this organism.[163]

[161] G. L. Pettipher and M. J. Latham, *J. Gen. Microbiol.*, 1979, **110**, 21.
[162] R. B. Needleman, H. J. Federoff, T. R. Eccleshall, B. Buchferer, and J. Marmur, *Biochemistry*, 1978, **17**, 4657.
[163] K.-E. Eriksson, *Biotechnol. Bioeng.*, 1978, **20**, 317.

The principal features of a mathematical model described for the enzymatic hydrolysis and fermentation of cellulose by *Trichoderma reesei*[164] are the assumption of two forms of cellulose (crystalline and amorphous), two sugars (cellobiose and D-glucose), and two enzymes (cellulase and β-D-glucosidase). An inducer–repressor–messenger RNA mechanism is used to predict enzyme formation, and pH effects are included. The model consists of 12 ordinary differential equations for 12 state variables and contains 38 parameters. The parameters were estimated from four sets of experimental data by optimization. The results appear satisfactory, and the computer programs permit simulation of a variety of system changes.

Trichoderma viride produces high yields of cellulase and especially β-D-glucosidase when grown in submerged culture on different carbon sources.[165] Cellulase synthesis was strongly repressed in the presence of D-glucose and only a low constitutive activity of β-D-glucosidase and 'carboxymethylcellulase', but no 'Avicelase', could be demonstrated when culturing *T. viride* on D-glucose.

The optimization of cellulase and β-D-glucosidase production by a basidiomycete species was studied, and cellulase and β-D-glucosidase (cellobiase) production by this and *Trichoderma viride* (and its mutants) in shake flasks were compared.[166] The former produced an active cellulase comparable to that of *T. viride* when tested on filter paper, carboxymethylcellulose, and cotton; however, it produced 20–26 times larger amounts of β-D-glucosidase. Both cellulase and β-D-glucosidase were obtained in good yield only when cellulose was the carbon source. The production of these enzymes was not repressed by readily assimilated carbon sources in the presence of cellulose. Only traces of cellulase and β-D-glucosidase were formed on D-glucose, D-fructose, maltose, and cellobiose although good growth was obtained on these substrates. These enzymes were not induced on sophorose, lactose, mannitol, or glycerol and growth was poor on these substrates. Cellobiose octa-acetate was a less effective inducer of cellulase and β-D-glucosidase than was cellulose.

Three kinds of α-D-glucosidase (I, II, and III) have been isolated from brewer's yeast.[167] α-D-Glucosidases I and II were homogeneous on disc electrophoresis, but α-D-glucosidase III was not. Their pH-optima were found to be in the range 6.3–7.1. Each of them was a typical α-D-glucosidase showing a preferential activity on phenyl α-D-glucopyranoside. α-D-Glucosidase I was called 'isomaltase' since it could hydrolyse isomaltose but not maltose. α-D-Glucosidases II and III showed no activity in isomaltose. The ratio of velocity of hydrolysis for phenyl α-D-glucopyranoside (K_m, 2.9 mM) and isomaltose (K_m, 28.6 mM) of α-glucosidase I was calculated to be 100:9 in that order, and those for phenyl α-D-glucopyranoside (K_m's, 0.8 mM and 3.6 mM) and maltose (K_m's, 14.3 mM and 71.4 mM) of α-D-glucosidase II and III were calculated to be 100:17 and 100:18, respectively.

β-D-Glucosidase has been immobilized with retention of enzymic activity by drying fibroin–enzyme solution on a horizontal plate, followed by an essential

[164] N. Peitersen and E. W. Ross, *Biotechnol. Bioeng.*, 1979, **21**, 997.
[165] D. Herr, *Biotechnol. Bioeng.*, 1979, **21**, 1361.
[166] J. G. Shewale and J. C. Sadana, *Can. J. Microbiol.*, 1978, **24**, 1204.
[167] K. Matsusaka, S. Chiba, and T. Shimomura, *Agric. Biol. Chem.*, 1978, **41**, 1917.

ethanol treatment,[168] by reaction with/adsorption on to a range of new polyphenolic resins,[169] and by chelation of organic (glass) supports activated by the surface deposition of an imperfectly crystallized film of TiO_2 – the enzyme may be linked to the activated support using 5-aminosalicylic acid (with or without diazotization), tannic acid, or tin(II) chloride, and by the silane–glutaraldehyde method on the same glass support.[170]

8 β-D-Glucuronidases

A pool of acid hydrolases exists within the acellular lining material of the alveoli and distal airways of the lungs.[25] These extracellular hydrolases, obtained using pulmonary lavage procedures, appear to be of a selected variety insofar as some hydrolases (β-D-2-acetamido-2-deoxyglucosidase and α-D-mannosidase) are highly active whereas others (β-D-glucuronidase and arylsulphatase) are barely detectable. The origins of these hydrolases were investigated (see p. 421).

Human β-D-glucuronidase, like many other glycoprotein lysosomal hydrolases, is subject to receptor-mediated endocytosis by fibroblasts.[171] Prior work demonstrated charge heterogeneity in β-D-glucuronidase and showed that high-uptake forms are more acidic than slowly internalized forms. Considerable indirect evidence implicated D-mannose 6-phosphate as an essential part of the recognition marker on high-uptake enzyme forms. The purification of β-D-glucuronidase from human spleen has now been reported and it was demonstrated enzymatically that D-mannose 6-sulphate is released on acid hydrolysis of pure enzyme. Furthermore, the D-mannose 6-phosphate content of the enzyme varies directly with its susceptibility to pinocytosis by fibroblasts. Enzyme forms resolved by CM-Sephadex® chromatography differed over an 18-fold range in uptake rate and in D-mannose 6-phosphate content. The most acidic forms had 4.4 mol of D-mannose 6-phosphate per mol of enzyme. The D-mannose 6-phosphate was released from the enzyme by treatment with *endo*-glycanase H with concomitant loss of susceptibility to adsorptive endocytosis. Thus, these studies provide direct evidence that D-mannose 6-phosphate is present on high-uptake enzyme forms, that it is present in the recognition marker for uptake, and that it is present on oligosaccharide that is released by *endo*-glycanase H.

With phenolphthalein β-D-glucopyranosyluronic acid as substrate in a human serum β-D-glucuronidase assay, centrifugation at high speed after addition of alkali was required to minimize blanks.[172] Optimal substrate concentration was 3–6 mM; excess substrate was inhibitory.

The intracellular localization of exogenously supplied human platelet β-D-glucuronidase in cultured skin fibroblasts derived from a β-D-glucuronidase-deficient patient was studied.[173] Four cellular fractions were obtained by

[168] S. Miyairi, M. Sugiura, and S. Fukui, *Agric. Biol. Chem.*, 1978, **42**, 1661.
[169] M. F. Chaplin and J. F. Kennedy, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2144.
[170] J. P. Cardoso, M. F. Chaplin, A. N. Emery, J. F. Kennedy, and L. P. Revel-Chion, *J. Appl. Chem. Biotechnol.*, 1979, **28**, 775.
[171] M. R. Natowicz, M. M. Y. Chi, O. H. Lowry, and W. S. Sly, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4322.
[172] A. W. Schrecker and M. A. Chirigos, *Clin. Chim. Acta*, 1978, **88**, 413.
[173] G. Bach and A. Liebmann-Eisenberg, *Eur. J. Biochem.*, 1979, **96**, 613.

differential speed centrifugation. Following incubation the exogenously supplied enzyme exhibited a distribution pattern identical to that of endogenous β-D-2-acetamido-2-deoxyhexosidase. Disruption of membranes by freezing and thawing caused a 35% increase of the enzyme activity, thus indicating a latent activity following the internalization. This indicated localization in the lysosomal fractions. Longer incubation periods led to an intracellular shift of the engulfed enzyme from the lighter lysosomal fraction to heavier particles.

The effects of various drugs on zymosan-induced release of β-D-glucuronidase (a lysosomal enzyme) and lipopolysaccharide-induced secretion of collagenase from cultured macrophages of guinea-pigs were investigated.[174] Dexamethasone, hydrocortisone, and prednisolone inhibited the release of β-D-glucuronidase. Dexamethasone, however, did not show such an effect when the drug was added to macrophage culture after particle uptake had been completed. In contrast, indomethacin and disodium *N*-(carboxyphenyl)-4-chloroanthranilate significantly enhanced the enzymic release. Aspirin and levamisole enhanced it slightly, but there were no distinct differences between the effects of these agents on lipopolysaccharide-induced secretion of collagenase in contrast to the case of β-D-glucuronidase release.

Seven renal glycoside hydrolases have been measured in four subcellular fractions prepared from highly inbred aglycosuric (*AV*-line) and glycosuric (*XA*-line) Chinese hamsters (*Cricetulus griseus*).[39] β-D-Glucuronidase was virtually absent in nuclear, lysosomal-mitrochondrial fractions and supernatant fractions of *AV*-kidneys showed higher activity than the *XA*s.

β-D-Glucuronidase from rat preputial glands was crystallized as thin sheets having *p6* symmetry in projection with $a = 20.2$ nm. A filtered image was produced by Fourier methods to a resolution of 2.2 nm by averaging information from six areas.[175] This suggests an approximately triangular molecular outline in projection, and this is taken to indicate a probable tetrahedral arrangement of the four sub-units of the β-D-glucuronidase molecule.

The injection into mice of a single dose of conduritol B epoxide, a covalent inhibitor of β-D-glucosidases, quickly produced changes in tissue levels of β-D-glucuronidase.[132] The specific activity of the enzyme decreased in liver, spleen, and kidney while brain showed little change. The inhibitor did not act on β-D-glucuronidase *in vitro*, so the effect of the inhibitor is complex, possibly a result of the loss of β-D-glucosidase activity. Since β-D-glucuronidase contains D-glucose, it was suggested that the transport of the enzyme between subcellular regions and tissues involves loss of part of the D-glucose moieties. Injection of a haemolysing agent, phenylhydrazine, produced an increased level of β-D-glucuronidase in liver and spleen within six days, but not in kidney. This enhancement was a little less in mice previously injected with the β-D-glucosidase inhibitor.

Procedures for the large scale isolation of β-D-glucuronidase from urine of androgen-treated mice carrying Gus^a, Gus^b, or Gus^h structural alleles have

[174] T. Okimura, H. Ohmori, Y. Kubota, and I. Yamamoto, *Biochem. Pharmacol.*, 1979, **28**, 2729.

[175] M. R. Dickson, M. Stewart, D. E. Hawley, and C. A. Marsh, *Biochem. J.*, 1979, **181**, 1.

included the use of immunoadsorption.[176] With respect to kinetic parameters, amino-acid composition, *N*-terminal amino-acid, reactivity with antibody, subunit structure, and electrophoretic mobility, urinary enzyme resembled the lysosomal (L form) enzyme present in liver, kidney, *etc.* However, it was slightly smaller (mol. wt. 6.9×10^4) than the L form (mol. wt. 7.1×10^4 by sodium dodecyl sulphate electrophoresis). Both forms of the enzyme were resolved into multiple forms (pI = 5.5–6.0) on isoelectric focusing. Analysis of peptides obtained from the variant forms of β-D-glucuronidase after cyanogen bromide treatment indicated that the genetic variation is restricted to a few amino-acids of the polypeptide chain.

The β-D-glucuronidase in chick embryo liver homogenate catalysed the release of D-glucuronic acid from 4-methylumbelliferyl β-D-glucopyranosyluronic acid and from the non-reducing terminals of chondroitin 4- and 6-sulphates.[177] Multiple kinetic forms of the enzyme were identified and the activities of the enzyme on tetra-, hexa-, octa-, and deca-saccharides of chondroitin 4- and 6-sulphates, measured using a new assay procedure which can detect the formation of 1 nmol of product, were similar, although rates were somewhat lower for the higher oligosaccharides. Highly purified β-D-glucuronidases from human placenta and from rat preputial gland also showed multiple kinetic forms when assayed using 4-methylumbelliferyl β-D-glucopyranosyluronic acid as substrate.

9 α-L-Iduronidases

The receptor-binding of 'high-uptake' forms of lysosomal enzymes to human diploid skin fibroblasts has been demonstrated directly by using a sensitive assay for the bound enzyme.[178] α-L-Iduronidase-deficient cells were incubated with human urinary α-L-iduronidase and the cell-associated enzyme was assayed with 4-methylumbelliferyl α-L-idopyranosiduronic acid. D-Mannose 6-phosphate greatly accelerated the dissociation of the bound enzyme, and during uptake of α-L-iduronidase the receptors were regenerated every few minutes, even in the absence of protein synthesis.

A fluorogenic substrate for α-L-iduronidase, 4-methylumbelliferyl α-L-idopyranosyluronic acid, has been synthesized and the enzyme activity has been measured in urine samples obtained from normal persons and patients suffering from mucopolysaccharidosis.[179] Urine samples derived from a patient with Scheie syndrome showed greatly reduced activity compared with a normal adult at a similar age. This patient exhibited a high level of urinary excretion of dermatan sulphate and heparan sulphate, which could be interpreted in terms of her low α-L-iduronidase activity. The use of the fluorogenic substrate has some advantages over existing methods because of the high sensitivity and the relative ease of handling, and it should be useful not only for diagnosis but also for following the purification process of the enzyme.

[176] A. J. Lusis and K. Paigen, *J. Biol. Chem.*, 1978, **253**, 7336.
[177] J. H. Glaser and H. E. Conrad, *J. Biol. Chem.*, 1979, **254**, 6588.
[178] L. M. Rome, B. Weissmann, and E. F. Neufeld, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 2331.
[179] M. Isemura, H. Kosaka, T. Ikenaka, R. Kido, and T. Yoshimura, *J. Biochem. (Tokyo)*, 1978, **84**, 627.

10 α- and β-D-Mannosidases

A pool of acid hydrolases exists within the acellular lining material of the alveoli and distal airways of the lungs.[25] These extracellular hydrolases, obtained using pulmonary lavage procedures, appear to be of a selected variety insofar as some hydrolases (β-D-2-acetamido-2-deoxyglucosidase and α-D-mannosidase) are highly active while others (β-D-glucuronidase and arylsulphatase) are barely detectable (see p. 421).

An abnormal, decreased thermostability of α-D-mannosidase has been found in crude extracellular fluids of patients with cystic fibrosis and carriers thereof.[180]

The 'acid' α-D-mannosidase in human plasma closely resembles liver acid α-D-mannosidase in its affinity for agarose-immobilized concanavalin A, molecular weight, and resolution into multiple components on DEAE-cellulose.[181] A combination of affinity chromatography and gel filtrations suggested that four forms of intermediate α-D-mannosidase, which differ either in their molecular weight or affinity for concanavalin A, exist in human plasma. A practical classification and nomenclature for the multiple forms of intermediate α-D-mannosidase in plasma based on molecular weight and affinity for concanavalin A is proposed. Multiple forms of intermediate α-D-mannosidase were also observed by ion-exchange chromatography on DEAE-cellulose, but there was not a simple correlation between these forms and those obtained with the other separation procedures. The form of intermediate α-D-mannosidase least abundant in plasma, approximately 7% of the activity, has very similar properties to the 'neutral' α-D-mannosidase in human liver. In contrast, the other three forms of 'intermediate' α-D-mannosidase, which account for over 90% of the activity, do not appear to be present in liver, except perhaps in trace amounts.

Decreased binding by the lectins concanavalin A and wheat-germ agglutinin was found for a number of 'acid' hydrolases (β-D-2-acetamido-2-deoxyhexosidase, α-L-fucosidase, α- and β-D-glucosidases, β-D-galactosidase, and α-D-mannosidase) from skin fibroblasts of three unrelated patients with mucolipidosis II (see p. 423).[29]

A search for lysosomal hydrolases and related enzymes has been made in haemolysates from human and rabbit red cells.[33] Apart from acid phosphatases, significant activities were found only for α-D-mannosidase, 'neutral' α-D-glucosidase, and β-D-2-acetamido-2-deoxyhexosidase. α-D-Mannosidase activity per cell in human red blood cells was 200-times lower than in white cells. The optimal pH was 5.5–6.0. Electrophoresis on cellulose acetate showed three bands. Haemolysates from four patients with mannosidosis were not deficient in α-D-mannosidase. Curves of pH activity and electrophoretic patterns were similar to those of controls. From its biochemical and genetic properties, it is concluded that red cell α-D-mannosidase differs from the lysosomal acid α-D-mannosidase.

Residual 'acid' α-D-mannosidase, varying in amount up to approximately 15% of normal values, can be measured in various organs of a calf with manno-

[180] T. Hosli and E. Vogt, *FEBS Lett.*, 1979, **104**, 271.
[181] S. Hirani and B. Winchester, *Biochem. J.*, 1979, **179**, 583.

sidosis.[182] The highest specific activity and relative proportion of residual activity were found in the liver. Chromatography on DEAE-cellulose showed that the residual activity was associated with two components, which were eluted at comparable positions with those found in normal tissues. The residual activity had a lower thermal stability and a higher K_m value for a synthetic substrate than did the normal enzyme. No differences in molecular weight or electrophoretic mobility between normal 'acid' α-D-mannosidase and the residual activity were observed by gel filtration and electrophoresis on cellulose acetate respectively. The isoelectric focusing profiles for the α-D-mannosidase in the normal and pathological livers were very similar. It is suggested that a mutant enzyme, resulting from a mutation in a structural gene, accounts for the residual 'acid' α-D-mannosidase in mannosidosis. The mutant enzyme, which cross-reacts with antiserum raised against normal bovine 'acid' α-D-mannosidase, is present at a decreased concentration compared with the normal enzyme. There is a correlation between the concentrations of residual activity and cross-reacting material in mannosidosis. α-D-Mannosidase with a pH optimum of 5.75 and which is activated by Zn^{2+} was also detected in the liver of the calf with mannosidosis. However, it is probably not a product of the defective gene because addition of Zn^{2+} indicated that it was also present in normal tissues.

α-D-Mannosidase activity in goat serum was observed to be distributed in sperm and seminal plasma.[183] In sperm the enzyme, present in soluble and bound forms, was located within the aerosome and the bound enzyme was associated with the denuded sperm. Purification of the enzyme 100-fold yielded a species homogeneous on gel electrophoresis (with and without sodium dodecyl sulphate) and on isoelectric focusing. The α-D-mannosidase (mol. wt. 2.2×10^5 by gel filtration and sodium dodecyl sulphate disc electrophoresis, pI 7.42, K_m's 4-nitrophenyl α-D-mannopyranoside 0.695 mM, methyl α-D-mannopyranoside 71.9 mM, pH optimum 4.0) exhibited some dependence upon Zn^{2+}, but studies with H_4edta *etc.* showed that the enzyme is not a zinc metalloenzyme but is activated by Zn^{2+}.

'Acid' α-D-mannosidase (mol. wt. 1.84×10^5, 8.1×10^4, and 4.54×10^4 by gel filtration, urea–gel filtration, and sodium dodecyl sulphate–polyacrylamide gel electrophoresis, respectively; pI 5.9; pH optimum 4.2) was purified more than 2000-fold – *via* conventional techniques, affinity chromatography on immobilized concanavalin A, and Pevikon electrophoresis – from porcine kidneys.[184] The final product contained the multiple forms A and B and had a quaternary structure. The immunoglobulin fraction of rabbit antiserum against the pure α-D-mannosidase leads in immunodiffusion and precipitation experiments to precipitation not only with α-D-mannosidase from porcine tissues but also (though with lower affinity) from bovine liver and human placenta, urine, and skin fibroblasts.

Seven renal glycoside hydrolases have been measured in four sub-cellular fractions prepared from highly inbred aglycosuric (*AV*-line) and glycosuric

[182] L. J. Burditt, N. C. Phillips, D. Robinson, B. G. Winchester, N. S. Van-de-Water, and R. D. Jolly, *Biochem. J.*, 1978, **175**, 1013.
[183] J. Singh, S. K. Bhatnagar, and S. R. Anand, *Mol. Cell. Biochem.*, 1978, **21**, 131.
[184] G. Mersmann and E. Buddecke, *Int. J. Biochem.*, 1977, **8**, 763.

(*XA*-line) Chinese hamsters (*Cricetulus griseus*).[39] α-D-Mannosidase was chiefly a particulate enzyme and its decrease in *XA* animals was evident in nuclear, lysosomal-mitochondrial, and mitochondrial-microsomal fractions.

When exponentially growing *Dictyostelium discoideum* amoebae were harvested from growth medium and suspended in a nutrient-free phosphate buffer, the cells released 20–30% of their total β-D-acetamido-2-deoxyglucosidase and α-D-mannosidase activities[45] (see p. 427).

β-D-Mannanase (β-D-mannosidases) purified from the germinated seeds of *Trifolium repens* by a procedure that included chromatography on hydroxyapatite, gel filtration on acrylamide/agarose (Ultragel 5/4), and preparative polyacrylamide gel electrophoresis,[185] has been completely resolved into two β-D-mannanases (I and II) with distinct specificities. The purified enzymes showed a single protein band when examined by polyacrylamide gel disc electrophoresis. β-D-Mannanase I, apparent mol. wt. 4.3×10^4, accounted for 49% of the total activity recovered from the final step of purification. β-D-Mannanase II, apparent mol. wt. 3.8×10^4, accounted for the remaining 51% of activity; molecular weight determinations were made by sodium dodecyl sulphate polyacrylamide gel electrophoresis. The substrate specificities of both enzymes were examined with the D-galacto-D-mannans of *T. repens* and of *Medicago sativa*, as well as with D-manno-oligosaccharides. The pH optimum was between pH 5.1 and 5.6 for both enzymes.

Strains of fungi imperfecti of genera *Aspergillus* and *Rhizopus* were tested for the ability to produce α-D-mannosidases.[186] The most suitable α-D-mannosidase producer of a total of 20 strains under study was *Aspergillus ravus* Link 69. The parameters studied during the cultivation included the growth rate expressed as cell dry weight. α-D-Mannosidase activity of the extracellular medium with 4-nitrophenyl α-D-mannopyranoside as substrate, and utilization of *Saccharomyces cerevisiae* D-mannan *via* its disappearance from the cultivation medium were investigated.

The isolation, purification, and characterization of α-D-mannosidase from cultures of *Aspergillus flavus* has been described.[187] The enzyme (pH optimum 4.2–4.5) was isolated from the growth medium of the mold at the beginning of the stationary phase of growth and partly purified by ion-exchange chromatography. The effect of temperature and cations on the activity of the enzyme and the effect of pH on its stability were examined (K_m for 4-nitrophenyl α-D-mannopyranoside 1.4 mM, the value of the sedimentation constant is $s^0_{20} = 4.5$ S, and the partial specific volume $q = 0.725$ cm^3 g^{-1}).

Reaction of α-D-mannosidase from *Phaseolus vulgaris* with *N*-bromosuccinimide or 2-hydroxy-5-nitrobenzylbromide resulted in loss of enzyme activity.[188] Spectral absorption and fluorescence studies, as well as amino-acid analysis, suggested that only L-tryptophan residues had been modified. No change in conformation could be detected by density gradient ultracentrifugation or

[185] H. Villarroya, J. Williams, P. Dey, S. Villarrova, and F. Peter, *Biochem. J.*, 1978, **175**, 1079.
[186] J. Augustin and D. Šikl, *Folia Microbiol.*, 1978, **23**, 349.
[187] J. Augustin, D. Šikl and J. Zemek, *Collect. Czech. Chem. Commun.*, 1978, **43**, 2812.
[188] E. Paus, *Biochim. Biophys. Acta*, 1978, **533**, 446.

circular dichroism of α-D-mannosidase modified by *N*-bromosuccinimide to virtually zero enzyme activity. The inhibition was partly offset by the substrate analogue α-methyl D-mannopyranoside and the competitive inhibitor D-mannono-1,4-lactone. Concomitantly, two L-tryptophan residues fewer were oxidized per molecule. Further, there was found evidence for the enzyme having a secondary structure dominated by β-pleated sheets.

Proteins of a crude enzyme preparation obtained from the cultivation medium of the basidiomycete *Phellinus abietis* were separated by gel filtration and ion-exchange chromatography.[189] The preparation contained a minimum of three enzymes capable of splitting α-D-mannosidic bonds: α-D-mannosidase, *exo*-D-mannanase, and *endo*-D-mannanase, which were separated. Some properties of the D-mannanase complex of the crude enzyme preparation, and of a partially purified α-D-mannosidase, were examined. The D-mannanase complex exhibited two pH optima, its temperature optimum being 45 °C. The pH optimum of purified α-D-mannosidase was at pH 5.0, the temperature optimum was at 60 °C; the enzyme had a relatively high heat stability. The K_m of α-D-mannosidase for 4-nitrophenyl α-D-mannopyranoside was 1.5×10^{-5} M. Pure α-D-mannosidase did not split D-mannan.

A glycoprotein which has been isolated from the cell walls of baker's yeast was shown to be a D-mannan–protein complex.[190] After immobilization by covalent attachment to macroporous agarose, this was used in the purification of an α-D-mannosidase from jack-bean meal.

11 Neuraminidases (Sialidases)

Modification of the cell surface with neuraminidase has been shown to increase the sensitivities of cells to diphtheria toxin and *Pseudomonas aeruginosa* endotoxin.[191]

Work on the search for a biochemical basis of six different types of sialidosis have centred on the use of two radiolabelled oligosaccharides prepared from human urinary oligosaccharides (12) and (13).[192]

α-Neu*N*Ac-(2 → 6)-β-D-Gal*p*-(1 → 4)-β-D-Glc*pN*Ac-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-D-[1-^{3}H]Glc*N*Ac-itol

(12)

α-Neu*N*Ac-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*pN*Ac-(1 → 2)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-D-[1-^{3}H]Glc*N*Ac-itol

(13)

These allowed distinction between types of neuraminidase activities. Results confirmed, *inter alia*, the hypothesis of a specific defect of α-(2 → 6)-neuraminidase in the case of a sialidosis B.

[189] Z. Zouchová, J. Kocourek, and V. Musilek, *Folia Microbiol.*, 1977, **22**, 98.
[190] M. Edward and R. J. Sturgeon, *Carbohydr. Res.*, 1977, **57**, 3.
[191] E. Mekada, T. Uchida, and Y. Okada, *Exp. Cell Res.*, 1979, **123**, 137.
[192] G. Strecker and J. C. Michalski, *FEBS Lett.*, 1978, **85**, 20.

A soluble neuraminidase is partially deficient in cultured fibroblasts derived from skin biopsies of four mucolipidosis IV patients.[193] Fibroblasts from two obligate heterozygotes also have sialidase activity lower than normal controls. Membrane-bound neuraminidase activity is not affected in this disease. Based on previous and present findings, we propose that this solubilized activity is probably of lysosomal origin. Neuraminidase activity in mucolipidosis IV cells is normal when neuraminlactose is used as substrate. Mixing cell homogenates from mucolipidosis IV patients and from controls results in the expected combined neuraminidase activity, indicating the absence of an internal inhibitor in the deficient cells. It is therefore suggested that the mutation in mucolipidosis IV specifically affects a lysosomal ganglioside neuraminidase, while the remaining non-lysosomal neuraminidases partially mask this deficiency.

The preparation of a sodium (4-methylumbelliferyl α-D-*N*-acetylneuraminate) substrate and its use in a sensitive fluorimetric assay of neuraminidase from human leucocytes, *Vibrio cholerae*, and cultured fibroblasts have been reported.[194] The fibroblast and leucocyte neuraminidases showed maximum activity at pH 4.2–4.4 and K_m values of 0.13 and 0.22 mM, respectively; the enzyme activity was considerably reduced in cultured fibroblasts of patients with mucolipidosis types I, II, and III. *V. cholerae* neuraminidase (pH optimum 4.6, K_m 1.5 mM) showed activation by calcium chloride, H_4edta, sodium chloride, and *N*-acetylneuraminic acid.

Use of a procedure devised for the preparation of enriched populations of subcellular organelles from homogenized bovine spleen showed that the distribution of neuraminidase activity was directed towards either endogenous substrate or exogenous ganglioside substrate – this suggests that it is enriched in the plasma membrane/microsomal fractions.[195] Neuraminidase activity towards exogenous sialoglycoproteins, isolated from erythrocyte membranes, was enriched in the least dense of the plasma membrane/microsomal containing fractions. The endogenous neuraminidase substrates were primarily the sialoglycolipids, haematoside and disialogangliosides. At the pH optimum, 3.8, and 37 °C, release of endogenous neuraminic acid was linear with time for 3 h after which 85% or more of the available endogenous substrate was hydrolysed.

Using lactose 6′-*O*-sulphate, *O*-α-*N*-acetylneuraminosyl-(2 → 3)-D-lactose 6′-*O*-sulphate, *O*-α-*N*-acetylneuraminosyl ?-*O*-sulphate-(2 → 3)-D-lactose 6′-*O*-sulphate, *O*-α-*N*-acetylneuraminosyl ?-*O*-sulphate-(2 → 6)-D-lactose and *O*-α-*N*-acetylneuraminosyl-(2 → 3)- and -(2 → 6)-lactose 6′-*O*-sulphates prepared by chemical sulphation of lactose, *N*-acetylneuraminosyl-lactose and its isomers by using pyridine–SO_3 reagent, significant kinetic differences were observed in the enzymic hydrolysis of the sulphated derivatives compared with unsubstituted substrates.[90] In the case of reactions catalysed by rat-liver lysosomal and *Clostridium perfringens* neuraminidases, the presence of an *O*-sulphate group in

[193] G. Bach, M. Zeigler, T. Schaap, and G. Kohn, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 1341.

[194] M. Potier, L. Mameli, M. Bélisle, L. Dallaire, and S. B. Melancon, *Anal. Biochem.*, 1979, **94**, 287.

[195] C. L. Schengrund, M. A. Repman, and J. T. Nelson, *Biochim. Biophys. Acta*, 1979, **568**, 377.

the *N*-acetylneuraminosyl moiety affected the reaction by decreasing the K_m and the V_{max}; its presence in the D-galactosyl moiety affected the reaction by decreasing the K_m and increasing the V_{max} and its presence in both *N*-acetylneuraminosyl and D-galactosyl moieties decreased the K_m and the V_{max} of the reaction. Mixed-substrate reaction kinetic data indicated competition between the sulphated and unsubstituted substrates for the same active sites on the neuraminidase molecule.

Neuraminidase isolated from the culture filtrate of *Arthrobacter ureafaciens* has been characterized in detail with respect to its action on glycolipids.[196] Strong electrolytes had a reversible inhibitory effect on the action of the enzyme on brain gangliosides in accordance with Debye–Hückel effect of ionic environment on ionic activity, and resulted in an acidic shift and a broadening of the pH optimum. Both ionic and non-ionic detergents markedly enhanced the activity of the thiol-sensitive enzyme on the gangliosides, and caused an acidic shift of the pH optimum. It was suggested that the hydrophobic ceramide moiety increases affinity of the lipid substrate to the enzyme, but inhibits hydrolysis of the substrate, possibly due to its hydrophobic interaction with hydrophobic portions of the enzyme molecule.

Neuraminidase I and neuraminidase II from *Arthrobacter ureafaciens* have been characterized (mol. wts. 5.10×10^4 and 3.90×10^4, respectively) by gel filtration on Ultrogel AcA 44.[197] Neuraminidases I and II were similar to each other in their enzymatic properties except for the substrate specificities towards gangliosides and erythrocyte stroma. Their optimal pH's were between 5.0 and 5.5 with *N*-acetylneuraminosyl-lactose or bovine submaxillary mucin as substrates, but with colominic acid as a substrate, the pH optimum was between 4.3 and 4.5. They were most active around 53 °C, were stable between pH 6.0 and 9.0, and were thermostable up to 50 °C. They did not require Ca^{2+} for activity and were not inhibited by H_4edta. They were inhibited only slightly or not at all by 4-chloromercuribenzoic acid or Hg^{2+}. Both neuraminidases I and II were able to hydrolyse the α-ketosidic linkage of *N*-glycolylneuraminic acid as well as that of *N*-acetylneuraminic acid, and were able to liberate substantially all of the neuraminic acid from various kinds of substrates. However, they cleaved only about 50% of the neuraminic acid from bovine submaxillary mucin. The saponification of bovine submaxillary mucin by mild alkali treatment, on the other hand, resulted in an increased susceptibility to the neuraminidases and brought about the complete liberation of neuraminic acid. Remarkable differences were observed between neuraminidases I and II as regards substrate specificities on gangliosides. The addition of sodium cholate markedly stimulated the enzymatic hydrolysis of gangliosides, and increased the maximum velocity constant of neuraminidase I two-fold and that of neuraminidase II 143-fold. Although neuraminidases I and II were able to hydrolyse (2 → 3), (2 → 6), and (2 → 8)-α-linkages, the initial rate of hydrolysis of *O*-α-*N*-acetylneuraminosyl-(2 → 6)-lactose was greater than that of the (2 → 3)-isomer.

[196] M. Saito, K. Sugano, and Y. Nagai, *J. Biol. Chem.*, 1979, **254**, 7845.
[197] Y. Uchida, Y. Tsukada, and T. Sugimori, *J. Biochem. (Tokyo)*, 1979, **86**, 1573.

Lipid-bound neuraminic acid in the murine melanoma cell is not totally inaccessible to an exogenous macromolecular probe, as formerly believed. Roughly 30% of the neuraminic acid bound to lipid, and an equal proportion of the neuraminic acid bound to protein is cleaved by the action of *Clostridium perfringens* neuraminidase when the purified enzyme is added to the suspension medium of intact murine melanoma cells freshly derived from the tumour.[198] Cleavage of lipid-bound neuraminic acid is indifferent to the presence of Ca^{2+} in the medium. However, maximum release from protein requires a physiological concentration of this divalent cation. Variation in ionic strength has no effect on release of neuraminic acid. These findings show that a restricted portion of the bound neuraminic acid may be released from the intact murine melanoma cell by the extracellularly supplied enzyme acting topographically.

Extracellular neuraminidase production by group A streptococci was examined in 92 strains.[199] Fourteen of these strains produced appreciable amounts of enzyme; 12 of the neuraminidase-producing strains belonged to T types 1, 4, and 12. Production of the enzyme paralleled bacterial growth in culture and was maximal in medium containing 0.2% D-glucose. The enzyme produced by one of these strains was partially purified by ammonium sulphate fractionation and filtration on Sephadex® G-200. Its molecular weight was estimated at 90 000. Activity was optimal at pH 5.7 and in the presence of 0.01 to 0.03 M calcium and magnesium cations. The enzyme was stable at temperatures of 4 and 37 °C for at least 24 hr but was inactivated within 10 min at temperatures of 50 and 65 °C. The enzyme hydrolysed 40% of the neuraminic acid in bovine submaxillary mucin, but was inactive on *O*-α-*N*-acetyl neuraminosyl-lactose, porcine submaxillary mucin, oligosaccharides derived from porcine mucin, or human orosomucoid. The K_m value of this enzyme with bovine submaxillary mucin as substrate was in the order of 3.6×10^{-4} M.

Neuraminidase activity in concentrated culture filtrates of group B streptococci was measured with bovine submaxillary mucin as substrate.[200] Group B streptococcal neuraminidase was not active on human alpha-1 acid glycoprotein and did not show increased activity on bovine submaxillary mucin that had been *O*-deacetylated by alkaline treatment. The enzyme was produced in a variety of media, including a chemically defined medium supplemented with bovine serum albumin or human serum albumin. Maximal levels of activity were present in filtrates from cells grown in a dialysable fraction of Todd-Hewitt broth harvested during the late exponential phase of growth. Dramatic decreases were seen when filtrates from the late stationary phase were assayed. The decrease in specific activity during the stationary phase was shown to be due to proteolytic digestion of neuraminidase and not to the elaboration of an extracellular neuraminic acid aldolase.

12 β-D-Xylosidases

The common identity of human 'acid' β-D-glucosidase and β-D-xylosidase has been recognized[54] (see p. 429).

[198] N. W. Barton and A. Rosenberg, *Biochim. Biophys. Acta*, 1979, **554**, 125.
[199] L. Davis, M. M. Baig, and E. M. Ayoub, *Infect. Immun.*, 1979, **24**, 780.
[200] T. W. Milligan, D. C. Straus, and S. J. Mattingly, *Infect. Immun.*, 1977, **18**, 189.

A β-D-xylosidase of a thermophilic fungus, *Malbranchea pulchella* was purified 99-fold from the culture filtrate after ammonium sulphate fractionation, ion-exchange chromatography, column electrophoresis, and gel filtration.[201] The purified enzyme was found to be homogeneous upon ultracentrifugal analysis, disc electrophoresis and gel filtration. The enzyme (mol. wt. 2.6×10^4 by gel filtration, 2.78 S, $E_{1\,cm}^{1\%}$ at 280 nm = 13.2, pH optimum 6.2–6.8, temperature optimum 50 °C) was inhibited by Hg^{2+}, Zn^{2+}, Cu^{2+}, *N*-bromosuccinimide, 4-chloromercuribenzoate and sodium dodecyl sulphate, while this activity was activated by Ca^{2+}.[202] The enzyme released D-xylose as the end product even from 10% w/v xylobiose solution without forming any D-xylo-oligosaccharides. The enzyme acted well on aryl β-D-xylopyranosides, but showed no activity on alkyl β-D-xylopyranosides, and it was practically free from D-glucosidase activity. The K_m and V_{max} values of this enzyme for xylobiose were calculated to be 2.86×10^{-3} M and 34.5 μmol mg^{-1} min^{-1}, respectively, and for phenyl β-D-xylopyranoside were 3.01×10^{-3} M and 16.2 μmol mg^{-1} min^{-1}, respectively.

A purification method for an extracellular β-D-xylosidase induced in *Penicillium wortmanni* has been described.[203] It includes diafiltration, acetone precipitation, and hydroxyapatite chromatography. The enzyme (mol. wt. 10^5, pI 5.0, pH optimum 3.3–4.0) is most stable at pH 5.0–6.0. Sulphydryl and histidine reagents are not inhibitory. The influence of added cations and anions is negligible. *N*-Bromosuccinimide oxidation of two to three L-tryptophan residues per molecule entails rapid inactivation. Glycon-specificity studies indicated strict requirements at C-2, C-3, C-4, and C-5, although α-L-arabinopyranosides are substrates. As the enzyme seems to hydrolyse D-xylo-oligosaccharides endwise, with retention of configuration in the reaction product, the enzyme is a true glycoside hydrolase, probably operating by a double-inversion mechanism.

Enzyme preparations active against crystalline cellulose, marble-milled filter paper, carboxymethylcellulose, hemicellulose, and D-xylan were obtained from cultures of *Ruminococcus flavefaciens*.[161] These preparations also contained swelling factor, pectin methylesterase, pectin lyase, and low levels of β-D-glucosidase and β-D-xylosidase. 'Carboxymethylcellulase' and D-xylanase activities were present in a high molecular weight complex, but substrate competition studies showed that different active sites were probably responsible for each activity. Analysis of products and viscosity changes during enzymic hydrolysis of cellulose and D-xylan indicated that the most active enzymes were of the *exo*-(1 → 4)-β-D-glycanase type. A variety of reducing sugars was released from cell walls of *Lolium perenne* (perennial ryegrass) by the enzyme preparations.

13 *endo*-β-D-2-Acetamido-2-deoxygalactanases

Both 2- and 4-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactopyranosides have been shown to be effective chromogenic substrates

[201] M. Matsuo, T. Yasui, and T. Kobayashi, *Agric. Biol. Chem.*, 1977, **41**, 1593.
[202] M. Matsuo, T. Yasui, and T. Kobayashi, *Agric. Biol. Chem.*, 1977, **41**, 1601.
[203] F. Deleyn, M. Claeyssens, J. van Beeumen, and C. K. De Bruyne, *Can. J. Biochem.*, 1978, **56**, 43.

for an *endo*-D-2-acetamido-2-deoxygalactanase, and were applied to the monitoring of purification of the enzyme from *Diplococcus pneumoniae*.[204] It was pointed out that a combination of β-D-galactosidase and α-D-2-acetamido-2-deoxygalactosidase would also liberate nitrophenol from the substrates. The enzyme had no action on several other glycosides indicating its strict specificity for the α-D-linkage in the substrate to the aglycone. The enzyme did not act when the aglycone was methyl, but showed activity against the glycosides of phenol, nitrophenols, L-serine, and L-threonine. The use of 4-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-galactopyranoside, a competitive inhibitor of the enzyme, as an affinity ligand for purification of the enzyme was described.

14 *endo*-β-D-2-Acetamido-2-deoxyglucanases

endo-β-D-2-Acetamido-2-deoxyglucanase activity towards an oligo-D-mannosidic type glycoamino-acid substrate was found in the soluble fraction of rat liver and kidney.[205] No evidence for a lysosomal form of the activity was found.

An enzyme from *Streptomyces griseus* previously described as *endo*-β-D-2-acetamido-2-deoxyglucanase L (A. L. Tarentino and F. Maley, *J. Biol. Chem.* 1974, **249**, 811; Vol. 9 in this series, Part II, p. 336) because of its apparent specificity for D-Man-(D-GlcNAc)$_2$-L-Asn, has been purified to homogeneity.[206] The enzyme has now been found to hydrolyse (D-GlcNAc)$_3$ to (D-GlcNAc)$_2$ plus D-GlcNAc, and (D-GlcNAc)$_4$ to 2(D-GlcNAc)$_2$, at twice the rate observed for D-Man-(D-GlcNAc)$_2$-L-Asn. Removal of the L-asparagine from the latter compound reduces the rate of hydrolysis by about 30-fold. Reduction of (D-GlcNAc)$_3$ to (14) eliminates this compound as a substrate for *endo*-β-D-2-acet-

D-Glc*p*NAc-(1 → 4)-D-Glc*p*NAc-(1 → 4)-D-Glc*N*Ac-itol

(14)

amido-2-deoxyglucanase L. However, the reduction of (D-GlcNAc)$_4$ does not affect its rate of hydrolysis. *endo*-β-D-2-Acetamido-2-deoxyglucanase L consists of a single polypeptide chain (mol. wt. $4.95 \pm 0.04 \times 10^4$) which on isoelectric focusing separates into two closely migrating bands; a major band with a pI of 4.25 and a minor one with a pI of 4.20. Both bands possess similar enzyme activities and amino-acid composition, but differ slightly in their tryptic peptide maps.

15 Agarases

An α-agarase has been isolated from a Gram-negative marine bacterium that liquefies agar.[207] The enzyme was purified by chromatography on diethylamino-

[204] J. Umemoto, K. L. Matta, J. J. Barlow, and V. P. Bhavanandan, *Anal. Biochem.*, 1978, **91**, 186.
[205] R. J. Pierce, G. Spik, and J. Montreuil, *Biochem. J.*, 1979, **180**, 673.
[206] R. B. Trimble, A. L. Tarentino, G. Evans, and F. Maley, *J. Biol. Chem.*, 1979, **254**, 9708.
[207] K. S. Young, S. S. Bhattacharjee, and W. Yaphe, *Carbohydr. Res.*, 1978, **66**, 207.

ethylcellulose (OH^- form) buffered at pH 7.2. The purified enzyme specifically cleaved (1 → 3)-α-L-linkages in agarose, yielding a homologous series of agaro-oligosaccharides. Agarotetraitol and agarohexaitol were characterized.

16 Alginases and Alginate Lyases

Alginate lyase from *Turbo cornutus* acted on sodium alginate and resulted in a rapid decrease in viscosity and a gradual increase in reducing power of the substrate solution.[208] The result of gel filtration of the reaction products indicated that uronic acid oligosaccharides were liberated as the final product. The fact that the enzyme attacked preferentially the D-mannuronate-rich moieties of the alginate molecule was demonstrated by an analysis with paper electrophoresis of the reaction products after acid treatment, which separated the uronic acid oligosaccharides from the uronic acid polysaccharides.

Alginate lyase has been purified from mid-gut gland of wreath shell.[209] The enzyme was homogeneous as judged by polyacrylamide gel electrophoresis in sodium dodecyl sulphate (mol. wt. 3.2×10^4 under these conditions). Absorption spectra of the reaction products indicated that the enzyme had lyase activity. The enzyme (pH optimum 8.8–9.2) was most stable at pH 5–6. Phosphate showed strong stabilizing and enhancing effects on the enzyme activity. Divalent cations behaved differently toward alginic acid and propylene glycol alginate, suggesting requirements for free carboxy-groups and a single glycosidic chain in the enzyme action.

17 α-Amylases

A flow-through viscometer developed for application as a sensor in automated analyses consisted of a glass capillary connected to the sample flow circuit with thin-walled tubes at both ends.[210] These tubes separate the fluid to be tested from a hydraulic fluid, and this construction ensures the absence of dead space and a minimal test volume. Development of a mathematical model describing the enzymatic degradation of macromolecules resulted in a reciprocal equation allowing rectilinear presentation of the calibration data. The application of the enzyme to the assay of amylase was described.

An automated assay for serum amylase was described.[211] Using the Auto-Analyzer II the method employs, as substrate, a solution of dyed amylopectin which is commercially available (DyAmyl-L). After incubation of serum and substrate at 55 °C, KOH ($1.0\,mol^{-1}$) is introduced and the coloured products of enzymic hydrolysis are separated by dialysis against phosphate buffer. Absorbance is measured at 540 nm and the enzymic activity read from a standard curve obtained using calibration sera Versatol E and EN. The system provides accurate and reproducible measurement of serum amylase. The results obtained from

[208] T. Muramatsu and M. Katayose, *Agric. Biol. Chem.*, 1979, **43**, 287.
[209] T. Muramatsu and M. Katayose, *Agric. Biol. Chem.*, 1977, **41**, 1939.
[210] J. Kuiper, J. A. Roels, and M. H. J. Zuidweg, *Anal. Biochem.*, 1978, **90**, 192.
[211] J. A. Carney, S. A. Osibeluwo, J. A. Clements, and R. F. Jay, *Ann. Clin. Biochem.*, 1977, **14**, 350.

assaying 100 'normal' sera are compared with those using another method of α-amylase estimation.

A kinetic, two-point method for the assay of α-amylase in serum, involving spectrophotometric measurement of a starch–iodine complex, has been described.[212] This approach avoids interferences by serum proteins and other substances that react with iodine. The method requires less than 4 min per assay, only 10 μl of sample is used, and precision and accuracy are comparable to those of established procedures.

Support for the hypothesis that reasons for the variety of human salivary α-amylase is due to deamidation by release of ammonia from L-asparagine and/or L-glutamine residues has been gained from work on NH_4^+ inhibition of deamidation, suggesting a non-enzymic mechanism and is congruent with the function of L-glutaminyl and L-asparaginyl residues in proteins effecting a timed alteration of chemical structure through deamidation.[213]

An anomalous ratio of salivary to pancreatic amylase activities has been observed in urine from juvenile diabetics.[214] Decreased pancreatic amylase activity in urine from those subjects appears to be a characteristic trait.

Storage of human saliva, serum, and duodenal secretion transformed the amylase fractions on cellulose acetate membrane electrophoresis into more-anionic forms. Incubation with lectins, proteases, D-glucosidases, neuraminidase, and some effectors did not modify this conversion, which was promoted by rising temperature and pH values.[215] Increasing concentrations of ammonium ions delayed the transformation of amylolytic fractions, thus indicating non-enzymatic deamidation as the reason for amylase isoenzyme development. A change of molecular weight could be excluded.

An investigation designed to determine whether human serum α-amylase isoenzyme measurements can aid in establishing the diagnosis of acute pancreatitis in renal failure patients has been reported.[216] Twenty-two haemodialysis patients were studied, four of whom had documented acute pancreatitis. Total serum-amylase and pancreatic-amylase isoenzyme concentrations were significantly higher ($p < 0.02$) in the pancreatitis patients than in the 18 patients without pancreatitis, but there was appreciable overlap between these two groups. In each of the four patients with pancreatitis, the percentage of serum amylase derived from the pancreas (range, 80–90%) was well above the highest value (67%) observed in the patients without pancreatitis. These results suggest that determination of the percentage of serum amylase represented by pancreatic-amylase isoenzyme is of assistance in establishing the diagnosis of acute pancreatitis in renal failure patients.

The enzymatic method of H. W. Schiwara (*Z. Klin. Chem. Klin. Biochem.*, 1972, **10**, 12) using the enzymatic reaction sequence α-amylase–α-D-glucosidase–hexokinase/D-glucose 6-phosphate dehydrogenase for the determination of

[212] L. B. Marshall and G. D. Christian, *Anal. Chim. Acta*. 1978, **100**, 223.
[213] K. Lorentz, *Clin. Chim. Acta*, 1979, **93**, 161.
[214] J. Kamaryt, J. Stejskal, and L. Osickova, *J. Clin. Chem. Clin. Biochem.*, 1978, **16**, 539.
[215] K. Lorentz and B. Flatter, *Enzyme*, 1979, **24**, 163.
[216] M. D. Levitt and C. Ellis, *J. Lab. Clin. Med.*, 1979, **93**, 71.

α-amylase was evaluated on the ABA-100 instrument.[217] The coefficient of variation for control sera and human pooled serum was 0.9–4.2% within series, and 1.4–6.6% day to day. Reference values for a healthy population (212 blood donors) in sera were 13–79 U l^{-1} (±2 SD), mean 46 U l^{-1}. In catch urines the values did not show a normal distribution; the minimal and maximal range for men was 58–385 U l^{-1}, for women 7–318 U l^{-1}. The kinetic curve of the enzymatic test was measured and the influence of D-glucose and linearity studied. In comparison with the enzymatic test, the chromogenic method Amylochrom Roche® was tested on the sera and urine of patients. The coefficient of correlation in sera was $r = 0.975$, and in urine $r = 0.965$.

Anomeric forms of D-glucose produced from phenyl α-maltoside, maltose, or phenyl α-D-glucopyranoside have been determined quantitatively by simultaneous measurements of optical rotation and reducing power, for eight kinds of D-glucose-producing (1 → 4)-α-D-glucosyl hydrolases [*exo*-(1 → 4)-α-D-glucoanases], including D-glucose-forming amylase from human urine, and α-D-glucosidases from pig serum, honey bee, buckwheat seed, rice seed, sugar-beet seed, flint-corn seed, and brewer's yeast.[123] All the eight enzymes studied were found to produce α-D-glucopyranose exclusively.

Three amylase phenotypes, AmIB, AmIC, and AmIBC, were detected by electrophoresis of blood serum from 329 Holstein cattle.[218] These phenotypes appear to be controlled by two alleles AmIB and AmIC at the amylase I locus (AmI). Frequencies were 0.518 and 0.482 for alleles B and C. The numbers of phenotypes correspond closely to expectation of Hardy–Weinberg equilibrium. No evidence of linkage between the AmI locus and other genetic marker loci of blood and milk was detected.

The isolation and characterization of porcine pancreatic α-amylase have been reported.[219] A large fluorescence enhancement of 2-(4-toluidinylnaphthalene)-6-sulphonate caused by amylose decreases as the substrate is degraded by amylases.[220] This property was used to follow the enzymatic hydrolysis of amylose and to analyse the action pattern of six kinds of 'amylases': porcine pancreatic, Taka-amylase A, two bacterial α-amylases, soybean β-amylase, and *Rhizopus riveus* glucoamylase. This method can substitute the conventional blue-value method, and is more sensitive for short-chain amylodextrins. The advantage of the new method over the blue-value method is that it is useful to monitor the hydrolysis of malto-oligosaccharides to which the blue-value method cannot be applicable, and that it enables continuous monitoring of the enzyme reactions.

A large fluorescence enhancement of 2-(4-toluidinylnaphthalene)-6-sulphonate (TNS) observed in the presence of amylose was utilized to monitor quantitatively the time course of porcine pancreatic α-amylase catalysed hydrolysis of amylose with a number-average degree of polymerization of 16.8.[221] The

[217] W. Hohenwallner, E. Wimmer, and R. Sommer, *J. Clin. Chem. Clin. Biochem.*, 1979, **17**, 349.

[218] C. L. T. Rowbridge and H. C. Hines, *J. Dairy Sci.*, 1979, **62**, 982.

[219] I. Klum, *Collect. Czech. Chem. Commun.*, 1979, **44**, 288.

[220] H. Kondo, H. Nakatani, and K. Hiromi, *Agric. Biol. Chem.*, 1977, **41**, 631.

[221] H. Kondo, H. Nakatani, K. Hiromai, and R. Matsuno, *J. Biochem. (Tokyo)*, 1978, **84**, 403.

slope of the plot of decrease in the relative fluorescence intensity of the TNS-amylose system (termed as the fluorescence value) *versus* the number of linkages hydrolysed (reducing value) in the course of enzyme-catalysed hydrolysis was shown to be useful to describe the degree of 'multiple attack', which is defined as the number of re-attacks on a long-chain substrate molecular per one encounter of the enzyme and the substrate. A parameter γ was defined as the ratio of the reciprocal of the slopes obtained at each pH to that at pH 10.5, where the multiple attack is not operating. The γ *versus* pH profile gave an apparent pK value of about 9, indicating that some ionizable groups participate in the multiple attack mechanism. Based on a reaction scheme involving a 'sliding' of the substrate molecule on the enzyme, which may contribute to the multiple attack mechanism besides binding, dissociation, and cleavage steps of the substrate, and on the assumption of the steady-state for the enzyme–substrate complex, rate equations were obtained to describe the time course of hydrolysis of a linear substrate. The product distribution with the progress of the reaction can be calculated theoretically, and is dependent on the number of multiple attack and the mode of sliding. By comparing the experimental data with the theoretical ones, it was suggested that the multiple attack occurs through the sliding by maltose unit of the retained fragment on the enzyme, which is one of the fragments produced by the initial cleavage of the substrate molecule. It was found that anions (chloride, bromide, and nitrate ions) which critically affect the enzyme activity have no effect on the degree of multiple attack.

The α-amylase activities of serum, liver, pancreas, submaxillary and parotid glands, saliva, duodenum, colon, lung, heart, spleen, kidney, and skeletal muscle of the domestic cat (*Felix catus*) have been determined.[222] As in most mammals, the highest α-amylase content was found in the pancreas. Levels in saliva and salivary glands were very low. α-Amylase activities of the serum, liver, and pancreas has a pH optimum of 7.0–7.5, and were reversibly inactivated by removal of chloride ion.

Poly(A)-rich mRNA prepared from rabbit pancreas directed the synthesis of a protein (mol. wt. 5.65×10^4), which, using a cell-free protein synthesizing system derived from wheat embryo, appeared to represent the product of α-amylase mRNA cell-free translation.[223] This protein, which was 1500 daltons larger than the purified rabbit pancreas α-amylase, was resolved by isoelectric focusing into discrete species similar to those observed for purified α-amylase. The protein, which on peptide analysis demonstrated considerable identity with the α-amylase, was selectively precipitated by glycogen, but with a much lower recovery than that observed for rabbit on porcine pancreatic α-amylases.

The involvement of calcium, ATP, and cyclic AMP-dependent protein kinase activity in the release of amylase from rat parotid glands has been examined.[224] The differential involvement of Ca^{2+}, ATP, and cyclic AMP-dependent protein kinase activity in amylase release induced by various agents such as tolbutamide

[222] R. L. McGeachin and J. R. Akin, *Comp. Biochem. Physiol. B*, 1979, **63**, 437.
[223] S. B. Ray, B. E. Rothenberg, and M. G. Rosenfeld, *J. Biol. Chem.*, 1979, **254**, 1196.
[224] J. C. Kusek, *Biochim. Biophys. Acta*, 1979, **583**, 295.

suggests that three types of reactions are involved in the release of amylase, dibutyryl cyclic AMP, elevation of the extracellular K^+ concentration, reduction of the H^+ concentration, La^{3+}, and caffeine.

The kinetics of $^{45}Ca^{2+}$ uptake, efflux and calcium potentiation of amylase release by slices of rat parotid glands have been examined.[225] Pretreatment of the tissue with 11.25 mM $^{45}Ca^{2+}$ medium increased the total tissue ^{45}Ca content. Lanthanum (1 mM) decreased tissue uptake, blocked the slow components of exchange and appeared to inhibit transcellular calcium movement. Neither dibutyryl cyclic AMP nor caffeine caused consistently significant effects on $^{45}Ca^{2+}$ kinetics, or total ^{45}Ca content. Carbamylcholine increased the initial rate of $^{45}Ca^{2+}$ uptake, but had no effect on total uptake. Elevation of the extracellular Ca^{2+} concentration to 11.25 mM during stimulation of amylase release resulted in an initial decrease in the rate of amylase release followed by a potentiation of release which developed slowly, requiring 40–50 min to reach the maximal response. The inability to detect release-related changes in either calcium influx or mobilization, and the lengthy times and high Ca^{2+} concentrations required to achieve calcium potentiation suggests that calcium does not couple amylase release.

Impaired release of amylase from the parotid glands of rats has been observed when the animals were treated with reserpine.[226]

α-Amylase biosynthesis and release in the rat parotid has been studied under various conditions.[227] Incorporation of L-[^{3}H]leucine into α-amylase, extracted from the tissue by immunoadsorbent, was measured and found to be time-dependent and totally inhibited by the protein synthesis inhibitor puromycin. Adrenaline, at a concentration that gave maximum stimulation of release, inhibited L-[^{3}H]leucine incorporation into both total protein and α-amylase. This effect was reversed by phentolamine. Adrenaline and isoproterenol stimulated biosynthesis of total protein and α-amylase. These effects were blocked by propanolol, as were the effects on release. Dibutyryl cyclic AMP mimicked the effects of isoproterenol and adrenaline on both α-amylase biosynthesis and release. All the above stimulatory effects on α-amylase biosynthesis were only observed if the tissue was pretreated with effector before pulse-labelling with L-[^{3}H]leucine. Insulin stimulated incorporation of L-[^{3}H]leucine into total protein and α-amylase when added to the tissue at the same time as the L-leucine. Carbamoylcholine decreased L-[^{3}H]leucine incorporation into total protein and amylase when both were added to the tissue simultaneously, but this effect was prevented by removal of effector and washing the tissue before addition of L-[^{3}H]leucine. Stimulation of β-adrenergic receptors increased both amylase release and biosynthesis, but stimulation of α-receptors can inhibit biosynthesis without inhibiting release. Cholinergic agents can also inhibit α-amylase biosynthesis, but stimulate release. Insulin at approximately physiological concentration can increase incorporation of L-leucine into amylase without stimulating release.

[225] J. C. Kusek, *Biochim. Biophys. Acta*, 1979, **583**, 309.
[226] J. R. Martinez and O. H. Peterson, *Experientia*, 1979, **35**, 1343.
[227] M. A. McPherson and C. N. Hales, *Biochem. J.*, 1978, **176**, 855.

Poly(A)-containing RNA from rat parotid gland directs the cell-free synthesis of several products in the reticulocyte lysate translation system including a polypeptide (mol. wt. 5.8×10^4) which is immunoreactive with endogenous α-amylase (mol. wt. 5.6×10^4).[228] The cross-reactivity and peptide homology suggested that the cell-free product may be a precursor of the mature α-amylase. While the α-amylase *N*-terminus is blocked the polypeptide is not; a partial sequence was obtained for the polypeptide. The highly hydrophobic nature of the polypeptide *N*-terminus suggested that, like the secreted polypeptides, the extra piece may play a role in the transport and secretion of the mature α-amylase.

Rat pancreas amylase activity was found to be irreversibly destroyed when subjected to polyacrylamide gel electrophoresis.[229] The measure of enzyme inactivation was dependent on the duration of electrophoresis. In the presence of urea the amylase denaturing effect was enhanced.

α-Amylase has been isolated and purified, to a state free from contaminating enzymes, from the barley malt, and ungerminated barley grains.[230] Starch granules, isolated from the same sources, served as substrates to study the mode of action of amylases on the digestion of starch granules *in vitro*. β-Amylase alone had a very small activity on starch granules and formed maltose as sole digestion products. α-Amylase played a major role in the digestion of starch granules and the combined action of α- and β-amylases was more effective than the action of α-amylase alone, but was less effective, on the same enzyme activity basis, than the crude extract from barley malt, which was thought to represent *in vitro* the enzyme system for the digestion of starch granules in the germinating barley grains. The possible roles to be played by a debranching enzyme, α-D-glucosidase and/or D-glucosyl- or D-glucano-transferases were discussed.

An amylase, previously detected in barley and described as a new barley amylase, has been further purified by immunoadsorption and ion-exchange chromatography.[231] Analysis by isoelectric focusing and immunochemical techniques showed that the enzyme preparation did not contain the normal α- and β-amylases usually found in barley and malt. The enzyme had a very low isoelectric point (*ca.* pH 3.0) and was identified as an α-amylase on the basis of its action pattern on amylose.

The effect of mercury incorporated into barley starch granules during their digestibility by barley malt α-amylase has been studied. The digestibility varied with the batch of starch samples.[232]

The determination of α-amylase activity from cotton leaves with amylopectin azure has been described.[233]

[228] M. Gorecki and E. P. Zeelon, *J. Biol. Chem.*, 1979, **254**, 525.
[229] J. Dijkhof and C. Poort, *Enzyme*, 1978, **23**, 410.
[230] I. Maeda, S. Kiribuchi, and M. Nakamura, *Agric. Biol. Chem.*, 1978, **42**, 259.
[231] A. W. MacGregor, J. Daussant, and M.-L. Niku-Paavola, *J. Sci. Food Agric.*, 1979, **30**, 1071.
[232] H. Taniguchi, I. Maeda, and M. Nakamura, *Agric. Biol. Chem.*, 1978, **42**, 661.
[233] C. W. Chang, *Microchem. J.*, 1979, **24**, 50.

The Perkin–Elmer model 191 grain amylase analyser has potential usefulness to the cereal industry for measurement of amylase.[234] For example, it can satisfactorily measure the potency of fungal α-amylase supplements. With amylopectin as substrate, it can measure various concentrations of α- or β-amylase. The analyser cannot, however, differentiate between the effects of these two enzymes, which are both normally present in cereals. This limits its present usefulness, particularly for measurement of α-amylase. Selective inactivation with $HgCl_2$ or heat-treatment cannot guarantee that β-amylase is completely destroyed or that α-amylase has not been affected. Manufacturer refinements to the prototype method and instrument such as developing a β-limit dextrin substrate and increasing the sensitivity of the analyser may ultimately provide a simple, rapid assay of α-amylase.

During the germination of rice seed, multiple forms of amylase molecules are synthesized in the endosperm tissues as detectable by isoelectric focusing on polyacrylamide gel.[235] Among three major amylase components, two of them were purified to an apparent homogeneity, and bands A and C were identified to be α- and β-amylase, respectively. Some of their enzymic properties have been studied.

The substrate specificity of rice α-D-glucosidase II had been studied.[139] The enzyme was active especially on nigerose, phenyl α-maltoside, and malto-oligosaccharides. The actions on isomaltose and phenyl α-D-glucopyranoside were weak, and on sucrose and methyl α-D-glucopuranoside, negligible. The α-D-glucans, such as soluble starch, amylopectin, β-limit dextrin, glycogen, and amylose, were also hydrolysed (see p. 455).

Chloroplasts isolated from spinach (*Spinacia oleracea*) plants grown under controlled light/dark and temperature regimes, contained the phosphorolytic and amylolytic pathways for starch breakdown.[140] The latter consists at least of α- and β-amylase and β-D-glucosidase ('maltase') (see p. 455).

Hard red winter wheat, oats, and pearl millet were germinated at 15 °C for periods up to 14 days.[236] Endosperm areas in kernels fractured with a razor blade and starches isolated from the malted flours were examined with a scanning electron microscope to determine the morphology of starch granules and the nature of α-amylolytic attack on granules. Free sugars content, damaged starch content, and α-amylase activity of the flours were determined to establish relationships between physical changes in the cereal grains and formation of α-amylase during germination. Starch granules in the three cereals were degraded during germination by α-amylase accompanied by production of free sugars and increases in the damaged starch of flours milled from the germinated grains. Although wheat starch granules had the highest α-amylase activity, this enzymic degradation was less than expected. Enzymic attack was evidenced as channels or pits on the surfaces of wheat and millet starch granules. Compound oat starch granules were relatively resistance to enzymic attack and no evidence of erosion

[234] J. E. Kruger, P. M. Ranum, and A. W. Macgregor, *Cereal Chem.*, 1979, **56**, 209.
[235] K. Okamoto and T. Akazawa, *Agric. Biol. Chem.*, 1978, **42**, 1379.
[236] D. R. Lineback and S. Ponipom, *Stärke*, 1977, **29**, 52.

of the granule surface was observed; small granula were released from the compound granules during germination. The most extensive degradation was obtained with millet starch, appearing as discrete holes leading to the interior of the granules. Concentric shells were visible in the interior of wheat and millet starch granules, but were not observed in oat starch granules.

Sensitivity of a test in which a commercially available substrate, Phadebas®, was used for the assay of α-amylase in sprouted wheat has been increased by the use of optimally buffered (pH 6.20) Cibacron Blue dyed amylose at 60 °C.[237] With the modified test, measurable α-amylase levels were in the range of 3–30 millidextrinizing units (mDU) as compared to the 10–90 mDU range in a test previously reported for bread wheats. The modification extends the use of the test to evaluation of sprout damage in wheats used in the manufacture of Japanese noodles and sponge cakes.

A semi-automated nephylometric determination of α-amylase activity in sprouted wheat kernels is dependent upon the decrease in turbidity of an amylopectin suspension when acted upon by α-amylase.[238] The rate of nephlos change is used as a measure of α-amylase activity.

The affinity chromatographies of bacterial and fungal α-amylases on (4-chloromercuribenzoyl)-cellulose have been examined.[239]

A moderately halophilic *Acinetobacter* sp., capable of producing dextrinogenic amylase, was isolated from sea-sands.[240] Maximum enzyme production was obtained when the bacterium was cultivated aerobically in media containing 1–2 M-NaCl or 1 M-KCl. Two kinds of amylase, amylases I and II, were purified from the culture filtrate to an electrophoretically homogeneous state by glycogen-complex formation, DEAE-Sephadex® A-50 chromatography, and Sephadex G-200 gel filtration. Both enzymes had maximal activity at pH 7.0 in 0.2–0.6 M-NaCl or KCl at 50–55 °C. The activities were lost by dialysis against distilled water. Molecular weights for amylases I and II were estimated to be 55 000 and 65 000 respectively by SDS-gel electrophoresis. The action pattern on amylose, soluble starch, and glycogen showed that the products were maltose and maltotriose.

The acid-stable α-amylase of *Aspergillus niger* and the acid-labile α-amylase of *A. oryzae* have been studied.[241] It was shown that in addition to the more stable acid-resistant properties, the α-amylase of *A. niger* possesses increased thermal stability in comparison with the α-amylase of *A. oryzae*. The molecular weight of the acid-stable α-amylase is 5.8×10^4. The amino-acid composition, as well as the *C*- and *N*-terminal amino-acids of both forms of α-amylases were determined. It was shown that the enzymes each contain one thiol group, which, being bound to Ca^{2+}, plays an important role in maintaining the catalytically active conformation of the enzyme.

[237] P. R. Mathewson and Y. Pomeranz, *J. Assoc. Off. Anal. Chem.*, 1979, **62**, 198.
[238] K. Pradad, C. A. Watson, and J. B. Carney Jun., *Cereal Chem.*, 1978, **56**, 43.
[239] J. Kucera, *Collect. Czech. Chem. Commun.*, 1978, **44**, 804.
[240] H. Onishi and O. Hidaka, *Can. J. Microbiol.*, 1978, **24**, 1017.
[241] G. I. Kvesitadze, R. S. Svandze, T. Sh. Buachidze, and M. V. Bendianishivili, *Biochemistry (Engl. Transl.)*, 1978, **43**, 1330.

O-6-Deoxy-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose, *O*-6-chloro-6-deoxy-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose, *O*-6-bromo-6-deoxy-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose, and *O*-6-deoxy-6-iodo-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose were prepared, taking advantage of the substrate specificities of Tala-amylase A (α-amylase *ex Aspergillus orzyae*) and glucoamylase, and the action of Taka-amylase A on these substrates was investigated.[242] The Michaelis constant K_m and the molecular activity k_0 were determined at 37 °C and pH 5.2 using the modified maltotrioses. The values of K_m and k_0 decreased upon modification of maltotriose and those of $k_0K_m^{-1}$ were in agreement with the comparative initial rates for the corresponding derivatives of phenyl α-maltoside at low substrate concentrations. This result suggested that a subsite of the enzyme may have a specific interaction with halogen atoms in the substrate. All halogenomaltotrioses examined showed substrate inhibition at high substrate concentrations.

The action pattern and mechanism of the Taka-amylase A-catalysed reaction were studied quantitatively and kinetically by product analysis using a series of malto-oligosaccharides from maltotriose (G_3) to maltoheptaose (G_7) labelled at the reducing end with D-[^{14}C]glucose.[243] A marked concentration dependency of the product distribution from the end-labelled oligosaccharides was found, especially with G_3 and G_4 as substrates. The relative cleavage frequency at the first glycosidic bond counting from the non-reducing end of the substrate increases with increasing substrate concentration. Further product analyses with unlabelled and end-labelled G_3 substrates yielded the following findings: (i) maltose is produced in much greater yield than D-glucose from unlabelled G_3 at high concentration (73 mM); (ii) malto-oligosaccharides higher than the starting substrate were found in the hydrolysate of labelled G_3; (iii) non-reducing end-labelled maltose (G*–G), which is a specific product of condensation, was found to amount to only about 4% of the total labelled maltose. Based on these findings, it was concluded that transglycosylation plays a significant role in the reaction at high concentrations of G_3, although the contribution of condensation cannot be ignored. A new method for evaluating subsite affinities was proposed; it is based on the combination of the kinetic parameter ($k_0K_m^{-1}$) and the bond-cleavage distribution at a sufficiently low substrate concentration, where transglycosylation and condensation can be ignored. This method was applied to evaluate the sub-site affinities of Taka-amylase A. Based on a reaction scheme which involves hydrolysis, transglycosylation, and condensation, the time courses of the formation of various products were simulated, using the Runge–Kutta–Gill method. Good agreement with the experimental results was obtained.

Soluble, heat-stable *Bacillus* species α-amylase has been used to liquefy barley starch to obtain slurries of various DE (dextrose equivalent) values up to ~37.[244]

Extension of earlier work on subsite mapping shows that a model for depolymerase action can be used to predict product ratios accurately according

[242] K. Omichi and Y. Matsushima, *J. Biochem. (Tokyo)*, 1978, **84**, 835.
[243] T. Suganuma, R. Matsuno, M. Ohnishi, and K. Hiromi, *J. Biochem. (Tokyo)*, 1978, **84**, 293.
[244] Y. Y. Linko, A. Lindroos, and P. Linko, *Enzyme Microb. Technol.*, 1979, **1**, 273.

to the extent of reaction.[245] The experimental product ratios for *Bacillus amyloliquefaciens* α-amylase acting on ^{14}C-reducing end-labelled malto-oligosaccharides (DP 3–10) were reported. The depolymerase computer model generated a 10-subsite map for the α-amylase with a catalytic site adjacent to subsite 7. The binding affinities of the subsites were then used as the sole input in another computer program to predict quantitatively the mole fraction of products *versus* the extent of hydrolysis for substrates of a variety of chain lengths; excellent agreement with experimental data for seven malto-oligosaccharides was obtained.

In order to study the regulation mechanism of the production of extracellular α-amylase *Bacillus subtilis*, regulator genes relating to the hyper-production of α-amylase *B. natto* IAM 1212, *B. subtilis* YN9, and *B. subtilis* var. *amylosaccharitics* were transferred into 6160-BC6 and RAN 1.[246] The introduction of genes, *pap*9, or *papSAC*, into the two recipient strains showed numerous changes in the extracellular protein compositions detected by disc gel electrophoresis in sodium dodecyl sulphate. These results suggest that the production and localization of the alkaline phosphatases are regulated independently from the genes relating to the hyperproduction of extracellular α-amylase and protease.

Extracellular α-amylase was purified to homogeneity from Marburg strain of *Bacillus subtilis*.[247] The enzyme is a single polypeptide chain of molecular weight approximately 67 000. Its *N*-terminal amino-acid sequence is (15).

Leu-Thr-Ala-Pro-Ser-Ile-Lys

(15)

A membrane-derived α-amylase was solubilized from membrane vesicles by treatment with Triton X-100 and was highly purified by chromatography on an anti-α-amylase-protein A-Sepharose column. Membrane-derived α-amylase was indistinguishable from the soluble extracellular enzyme by sodium dodecyl sulphate-gel electrophoresis and radioimmunoassay. The membrane-derived enzyme contains phospholipid. Approximately 30 to 80% of the phospholipid was extracted from the purified enzyme by chloroform–methanol. The extracted phospholipid was predominantly phosphatidylethanolamine. Treatment with phospholipase D released phosphatidic acid. Membrane-bound α-amylase was latent in membrane vesicles. Release of membrane-bound α-amylase from vesicles by an endogenous enzyme was maximal at pH 8.5, was inhibited by metal chelators and di-isopropyl fluorophosphate and was stimulated by Ca^{2+} and Mg^{2+}. The amount of membrane-bound α-amylase was related to the level of secretion.

The action of *Bacillus subtilis* α-amylase on 6-*O*-methylamylose and 6-deoxyamylose has been investigated, the products being characterized by mass spectrometry.[248] A modified D-glucose monomer was not found in any of the

[245] E. M. Torgerson, L. C. Brewer, and J. A. Thoma, *Arch. Biochem. Biophys.*, 1979, **196**, 13.
[246] K. Hitotsuyanagi, K. Yamane, and B. Maruo, *Agric. Biol. Chem.*, 1978, **42**, 585.
[247] P. Mantsala and H. Zalkin, *J. Biol. Chem.*, 1979, **254**, 8540.
[248] C. E. Weill, M. Kaminsky, and J. Guerrera, *Carbohydr. Res.*, 1979, **73**, 337.

hydrolysates, suggesting that a D-glucopyranosyl residue that has a modified hydroxymethyl group is not hydrolysed if the modified residue is alone on the reducing side of the cleavage site. Disaccharides containing one modified D-glucopyranosyl residue, and trisaccharides having one or two such residues, are formed during the reaction, indicating that the modified units can occupy sites to the 'right' of the cleavage site as long as there is also at least one modified D-glucopyranose residue present.

α-Amylase K is a novel thermostable α-amylase from *Bacillus subtilis* which produces maltohexaose from starch.[249] Various methods showed the existence of two forms which did not differ significantly in action and which may therefore be isoenzymes. α-Amylase K is stable at 60 °C in the presence of additional calcium chloride with 80% activity remaining after 6 days. It is completely deactivated after 10 min at 90 °C and inhibited by tris-(hydroxymethyl)-aminomethane above pH 5.0. The purified α-amylase K fractions are less thermostable than the original enzyme solution. α-Amylase K is stable to freeze drying but reconstituted enzyme solution was less active at pH-values below 5.8.

The action pattern of α-amylase K, a novel amylase from a strain of *Bacillus subtilis*, on amylopectin, glycogen, and a number of starches is reported.[250] It was found that the predominant component of the hydrolysis mixture was maltohexaose under most conditions with varying amounts of maltoheptaose, maltopentaose, and maltotriose being present depending on the time of hydrolysis. Michaelis constants and maximum rates of reaction were determined for a number of substrates which indicated that the enzyme was most active against amylopectin. Conditions for large scale industrial use of the enzyme, including high substrate concentration, and the absence of buffer from the hydrolysis mixture were discussed. A thin layer chromatographic technique for the rapid separation and identification of the hydrolysis products (up to maltodecaose) is reported.

The mechanism of starch degradation by the yeast *Lipomyces starkeyi* was studied in strain CBS 1807.[251] It was shown that the amylolytic system is associated with cell walls. It is equally well induced by starch or maltose. The enzyme exhibits considerable α-amylase type activity and also liberates a small amount of D-glucose. Enzyme synthesis occurs during the exponential growth phase. The regulation of the synthesis of the enzyme is discussed.

A large amount of amylase inhibitors, of at least four kinds, were produced by *Streptomyces* sp. No. 280 when cultivated on 3% oatmeal medium and it was found that the molecular weight of the inhibitors were transformed to small molecules during the cultivation time.[252] The transformation of the amylase inhibitor was found to result from degradation of its carbohydrate moiety by α-amylase in the culture broth. The amylase inhibitor was hydrolysed partially by the action of Taka-amylase A or hog pancreatic α-amylase. With hydrolysation of amylase inhibitor by α-amylase, neutral sugars (mainly maltose) were

[249] J. F. Kennedy and C. A. White, *Stärke*, 1979, **31**, 93.
[250] J. F. Kennedy, C. A. White, and C. L. Riddiford, *Stärke*, 1979, **31**, 235.
[251] G. J. Moulin and P. Galzy, *Agric. Biol. Chem.*, 1979, **43**, 1165.
[252] H. Chafn and S. Ueda, *Agric. Biol. Chem.*, 1979, **43**, 2265.

liberated from the amylase inhibitor and a modified inhibitor was newly formed, but amylase inhibitory activity against glucoamylase was not changed. The inhibitory activity against muscle phosphorylase *a*, however, was almost completely lost.

The *Streptomyces aureofaciens* extracellular proteolytic system has been split into four fractions by column chromatography giving three purely caseinolytic fractions and one fraction active toward both starch and casein.[253] The caseinolytic and amylolytic fraction was further fractionated by DEAE-Sephadex® A-50 chromatography into one purely amylolytic fraction and another showing both activities, was refractioned into four new fractions by DEAE-cellulose chromatography. These fractions were found to be heterogeneous by polyacrylamide gel electrophoresis, three of them acted on both starch and casein, and a fourth was only caseinolytic.

Amylase inhibitor was purified by about 25-times from culture filtrate of *Streptomyces diastaticus* through adsorption on active carbon and ion-exchange chromatographies and gel filtration, EI-complex formation with bacterial α-amylase, isolation of complex by gel filtration, dissociation from complex by acid denaturation, and ion-exchange and gel filtration chromatographies.[254] The homogeneous product inhibited α-amylases and glucoamylase, but not β-amylases and other glucoside hydrolases. S-AI mol. wt. 1.5×10^3 by gel filtration was a very stable substance, as it retained 100% of its original activity after being kept for 30 min at 100 °C in a pH range between 3.0 and 10.0. The inhibitor was thought to be an oligosaccharide which was composed mainly of glucose (*ca.* 85%). Amylase inhibitor was hydrolysed by β-amylase from the non-reducing terminal and released two moles of maltose per mole successively.

An α-amylase found in the culture filtrate of a strain of *Thermoactinomyces vulgaris* was purified by ammonium sulphate fractionation, and ion-exchange chromatographies.[255] The purified enzyme showed a single band on disc gel electrophoresis. The pH optimum, temperature optimum, and isoelectric point were pH 5.0, 70 °C, and pI 5.2, respectively. The α-amylase was stabilized by Ca^{2+} and was found to hydrolyse pullulan to panose. Therefore, the hydrolytic pattern of this enzyme is different from those of pullulanase and isopullulanase.

Torulopsis ingeniosa yeast was found to possess an α-amylase strongly attached to the cell wall, its pH optimum being 5.5, its optimum temperature was at 50 °C, and it was highly sensitive to thermal inactivation.[256] The enzyme was found to be induced by starch, but the synthesis was not subject to a D-glucose effect.

In a study of the production of α-amylase by yeasts and yeast-like organisms, of a total 177 strains, only eight were found to be capable of producing the enzyme.[257]

[253] C. Laluce and R. Molinari, *Biotechnol. Bioeng.*, 1979, **21**, 915.
[254] K. Ohyama and S. Murao, *Agric. Biol. Chem.*, 1977, **41**, 2221.
[255] M. Shimizu, M. Kanno, M. Tamura, and M. Suekane, *Agric. Biol. Chem.*, 1978, **42**, 1681.
[256] G. Moulin and P. Galzy, *Folia Microbiol.*, 1978, **23**, 423.
[257] J. Augustin, J. Zamek, A. Kockova-Kratochvilova, and L. Kuniak, *Folia Microbiol.*, 1978, **23**, 353.

α-Amylases have been immobilized with retention of enzymic activity by adsorption on to Duolite® S-761, a phenol–formaldehyde absorbent, followed by glutaraldehyde cross-linking,[258] by reaction with carboxymethylcellulose acid chloride,[259] by coupling to a variety of cellulose derivatives prepared by bonding aliphatic and aromatic diamines and amino-acids on to cellulose *via* epichlorohydrin,[239] by reaction with/adsorption on to a range of new poly-phenolic resins,[169] and by reaction with hydrous titanium(IV) oxide-coated magnetic iron oxide.[260]

18 β-Amylases

The Perkin-Elmer Model 191 grain amylase analyser has potential usefulness to the cereal industry for measurement of amylase.[234] For example, it can satisfactorily measure the potency of fungal α-amylase supplements. With amylopectin as substrate, it can measure various concentrations of α- or β-amylase. The analyser cannot, however, differentiate between the effects of these two enzymes, which are both normally present in cereals. This limits its present usefulness, particularly for measurement of α-amylase. Selective inactivation with $HgCl_2$ or heat-treatment cannot guarantee that β-amylase is completely destroyed or that α-amylase has not been affected. Manufacturer refinements to the prototype method and instrument such as developing a β-limit dextrin substrate and increasing the sensitivity of the analyser may ultimately provide a simple, rapid assay of α-amylase.

β-Amylase has been isolated and purified, to a state free from contaminating enzymes, from barley malt and ungerminated barley grains, respectively.[230] Starch granules, isolated from the same sources, served as substrates to study the mode of action of amylases on the digestion of starch granules *in vitro* (see p. 480).

The crystallization of Japanese radish (*Raphanus sativus*) β-amylase, has been described.[261] Preliminary crystallographic data were reported, using a 40% saturated ammonium sulphate solution containing phosphate buffer, H_4edta, and 2-mercaptoethanol.

During the germination of rice seed, multiple forms of amylase molecules are synthesized in the endosperm tissues, which were detectable by isoelectric focusing on polyacrylamide gel.[235] Among three major amylase components, two were purified to an apparent homogeneity, and bands A and C were identified to be α- and β-amylase, respectively. Some of their enzymic properties have been studied.

In a study of the enzyme mechanism of starch breakdown in germinating rice seeds, the localization of the β-amylase by an immunohistochemical process was described.[262]

[258] B. T. Hofreiter, K. L. Smiley, J. A. Boundy, C. L. Swanson, and R. J. Fecht, *Cereal Chem.*, 1978, **55**, 995.
[259] S. Dumitriu, V. Bulacovschi, D. Constantinescu, and A. Rugina, *Cellul. Chem. Technol.*, 1979, **13**, 391.
[260] J. F. Kennedy and C. A. White, *Stärke*, 1979, **31**, 375.
[261] S. Aibara, H. Yamashita, and Y. Morita, *Agric. Biol. Chem.*, 1978, **42**, 179.
[262] K. Okamoto and T. Akazawa, *Plant Physiol.*, 1979, **64**, 337.

An active β-amylase was purified from germinated rice seeds by precipitation with ammonium sulphate, acid treatment, ion-exchange, chromatographies, and gel filtrations.[263] The purified enzyme was homogeneous in disc electrophoretic analysis. The enzyme (mol. wt. 5.3×10^4 by thin-layer gel filtration and polyacrylamide gel electrophoresis, pI 5.0 by disc electrofocusing, pH optimum 5.5–6.5, K_m for soluble starch 3 mg ml^{-1}) was inhibited by thiol reagents or heavy metal ions. The active β-amylase was oxidatively dimerized by treatment with 0.3 M ferricyanide in 3 M-urea. The dimerized enzyme was thought to be one of the inert β-amylases in ungerminated rice seeds.

β-Amylase has been prepared from defatted hawk eye soybean flour.[264] The enzyme-concentration dependence of the initial velocity for the hydrolytic reaction was investigated at pH 5.4 in a range of the enzyme concentrations and it was found that the initial velocity was proportional to the enzyme concentration in this range. The hydrolyses of maltodextrin ($\overline{DPn}$ = 74.4) and soluble starch catalysed by soybean β-amylase were investigated in the pH range from 3.0 to 9.1 at 25 °C, and K_m and V_{max} for each substrate were determined at each pH. The pH–rate profile showed a bell-shaped curve, and the pH optimum was at 5.85. From Dixon plots of V and VK_m^{-1}, the pK values were found to be 3.5 and 8.2 for the free enzyme, and 3.5 and 8.5 for the enzyme–substrate complex. The pH–rate profile in the presence of 25% methanol (v/v) was also obtained at alkaline pH. The pK_e values were the same as those in the absence of methanol. Based on these results, it was estimated that in the enzyme molecule the ionizable acidic group was an amino-group and the basic group was a carboxy-group.

A unique demonstration of the capacity of a carbohydrase to utilize both anomeric forms of a substrate has been presented.[265] Crystalline sweet potato β-amylase has been found to act upon α- and β-maltosyl fluorides. The reaction with β-maltosyl fluoride was distinguished by an unusual relationship of rate to substrate concentration, indicative of the need for two molecules of substrate to be bound to enzyme in order for reaction to occur. In addition, certain compounds with an α-D-glucopyranosyl terminal, *e.g.* methyl β-maltoside and 4-nitrophenyl α-D-glucopyranoside, greatly enhanced the rate of utilization of low levels of β-maltosyl fluoride by the enzyme. The reaction pathway that accounts for these results involves two steps: maltosyl transfer from β-maltosyl fluoride to O-4 of a second molecule of substrate (or alternative acceptor) to form a (1 → 4)-α-D-higher saccharide, then rapid hydrolysis of the latter to give maltose. These results support the concept that glycoside hydrolases and glycosyl transferases are inter-related catalysts of glycosylation, *i.e.* glycosylases.

β-Maltosylamine has proved to be an effective specific inhibitor of sweet potato β-amylase.[266] This result extends the observation that 1-aminoglycosides are specific inhibitors of glycoside hydrolases, and also demonstrates that enzymes acting with inversion, as well as those acting with retention, of anomeric configuration can be inhibited by glycosylamines. β-Maltosylamine, which acted as a reversible inhibitor, appeared to be directed to the active-site

[263] H. Matsui, S. Chiba, and T. Shimomura, *Agric. Biol. Chem.*, 1977, **41**, 841.
[264] Y. Nitta, T. Kunikata, and T. Watanabe, *J. Biochem. (Tokyo)*, 1979, **85**, 41.
[265] E. J. Hehre, C. F. Brewer, and D. S. Genghof, *J. Biol. Chem.*, 1979, **254**, 5942.
[266] D. E. Walker and B. Axelrod, *Arch. Biochem. Biophys.*, 1979, **195**, 392.

since it protected the essential thiol group of the enzyme from inactivation by *N*-ethylmaleimide.

Chloroplasts isolated from spinach (*Spinacia oloracea*) plants grown under controlled light/dark and temperature regimes, contained the phosphorolytic and amylolytic pathways for starch breakdown.[140] The latter consists at least of α- and β-amylase and β-D-glucosidase ('maltase'). Only low amylolytic activity was observed in chloroplasts isolated during the light phase. In chloroplasts prepared during the dark phase, this activity was almost twice as high. These diurnal oscillations of the amylolytic activity were maintained when the plants were kept in prolonged darkness or continuous light. The amylolytic system exhibited a sharp pH optimum between 5.8 and 6.0. Phosphorylase activity, when assayed with saturating concentrations of inorganic phosphate, did not show diurnal fluctuations.

In a study of the preservation of bacterial β-amylase (ex *Bacillus aureus*) the effect of metal ion, maltose and dextrin *etc.* was studied.[267] The activity of the enzyme decreased to 9.3% of initial activity after one month. However, it was found that when metal ions, such as Ni^{2+}, Co^{2+} or Zn^{2+}, exist in enzyme solution, the enzyme activity was effectively preserved. Maltose, dextrin, and other metal ions tested were not effective for preserving the enzyme. Ni^{2+} and Co^{2+} were more effective in preserving the enzyme compared with Zn^{2+}. Mn^{2+} was also effective for preserving the enzyme at a concentration of 2×10^{-2} M. It is not made clear yet why the β-amylase is stabilized by presence of these metal ions.

An amylase inhibitor purified from the culture filtrate of *Streptomyces diastatians* inhibited α-amylase and glucoamylases (see p. 486).[254]

β-Amylase has been immobilized with retention of enzymatic activity by reaction with/adsorption on to a range of new poly-phenolic resins.[169]

19 Amylo-1,6-D-glucosidases

The first isolation of 'glycogen debranching enzyme', termed by the authors '1,4-glucan-4-glucosyltransferase plus amylo-1,6-glucosidase', from chicken pectoralis muscle in a homogeneous form has been reported.[268] The physicochemical properties of the enzyme were compared with those of the homologues 7 S myofibrillar protein (mol. wt. 1.65×10^5) and it was concluded that the latter represents a glycogen debranching enzyme. The properties of the enzyme were also compared with those from rabbit, dog, fish, and baker's yeast.

20 Arabinanases

Wild type *Bacillus subtilis*, when grown on beet arabinan, secretes into its culture medium an *endo*-arabinanase and two arabinosidases.[56] A procedure has been developed for the purification of the *endo*-arabinanase (see p. 429).

[267] Y. Takasaki, *Agric. Biol. Chem.*, 1979, **43**, 1599.
[268] C. W. Heizmann and H. M. Eppenberger, *FEBS Lett.*, 1979, **105**, 35.

21 Cellulases

Cellulose, a vast and renewable resource and a potential source of food, is currently very much underutilized.[269] A review deals with the molecule itself, the uses to which it might be put, and cellulases, the enzymes that may convert potential to reality.

A procedure for automatic determination of cellulase by viscometry has been described.[270]

The applications of aqueous, low pressure liquid chromatography on Aminex SOW-X4 to the assay of cellulolytic enzyme activity and cellulo-oligosaccharides has been discussed.[271] The advantages of this method are speed of analysis (less than 20 min), small sample size (20 μl), good resolution, and a tolerance of the system to the presence of extraneous salts and proteins. Examples showing the use of this tool are given.

A new procedure described for the determination of cellulase activity is based on incubation of the enzyme with finely divided cellulose at pH 6.9 and determination of the D-glucose and cellobiose liberated as their trimethylsilyl derivatives by g.l.c.[272] The method, although generally applicable, was specifically developed for measurement of cellulase activity in mixed enzyme preparations from sheep rumen contents; the coefficient of variation of the assay was 2.4–4.5%.

Production of enzymes degrading plant cell walls was studied using media containing cellobiose or ammonium ions as limiting nutrients.[273] Cellulase ('carboxymethylcellulase'), xylanase and pectin lyase were primarily cell-associated during exponential growth in batch culture but accumulated in the supernatant during the stationary phase. Activities of carboxymethylcellulase and xylanase were higher in cellobiose-limited than in NH_4^+-limited continuous cultures, were inversely related to the growth rate, and became progressively more cell-associated as the growth rate increased. The proportion of fermentation products in cellobiose-limited continuous cultures was dependent on the growth rate and the calculated cell yields per mol ATP varied between 11.92 and 16.39. These results were discussed in relation to the *in vivo* growth and metabolism of *Ruminococcus flavefaciens*.

Pea epicotyl extract reduces the viscosity of a solution of Capucine xyloglucans.[274] Results of comparison of the action of this extract with that of a commercial cellulase (Onozuka) were in favour of a hypothesis according to which the cellulase from the extract could hydrolyse the substrate.

The decomposition of carboxymethylcellulose by intestinal bacteria of cockroaches has been examined.[275]

[269] M. P. Coughlan and M. A. Folan, *Int. J. Biochem.*, 1979, **10**, 103.
[270] H. Bartels, R. Hoesly, and G. Rommele, *Z. Anal. Chem.*, 1979, **295**, 406.
[271] M. R. Ladisch, A. W. Anderson, and G. T. Tsao, *J. Liq. Chromatogr.*, 1979, **2**, 745.
[272] G. B. Storer, J. M. Gawthorne, G. L. Francis, and R. J. Illman, *Anal. Biochem.*, 1979, **92**, 270.
[273] R. C. Hignett, A. L. Roberts, and M. J. Latham, *J. Gen. Microbiol.*, 1979, **110**, 29.
[274] R. Guignard and P.-E. Pilet, *C. R. Hebd. Seances Acad. Sci., Ser. D.*, 1978, **286**, 855.
[275] D. L. Cruden and A. J. Markovetz, *App. Envir. Microbiol.*, 1979, **38**, 369.

The cellulase activities of, and degradation of cellulase and lignin, and formation of humus by cellulolytic fungi have been studied.[276]

Filtrates from cultures of different ages of *Botryodiplodia theobromae* have been fractionated by gel filtration, ion-exchange chromatography, and polyacrylamide gel electrophoresis.[148] Five cellulases (C1, C2, C3, C4, and C5) were found, and their molecular weights, estimated by gel filtration, were 4.60×10^4–4.80×10^4 (C1), 3.00×10^4–3.50×10^4 (C2), 1.50×10^4–1.80×10^4 (C3), 1.00×10^4–1.10×10^4 (C4), and 0.48×10^4–0.55×10^4 (C5). Cellulase C5 was absent from old culture filtrates. Cellulase C1 had little or no activity on carboxymethylcellulose (viscometric assay), but degraded cotton flock and cellulose powder to give cellobiose only. The other components (C2–C5) produced cellobiose and smaller amounts of D-glucose and cellotriose from cellulosic substrates and were more active in lowering the viscosity of carboxymethylcellulose. The ratio of activities assayed by viscometry and by the release of reducing sugars from carboxymethylcellulose increased with decrease in the molecular weights of cellulases C2–C5. Cellobiose inhibited the activities of the cellulases, but D-glucose stimulated at low concentrations although it inhibited at high concentrations. Cellulases C2–C5 acted in synergism with C1, particularly in the presence of β-D-glucosidase.

The cellulolytic system from culture filtrates of *Clostridium thermocellum*, when partially purified by preparative electrophoresis, showed a single band on analytical polyacrylamide gel electrophoresis.[277] However, sodium dodecyl sulphate polyacrylamide gel electrophoresis indicated that at least five proteins were present. Neutral carbohydrate was present in the purified fraction and it was suggested that the cellulase was present as a complex.

The cellulolytic and pectinolytic enzymes associated with healthy and *Glycosporium papaya* rot-infected papaya (*Carica papaya*) fruit, and secreted in papaya pulp medium by the pathogen have been investigated.[278]

A cellulase component of 'Avicelase' type was obtained from 'Driselase', a commercial enzyme preparation from a wood-rotting fungus *Irpex lacteus* (*Polyporus tulipiferae*).[279] It showed a single band on sodium dodecyl sulphate polyacrylamide electrophoresis. The amino-acid composition of this cellulase resembled those of cellulase components of *endo*-type from the same fungus. However, it produced exclusively cellobiose from carboxymethylcellulose as well as from water-insoluble celluloses such as Avicel or cotton at earlier stages of hydrolysis. In addition, the hydrolysis of carboxymethylcellulose practically stopped after an initial rapid stage. The cellulase showed a strong synergistic action with an *endo*-cellulase of higher randomness (typical carboxymethylcellulose type) in the hydrolysis of carboxymethylcellulose as well as of Avicel. In contrast to cello-triose and -tetraose, cello-pentaose and -hexaose were attacked very rapidly, and only cellobiose was produced. These results suggest

[276] K. K. Kapoor, M. K. Jain, M. M. Mishra, and C. P. Singh, *Ann. Microbiol.*, 1978, **129B**, 621.

[277] N. Ait, N. Creuzet, and P. Forget, *J. Gen. Microbiol.*, 1979, **113**, 399.

[278] P. K. Rai, *Curr. Sci. (India)*, 1977, **46**, 21.

[279] T. Kanda, S. Nakakubo, K. Wakabayashi, and K. Nisizawa, *J. Biochem. (Tokyo)*, 1978, **84**, 1217.

that the cellulase is an *exo*-type component. However, it mutarotated the products from cellopentaitol in the same direction as *endo*-cellulases. It represented a relatively large portion of the total cellulase activity, and may play an important role in the degradation of native cellulose *in vivo*.

The filter paper disintegrating activity (FD) and the plant tissue macerating activity (MA) in the enzyme preparation ('Driselase') produced by *Irpex lacteus* have been studied.[280] Optimum pH value of FD was 3.5–5.0 and that of MA was 4.0–6.0. Optimum temperature of FD was 45 °C and that of MA was 40–45 °C. Both FD and MA were stable in a wide pH range investigated and they were thermostable up to 45 °C. FD was remarkably inhibited by Ag^{+}, Hg^{2+}, and Sn^{2+}; on the other hand, MA was not affected by any of the metal ions tested. 'Driselase' exhibited high cellulase, hemicellulase, and pectinase activities, but its excellent points were characterized by multiple enzyme systems that were able to be represented by *e.g.* MA and soybean meal distintegrating activity. Using various grains, a microscopical examination of how tissues were macerated and cell walls were disintegrated with Driselase was conducted.

The effects of dietary supplementation by enzyme preparation (Driselase) produced by *Irpex lacteus* on feeding animals has been studied.[281] When 0.01–0.03% of Driselase were supplemented to broiler diets which contained about 60% corn, body weight gain and feed conversion were significantly improved at 10% and 5% level, respectively. When 0.01–0.03% of Driselase were supplemented to broiler diets which contained about 60% of barley, body weight gain, and feed conversion were also improved significantly at 1% level each. Driselase supplementation improved feed efficiency of barley to the level of corn. In the case of piglets when 0.01% of Driselase was supplemented to artificial milk, body weight gain increased significantly at 5% level. Feed conversion was improved about 9%.

Germinating microcysts of *Polysphondylium pallidum* possess the ability to digest carboxymethylcellulose to its D-glucose sub-units.[156] This carboxymethyl cellulase activity, which is cyclohexamide-sensitive, increases several-fold intracellularly during germination and is excreted into the extracellular medium. Augmentation of the extracellular level of cellulase enhances emergence of amoebae from microcysts, suggesting that this activity is critical for germination. The inhibition of β-D-glucosidase activity with D-glucono-1,4-lactone also inhibits emergence, implicating that enzyme in germination as well.

Enzyme preparations active against crystalline cellulose, marble-milled filter paper, carboxymethylcellulose, hemicellulose, and xylan were obtained from cultures of *Ruminococcus flavefaciens*.[161] These preparations also contained swelling factor, pectin methylesterase, pectin lyase and low levels of β-D-glucosidase and β-D-xylosidase. 'Carboxymethylcellulase' and xylanase activities were present in a high molecular weight complex, but substrate competition studies showed that different active sites were probably responsible for each activity. Analysis of products and viscosity changes during enzymic hydrolysis of

[280] S. Noguchi, G. Shimura, M. Kawai, Y. Suga, and H. Samejima, *Agric. Biol. Chem.*, 1978, **42**, 339.

[281] Y. Suga, M. Kawai, S. Noguchi, G. Shimura, and H. Samejima, *Agric. Biol. Chem.*, 1978, **42**, 347.

carboxymethylcellulose and xylan indicated that the most active enzymes were of the *exo*-(1 → 4)-β-D-glycanase type. A variety of reducing sugars was released from cell walls of *Lolium perenne* (perennial ryegrass) by the enzyme preparations.

The enzyme mechanisms involved in degradation of cellulose by the white-rot fungus *Sporotrichum pulverulentum* have been reviewed.[163] The hydrolytic enzymes involved include (i) five *endo*-(1 → 4)-β-D-glucanases, (ii) one *exo*-(1 → 4)-β-D-glucanase, and (iii) one or several β-D-glucosidases. Regulatory mechanisms of the extracellular enzyme activities, such as monosaccharide levels causing catabolite repression of the *endo*-D-glucanases, were investigated. The enzymes used by *S. pulverulentum* in cellulose hydrolysis were compared with those used by *Trichoderma viride*. The involvement of cellobiose oxidase and a cellobiose : quinone oxidoreductase in the degradation was also discussed.

A strain of *Sporotrichum thermophile*, grown in submerged culture at 44 °C, degraded crystalline cellulose by the secretion of cellulolytic enzymes into the culture medium.[282] Dialysed culture filtrates, after growth on cellobiose, were also effective in degrading crystalline cellulose (Avicel). Cellulase, measured viscosimetrically with carboxymethylcellulose as substrate, and therefore referred to as 'carboxymethylcellulase', was induced by cellulose, D-gluco-D-mannan, cellobiose and, to a limited extent, by laminaribiose and cellobionic acid. Low 'carboxymethylcellulase' activities were also detectable when the organism was grown on other carbon sources. In culture media supplemented with readily metabolized non-inducing substrates, such as D-glucose, 'carboxymethylcellulase' activity did not increase after their exhaustion, indicating that no derepression of constitutive 'carboxymethylcellulase' occurred. 'Carboxymethylcellulase' synthesis induced during growth on cellobiose was strongly inhibited, although not completely suppressed, by D-glucose and other carbon sources. In cultures grown on D-glucose and cellobiose, D-glucose was utilized first and 'carboxymethylcellulase' synthesis was repressed; cellobiose utilization occurred only after D-glucose exhaustion and triggered 'carboxymethylcellulase' formation. When D-glucose was added to a culture growing on cellobiose, further utilization of the latter was prevented and 'carboxymethylcellulase' synthesis was inhibited. The use of D-glucose analogues gave some indications that cellobiose did not compete with D-glucose for the same transport carrier and that D-glucose catabolism was a pre-requisite for the inhibition of 'carboxymethylcellulase' synthesis.

Talaromyces emersonii when grown on cellulose (purified material from spruce, beet-pulp, or newspaper)-containing media produced a complete extracellular cellulase complex.[283] The enzyme catalysed extensive degradation of soluble and a variety of insoluble (crystalline) celluloses both pure and 'waste'. With respect to potential practical applications of cellulolytic organisms, *Talaromyces emersonii* appeared to compare favourably with *Trichoderma viride*.

[282] G. Canevascini, M. R. Coudray, J. P. Rey, R. J. G. Southgate, and H. Meier, *J. Gen. Microbiol.*, 1979, **110**, 291.
[283] M. A. Folan and M. P. Coughlan, *Int. J. Biochem.*, 1979, **10**, 505.

The 'carboxymethylcellulase' and 'Avicelase' (cellulase) activities of *Thermoactinomyces* sp., strain YX, were produced simultaneously with cell growth.[284] Throughout the growth phase these activities were primarily extracellular, with up to 50% absorbed to residual cellulosic substrate at any one time. On the other hand, the β-D-glucosidase activity was associated with the culture solids throughout the entire fermentation and appears to be intracellular. Preparative isoelectric focusing of the culture filtrate, in the pH range 3–5, separated three major fractions with cellulolytic activities towards both carboxymethylcellulose and Avicel. Based on the 'carboxymethylcellulase' and 'Avicelase' activities of these separated fractions, it was not possible to discriminate between *endo-* and *exo*-D-glucanases produced by the *Thermoactinomyces* sp. Analytical isoelectric focusing of culture filtrates obtained throughout the growth phase of *Thermoactinomyces* indicated that all the extracellular cellulolytic enzymes are produced and released into the culture filtrate simultaneously, with no evidence of a sequential appearance of the various enzymes or isoenzymes.

The development of an agar plate screening technique has allowed the isolation of a range of mutants of *Trichoderma reesei* capable of biosynthesizing cellulase under conditions of high catabolite repression.[285] Commercial aspects of the production and isolation of hyper-cellulase-producing mutants of *T. reesei* were discussed.

By employing a two-stage continuous culture system, some of the more important physiological parameters involved in cellulase biosynthesis have been evaluated with an ultimate objective of designing an optimally controlled cellulase process.[286] The two-stage continuous culture system was run for a period of 1350 h with *Trichoderma reesei*. The temperature and pH were controlled at 32 °C and pH 4.5 for the first stage (growth) and 28 °C and pH 3.5 for the second stage (enzyme production). Lactose was the only carbon source for both stages. Enzyme was produced only in the second stage. Volumetric and specific enzyme productivities obtained were 90 IU l^{-1} h^{-1} and 8 IU g biomass^{-1} h^{-1}.

A new procedure using high-performance liquid chromatography for the rapid separation of cellulase proteins has been described.[287] The cellulase components of *Trichoderma reesei* are fractionated on an anion-exchange column using a phosphate buffer at pH 6.2. Activities of the individual components obtained from *T. reesei* QM6a, a wild strain, and several mutant strains have been determined. Each system examined contained β-D-glucosidase, at least two *exo*-(1 → 4)-β-D-glucanases and five *endo*-(1 → 4)-β-D-glucanases with the *endo*-(1 → 4)-β-D-glucanases accounting for 20–36% and the *exo*-(1 → 4)-β-D-glucanases for 64–80% of the soluble protein.

The principal features of a mathematical model described for the enzymatic hydrolysis and fermentation of cellulose by *Trichoderma reesei* are the assumption of two forms of cellulose (crystalline and amorphous), two sugars

[284] B. G. R. Hagerdal, J. D. Ferchak, and E. K. Pye, *Appl. Envir. Microbiol.*, 1978, **36**, 606.
[285] B. S. Montencourt and D. E. Eveleigh, *Appl. Envir. Microbiol.*, 1977, **34**, 777.
[286] D. Ryu, R. Andreotti, M. Mandels, B. Gallo, and E. T. Reese, *Biotechnol. Bioeng.*, 1979, **21**, 1887.
[287] F. H. Bissett, *J. Chromatogr.*, 1979, **178**, 515.

(cellobiose and D-glucose), and two enzymes (cellulase and β-D-glucosidase) (see p. 462).[164]

Trichoderma viride produces high yields of cellulase and especially β-D-glucosidase when grown in submerged culture on different carbon sources.[165] Cellulase synthesis was strongly repressed in the presence of D-glucose and only a low constitutive activity of β-D-glucosidase and 'carboxymethylcellulase', but no 'Avicelase', could be demonstrated when culturing *T. viride* on D-glucose. With carboxymethylcellulose as a substrate, the secretion of enzyme as well as growth depended on the degree of substitution, but in general carboxymethylcellulose cannot be regarded either as a powerful inducer or as a carbon source. With insoluble cellulose, maximum enzyme production and activities were obtained using an alkali-treated cellulose powder. On this substrate the excretion of soluble protein into the culture broth increased and the protein concentration corresponded to cellulolytic activities.

The optimization of cellulase and β-D-glucosidase production by a basidiomycete species was studied and cellulase and 'cellobiase' production by this and *Trichoderma viride* (and its mutants) in shake flasks were compared.[166] The former produced an active cellulase comparable to that of *T. viride* when tested on filter paper, carboxymethylcellulose, and cotton; however, it produced 20–26 times larger amounts of 'cellobiase'. Both cellulase and β-D-glucosidase were obtained in good yield only when cellulose was the carbon source. The production of these enzymes was not repressed by readily assimilated carbon sources in the presence of cellulose. Only traces of cellulase and β-D-glucosidase were formed on D-glucose, D-fructose, maltose, and cellobiose although good growth was obtained on these substrates. These enzymes were not induced on sophorose, lactose, mannitol, or glycerol and growth was poor on these substrates. Cellobiose octa-acetate was a less effective inducer of cellulase and β-D-glucosidase than was cellulose.

Cellulase produced by *Trichoderma viride* was found to act on carboxymethylcellulose (K_m 4.9 g l^{-1}) showing a pH optimum at 4.5 and a temperature optimum at 55 °C. Ag^+, Hg^{2+}, Zn^{2+}, Cu^{2+}, and N_3^- ions were inhibitory.[288]

Cellulolytic activity of the termite flagellate *Trichomitopsis termopsidis* has been demonstrated *in vivo* and *in vitro*.[289] Homogenates of axenically-cultivated flagellates hydrolysed carboxymethylcellulose enzymically, indicating that *T. termopsidis* does not depend on living extracellular or endosymbiotic bacteria for cellulose degradation. Cellulolytic activity of the flagellate was also determined by comparing the longevities of defaunated termites (*Zootermopsis*) refaunated by feeding with either *T. termopsidis* or with heat-killed flagellates. The termites refaunated greatly outlived members of the other group, supporting the view that cellulose digestion by the intestinal flagellate is responsible for the nutrition of termites.

The phytopathogen *Xanthomonas campestris* when grown on several polysaccharide substrates, including D-galacto-D-mannan, produced β-D-mannanases and 'carboxymethylcellulases' which were present as cell-wall- or membrane-

[288] J. K. Gupta and Y. P. Gupta, *Folia Microbiol.*, 1979, **24**, 269.
[289] M. A. Yamin and W. Trager, *J. Gen. Microbiol.*, 1979, **113**, 417.

bound constituents, and also in the extracellular medium.[290] Cell-bound β-D-mannanases could be partially solubilized by Triton X-100, but this treatment could not release cell-bound cellulase. Cell-free and -bound β-D-mannanases and 'carboxymethylcellulases' were found to be of the *endo*-enzyme type.

22 Chitinases

A glycol-chitin-splitting enzyme without lysozyme (muramidase) activity has been found in calf serum.[291] The enzyme also degrades colloidal chitin and is thus a true chitinase, {poly[1,4-β-(2-acetamido-2-deoxy-D-glucoside)] glycanohydrolase} without *exo*-β-D-2-acetamido-2-deoxyglucanase effect. The enzyme purified 1000-fold by ion-exchange chromatography and gel filtration showed optimal activity between pH 1.5–2.0 with glycol chitin as substrate and between pH 3–6 in a rather broad optimum with colloidal chitin. The optimal stability of the enzyme (pI 5.3, mol. wt. $4.7 \pm 0.3 \times 10^4$) is in the pH interval 3.0–6.5 when tested by incubation with glycol chitin at 50 °C for 60 min. The optimal temperature for the degradation of glycol chitin is 40 °C when assayed at pH 1.5 and 51 °C when assayed at pH 3.5. The enzyme is activated by moderate heating at pH < 6.5.

23 Chitosanases

The lysis of cell wall and the preparation of spheroplast of *Mucor mucedo*, which belongs to the same *Mucorales* as *Rhizopus*, by a purified chitosanase from *Bacillus* R-4 has been described.[292]

24 Chondroitin Lyases

Immunochemical identification certified the electrophoretically separable components of *Arthrobacter* chondroitin lyase to be the multiple forms of chondroitin lyase AC.[293] The electrophoretic patterns of the enzyme preparations obtained at various cultivation stages showed that condroitin lyase III is produced at first and the multiple forms of the chondroitinase occur during cultivation of the strain. In mature culture, chondroitin lyase I was the main component among the multiple forms. From the conversion experiments using the supernatant fluid of chondroitinase-free culture broth and UDP-D-glucose and using glycoside hydrolases from *Charonia lampas*, chondroitin lyase I and chondroitin lyase II, respectively, seemed to arise from D-glucosylation of chondroitin lyase III and from de-glycosylation of chondroitin lyase III or I.

While an isolated soil bacterium, *Arthrobacter aurescens*, had been found to secrete a chondroitin lyase AC into the culture medium, it was recognized that the condroitinase preparation obtained from the broth of the strain cultured in a jar fermenter contained at least three electrophoretically separable components

[290] R. F. H. Dekker and G. P. Candy, *Arch. Mikrobiol.*, 1979, **122**, 297.
[291] G. Lundblad, M. Elander, J. Lind, and K. Slettengren, *Eur. J. Biochem.*, 1979, **100**, 455.
[292] Y. Tominaga and Y. Tsujisaka, *Agric. Biol. Chem.*, 1979, **43**, 2607.
[293] K. Hiyama and S. Okada, *Agric. Biol. Chem.*, 1978, **42**, 1411.

of the enzyme.[294] Each component (named chondroitin lyases I, II, and III, respectively) was separated from the others by use of isoelectric focusing and then the enzymic properties of each component were examined. The pI value of each component was different (I, 5.5; II, 5.9; III, 6.4). However, no difference could be detected in pH-stability (pH 5.0–7.5), optimum pH (pH 6.0), thermal stability (below 45 °C), optimum temperature (50 °C), substrate specificity (chondroitin AC lyase), mode of action (*endo* type, the degree of multiple attack was 3.0–3.1), and the dissociation constant of the enzyme–substrate complex (K_m for chondroitin 6-sulphate was $3.3–3.6 \times 10^{-4}$M). Thus the electrophoretically separable components with the chondroitinase activity were thought to be the multiple forms of the enzyme, chondroitin lyase AC. There was also no appreciable difference in the molecular weight values of those chondroitinase components; however, considerable difference was detected in the total carbohydrate content of those components.

Chondroitin lyase B and chondroitin lyase C have been separated from an extract of *Flavabacterium heparinum* induced with chondroitin 6-sulphate by using column chromatography on hydroxyapatite.[295] Chondroitin lyase C was eluted together with the activities of hyaluronidase, 'Δ4,5glycosiduronase', (α-L-threoenouronidase) and sulphatase. The latter two activities were eliminated exclusively by passing the crude chondroitinase C fraction through a phosphonocellulose column pre-equilibrated with phosphate buffer. Chondroitin lyase C was then purified by affinity chromatography using dermatan sulphate-bound AH-Sepharose® 4B coated with the same glycosaminoglycan. Purification of the enzyme was achieved 18-fold and in 73% yield. On the other hand, the activities of 'Δ4,5glycosiduronase' (α-L-threoenouronidase) and sulphatase were decreased to 50 and 60%, respectively, as compared with those in the crude chondroitinase B fraction, after passing the fraction through a column of phosphono-cellulose pre-equilibrated with phosphate buffer. The remaining activities of these two enzymes were then eliminated from chondroitin lyase B by affinity chromatography with heparin-bound AH-Sepharose® 4B coated with dermatan sulphate. In the affinity chromatography used, non-covalent coating of the glycosaminoglycan-bound (covalently) AH-Sepharose® 4B with the same or another glycosaminoglycan was found to be important.

25 Dextranases

A bacterial 'isomalto-dextranase', described previously as a new type of dextranase different from the known 1,6-α-D-glucan 6-glucanohydrolase [EC 3.2.1.11], was found to be a configuration-retaining *exo*-D-glucanase so far as judged from the downward mutarotation shown by products in a dextran digest, and from the lower activity of the enzyme on less highly polymerized isomaltodextrins.[296] The dextranase was observed to cause not only trans-isomaltosylation (isomaltotetraose formation in dextran digests, isomaltotriose formation in dextran digests containing D-glucose and trans-isomaltosylation among iso-

[294] K. Hiyama and S. Okada, *Agric. Biol. Chem.*, 1977, **41**, 1279.
[295] N. Ototani and Z. Yosizawa, *Carbohydr. Res.*, 1979, **70**, 295.
[296] T. Sawai and Y. Niwa, *Agric. Biol. Chem.*, 1975, **39**, 1077.

maltodextrins) but also isomaltose condensation to isomaltotetraose in concentrated solutions. These activities shown by the isomalto-dextranase are in keeping with the novel concept that carbohydrate hydrolases are catalysts of glycosylation (glycosyl–hydrogen interchange). The relative ease of isomaltose condensation catalysed by the enzyme appears due to the exergonic nature of the reaction. A free energy change value of *ca.* -1200 cal mol^{-1} was obtained for the condensation.

In a model experiment, glucoamylase was shown to inhibit α-D-glucan synthesis as catalysed by potato phosphorylase.[297] Both glucoamylase (*ex Rhizopus niveus*) and *endo*-dextranase (*ex Chaetomium gracile*) inhibited dextran synthesis with dextransucrases of *Leuconostoc mesenteroides.* The inhibition was ascribed to competition between glucoamylase and dextransucrase for the D-glucosyl groups at the non-reducing end of dextran. The inhibition caused by *endo*-dextranase may have resulted from rapid and random hydrolysis of acceptor dextrans. Moreover, significantly low units of glucoamylase, as compared with *endo*-dextranase, effectively inhibited dextran synthesis. These results suggested that biosynthesis of dextran occurs by the addition of D-glucosyl groups at the non-reducing end of the growing dextran. The measurement of initial velocity suggested that the ping-pong Bi–Bi mechanism proposed for the levansucrase of *Bacillus subtilis* is also applicable to dextransucrase.

A new type of dextranase, (1 → 2)-dextran debranching enzyme, has been detected in the culture fluid of a Gram-negative bacterium, *Flavobacterium* species. The optimum conditions for enzyme production was studied.[298] The debranching enzyme was concentrated from the culture supernatant, treated at 55 °C to inactivate the concomitant dextranase activity, and purified by ion-exchange chromatography on DEAE-cellulose. Further purification was performed by biospecific adsorption chromatography using Sephadex® G-150, from which the enzyme was eluted with solution of clinical dextran, and by polyacrylamide preparative gel electrophoresis. The purified enzyme (mol. wt. 1.25×10^5) was electrophoretically homogeneous. About a 2700-fold increase in specific activity was achieved with a yield of 14.2%.

The action patterns of a purified, intracellular dextranase and three intracellular α-D-glucosidases from *Pseudomonas* UQM733 on pure isomalto-oligosaccharides have been studied.[158] The α-D-glucosidases had optimal activity on isomaltotetraose and were therefore classified as 'oligoglucanases'. They have been used to determine the structure of two branched isomalto-oligosaccharides obtained by enzymic degradation of dextran.

The intracellular enzymes of *Pseudomonas* UQM733 which act on dextran have been re-investigated mainly by isoelectric focusing.[157] At least three dextranases were shown to be present, and one of them (D_4) has been purified and shown to be very similar to one of the extracellular *endo*-dextranases (D_1).

Glucoamylase from *Rhizopus niveus* has been shown to have an *exo* action on (1 → 3)-α- and (1 → 6)-α-D-glucan linkages as well as on malto-oligosaccharides.[299]

[297] M. Kobayashi and K. Matsuda, *Carbohydr. Res.*, 1978, **66**, 277.
[298] Y. Mitsuishi, M. Kobayashi, and K. Matsuda, *Agric. Biol. Chem.*, 1979, **43**, 2283.
[299] M. Kobayashi and K. Matsuda, *Agric. Biol. Chem.*, 1978, **42**, 181.

Dextranase has been immobilized with retention of enzymic activity by covalent reaction with porous titanium(IV) oxide particles coated with diazotized 1,3-diaminobenzene.[300]

26 β-D-Galactanases

endo-β-D-Galactanase (*endo*-β-D-galactosidase), an enzyme that hydrolyses 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose linkages in glycoconjugates, has been used to probe the plasma membrane of human erythrocytes.[301] Coomassie Blue staining of stroma components separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis indicates that treatment of red cells with *endo*-β-D-galactanase converts Protein 3, the anion transporter of the erythrocyte, to a more compact staining band. No other components detected by Coomassie Blue staining are affected. Following labelling of red cells with D-galactose oxidase followed by sodium borotritide, 45–50% of the [^{3}H]galactose residues can be released by *endo*-β-D-galactanase. In contrast, only 5% of the label incorporated by treatment with periodate followed by sodium borotritide can be removed. D-[^{3}H]Galactose residues are released from three components: Protein 3, Band 4.5, and the megaloglycolipids. The susceptibility of these components to *endo*-β-D-galactanase, together with the high content of D-galactose and 2-acetamido-2-deoxy-D-glucose present in Protein 3 and the megaloglycolipids, suggests that the erythrocyte membrane contains several components with 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (*N*-acetyl-lactosamine) repeating units, a structure commonly found in connective tissue glycoconjugates.

Extra- and intra-cellular glycan hydrolases were isolated from *Aspergillus flavus* and partially characterized.[302] Both preparations exhibited β-D-galactanase activity. Gel chromatography of the extracellular enzyme preparation revealed one protein fraction containing β-D-galactanase activity and a second one exhibiting mainly β-D-xylanase activity. Electrophoresis in starch gel and disc electrophoresis in polyacrylamide gel showed that the preparation obtained from the cultivation broth contained five protein fractions, whereas two protein fractions could be detected in the intracellular preparation. Hydrolysis of a partially degraded polysaccharide of peach gum by the above preparations yielded D-galactose as the main product and traces of D-mannose, L-arabinose, D-xylose, and a number of oligosaccharides.

27 *endo*-(1 → 3)-α-D-Glucanases

A (1 → 3)-α-D-glucanase was detected in the culture supernatant of a microorganism, a strain of *Streptomyces* tentatively named KI-8, which was isolated from soil on agar medium containing (1 → 3)-α-D-glucan as sole carbon source.[303] This enzyme required a (1 → 3)-α-D-glucosidic linkage as an inducer. The

[300] J. F. Kennedy, S. A. Barker, and I. M. Kay, *Carbohydr. Res.*, 1980, **80**, 25.
[301] T. J. Mueller, Y. T. Li, and M. Morrison, *J. Biol. Chem.*, 1979, **254**, 8103.
[302] A. Kardosova, J. Rosik, and J. Kubala, *Folia Microbiol.*, 1978, **23**, 97.
[303] K. Imai, T. Kikuta, M. Kobayashi, and K. Matsuoa, *Agric. Biol. Chem.*, 1977, **41**, 1339.

optimum conditions for enzyme production were studied. The enzyme was purified by fractional salt precipitation, column chromatography on DEAE-cellulose and P(phospho)-cellulose. To eliminate the concomitant (1 → 3)-β-D-glucanase activity, partially purified enzyme preparation was passed through a column packed with pachyman. Final purification was accomplished by the adsorption chromatography using Sephadex® G-150 from which (1 → 3)-α-D-glucanase was eluted with a solution of (1 → 3)-α-D-gluco-oligosaccharides. The purified enzyme was electrophoretically homogeneous and had a molecular weight of approximately 7.8×10^4 by sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

Some properties of purified *endo*-(1 → 3)-α-D-glucanase isolated from the culture supernatant of the soil micro-organism *Streptomyces* KI-8 have been examined.[304] The optimum pH and temperature were pH 5.4 and 60 °C, respectively. The enzyme was stable up to 50 °C on heating for 10 min and hydrolysed the substrate (1 → 3)-α-D-glucan into D-glucose and nigerose by an *endo*-type of action. Nigerotriose, nigerotetraose, and nigeropentaose were hydrolysed into D-glucose and nigerose, whereas nigerose was not attacked. The degree of hydrolysis of pseudonigeran, *Lentinus* (1 → 3)-α-D-glucan, mutan IG-1 (less soluble fraction) and IG-2 (more soluble fraction) by the (1 → 3)-α-D-glucanase were 28.5%, 14.3%, 8.8%, and 10.0%, respectively; K_m values were 1.12, 1.98, 8.00, and 5.00 mg ml^{-1} respectively.

28 *exo*-(1 → 3)-α-D-Glucanases

exo-(1 → 3)-α-D-Glucanase has been purified to homogeneous state from culture broth of *Cladosporium resinde*.[305] The enzyme was purified through salting-out with ammonium sulphate, followed by ion-exchange chromatographies and gel filtration. A pepsin inhibitor from *Streptomyces* was added on each step of purification to prevent proteolytic cleavage of the (1 → 3)-α-D-glucanase protein by contaminating enzymes. The purified enzyme showed no action on carboxymethylcellulose, (1 → 3)-β-D-glucan, dextran, starch, pullulan, or maltose. The enzyme was most active at pH 4.5 and 47 °C, stable at 4 °C at pH 3.5–7.0, and not inactivated up to 47 °C on heat-treatment for 30 min at pH 4.5. The enzyme [mol. wts. 8.7×10^4 and 8.8×10^4 by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and gel filtration, respectively; pI 3.2, K_m for (1 → 3)-α-D-glucan 13 mM (as anhydroglucose units)] was found to liberate α-D-glucose successively from the non-reducing ends of (1 → 3)-α-D-glucans. It acted on pseudonigeran, (1 → 3)-α-D-glucans from *Lentinus edodes* and *Polyporus betulinus*, as well as on insoluble D-glucans produced by *Streptococcus mutans* and *Streptococcus salivarius*. Oligosaccharides of the nigerose series with the degree of polymerization above four were rapidly hydrolysed by the enzyme, and nigerose was also attacked at high enzyme concentrations.

29 *endo*-(1 → 3)-β-D-Glucanases

The crystalline style of the gastropod *Telescopium telescopium* has been shown to contain two *endo*-(1 → 3)-β-D-glucanases and a β-D-glucosidase.[135] The two

[304] K. Imai, M. Kobayashi, and K. Matsuda, *Agric. Biol. Chem.*, 1977, **41**, 1889.
[305] A. Tsunoda, Y. Sakano, and T. Kobayashi, *Agric. Biol. Chem.*, 1978, **42**, 1045.

D-glucanases (I and II) have been purified and shown to be *endo*-enzymes. Both enzymes attack laminaran, carboxymethylpachyman and lichenin, but have no action towards carboxymethylcellulose. The main products of hydrolysis of laminaran were D-glucose and (1 → 3)-linked oligosaccharides of DP 2, 3, and 4. D-Glucanases I and II were similar to each other, although they differed in molecular weight and kinetic properties.

Saccharomyces cerevisiae S288C produced two laminarinases [(1 → 3)-β-D-glucanases] which were separated by diethylaminoethyl-Sephadex® column chromatography; one was an *endo*-(1 → 3)-β-D-glucanase, and the other was an *exo*-(1 → 3)-β-D-glucanase, active not only on laminaran but also on pustulan [(1 → 6)-β-D-glucan] and on 4-nitrophenyl β-D-glucopyranoside.[306] A mutant defective in the production of this last enzyme was isolated, and the mutation was named *exbl-1*. The selection procedure was based on the capacity of *exo*-(1 → 3)-β-D-glucanase to hydrolyse synthetic D-glucosides. The level of *endo*-(1 → 3)-β-D-glucanase in cell extracts of the mutant was normal, but the *exo*-(1 → 3)-β-D-glucanase could not be detected by column chromatographic analysis of these extracts. The mutant phenotype, recessive in heterozygous diploids, was stable through successive meioses and showed a Mendelian segregation, indicating that the mutation affected a single gene, which was named *EXB 1*. The lack of production of *exo*-(1 → 3)-β-D-glucanase persisted through all the phases of growth, but growth itself was not impaired by the enzyme deficiency.

Bacillus circulans was grown on cells of six species of yeast as inducer substrates, and the courses of progress of β-D-glucanase production were compared.[307] Also, the isoelectric focusing patterns of (1 → 3)-β-D- and (1 → 6)-β-D-glucanases were determined. Conspicuous differences were observed among the isoelectric focusing patterns of the crude enzymes produced with different yeast species as inducer substrates. Lyses of the cell walls of *Pyricularia oryzae* P_2 and several species of yeasts by the enzyme systems were compared. The lysis of the cell walls of *Debaryomyces hansenii* was stronger in the order of the complexity (number of major peaks *etc.*) of the (1 → 3)-β-D-glucanases in isoelectric focusing patterns.

The *endo*-(1 → 3)-β-D-glucanase secreted into the culture medium by cells of *Candida utilis* was isolated and purified to homogeneity on polyacrylamide gel electrophoresis and in ultracentrifugation studies ($s_{20,W}$ = 1.97 S).[308] The purified enzyme represented only 0.001% of the total (1 → 3)-β-D-glucanase activity, the remainder being due to an *exo*-(1 → 3)-β-D-glucanase, and behaved as an acidic glycoprotein (pI 3.3) in isoelectric focusing experiments. Studies on the hydrolysis of different substrates showed that the enzyme (mol. wt. 2.1 × 10^4 by gel filtration and polyacrylamide-gel electrophoresis) was only able to break down (1 → 3)-β-D-linkages, by an *endo* splitting mechanism. D-Glucono-1,5-lactone, D-glucurono-6,3-lactone, and heavy-metal ions such as Hg^{2+} were

[306] T. Santos, F. Del Ray, J. Conde, J. R. Villanueva, and C. Nombela, *J. Bacteriol.*, 1979, **139**, 333.
[307] H. Tanaka, K. Itakura, and K. Toda, *Agric. Biol. Chem.*, 1978, **42**, 1631.
[308] T. G. Villa, V. Notario, and J. R. Villanueva, *Biochem. J.*, 1979, **177**, 107.

inhibitors of the enzyme activity. The function of this *endo*-(1 → 3)-β-D-glucanase in *C. utilis* was discussed.

The influence of carbon and nitrogen sources on the release of (1 → 3)-β-D-glucanase activity during the autolysis of *Neurospora crassa* have been examined.[51]

D-Glucose repressed the synthesis of (1 → 3)-β-D-glucanase in *Neurospora crassa*.[309] The production of enzyme during growth in D-glucose-supplemented medium was negligible, but if deprived of carbon source the fungus actively synthesized high levels of enzyme when growth ceased. Similar results were obtained for (1 → 6)-β-D-glucanase, but less enzyme was produced. A different pattern of production of these enzymes was found in *Trichoderma viride* and *Saccharomyces cerevisiae*. The enzymes were produced in D-glucose-supplemented medium with increasing specific activity during growth. Resting cells deprived of D-glucose either failed to produce D-glucanases or produced them in small quantities.

Oerskovia sp. CK produced three types of (1 → 3)-β-D-glucanases designated as F-L, F-0, and F-2.[310] F-L showed high lytic activity to viable yeast cells and weak activity to yeast D-glucan. F-0 and F-2 had little or no lytic activity and strong (1 → 3)-β-D-glucanase activity. F-0 or F-2 showed high lytic activities to yeast cells pretreated with small amounts of F-L which did not lyse the cells. Lytic activity of F-0 or F-2 also increased when cells were treated with alkaline pH or with both reducing agents and pH. From these results, it is supposed that the ineffectiveness of F-0 or F-2 on the lysis of year cells might be attributed to a spatial inaccessibility of enzymes to the yeast D-glucan layer. However, the treatment of F-L, alkaline pH, and reducing agents would bring about a modification of cells to give F-0 or F-2 access to the cell wall D-glucan and consequently the lysis of cells would occur.

Extracellular polysaccharides from *Rhizobium trifolii*, U226, Coryn and Bart A; *Rhizobium phaseoli*, U453; *Rhizobium leguminosarum*, U331; and *Rhizobium meliloti*, U27, after chemical modification, became substrates for certain β-D-glucanases.[311] The *Streptomyces endo*-(1 → 4)-β-D-glucanase hydrolyses reduced and deacetylated rhizobial polysaccharides, both before and after removal of carboxyethylidene substituents, to produce a series of oligosaccharides. The *Rhizopus arrhizus endo*-(1 → 3)-β-D-glucanase hydrolyses only fully modified polysaccharides to yield, in the case of *R. meliloti* U27, laminarabiose, and, in all other instances, a disaccharide identified as 3-*O*-β-D-galactopyranosyl-D-glucose. The same disaccharides were released by the *Rhizopus* enzyme from oligosaccharides produced by the action of the *Streptomyces* enzyme on fully modified polysaccharides. The results are discussed in relation to the available data for the structure of the polysaccharides and the specificity of the enzymes.

The intracellular and cell-wall associated (1 → 3)-β-D-glucanases of *Saprolegnia* species have been investigated.[312]

[309] F. Delrey, I. Garcia-Acha, and C. Nombela, *J. Gen. Microbiol.*, 1979, **110**, 83.
[310] T. Obata, K. Fujioka, S. Hara, and Y. Namba, *Agric. Biol. Chem.*, 1977, **41**, 671.
[311] M. A. Anderson and B. A. Stone, *Carbohydr. Res.*, 1978, **61**, 479.
[312] M. Febre, *Mycopathologia*, 1979, **67**, 89.

29a *exo*-(1 → 3)-β-D-Glucanases

Saccharomyces cerevisiae S288C produced two laminarinases [(1 → 3)-β-D-glucanases] which were separated by diethylaminoethyl-Sephadex® column chromatography; one was an *exo*-(1 → 3)-β-D-glucanase active not only on laminaran but also on pustulan [(1 → 6)-β-D-glucan] and on 4-nitrophenyl β-D-glucopyranoside (see p. 501).

30 *exo*-(1 → 4)-α-D-Glucanases

Anomeric forms of D-glucose produced from phenyl α-maltoside, maltose, or phenyl α-D-glucopyranoside have been determined quantitatively, by simultaneous measurements of optical rotation and reducing power, for eight kinds of D-glucose-producing (1 → 4)-α-D-glucan hydrolases [*exo*-(1 → 4)-α-D-glucanases], including D-glucose-forming amylase from human urine, and α-D-glucosidases from pig serum,[123] honey bee, buckwheat seed, rice seed, sugar-beet seed, flint-corn seed, and brewer's yeast. All the eight enzymes studied were found to produce α-D-glucopyranose exclusively.

The substrate specificity of flint corn α-D-glucosidase has been investigated.[138] The enzyme was active especially on nigerose, phenyl α-maltoside and malto-oligosaccharides. The action on isomalto-oligosaccharides was very weak. The α-D-glucans, such as amylopectin β-limit dextrin, glycogen, and amylose besides soluble starch, were also hydrolysed, though no activity on dextran was observed (see p. 455).

The substrate specificity of rice α-D-glucosidase II has been studied.[139] The enzyme was active especially on nigerose, phenyl α-maltoside, and malto-oligosaccharides. The actions on isomaltose and phenyl α-D-glucopyranoside were weak, and on sucrose and methyl α-D-glucopyranoside, negligible. The α-D-glucans, such as soluble starch, amylopectin, β-limit dextrin, glycogen, and amylose, were also hydrolysed (see p. 455).

The anomeric configuration of the products of reactions catalysed by D-glucosidases from bovine liver and the mold fungus *Aspergillus awamori* has been determined.[126] It was found that the enzyme from bovine liver is an α-D-glucosidase and that from *A. awamori* an *exo*-D-glucanase (see p. 451).

The extracellular maltotetraose-forming amylase of *Pseudomonas stutzeri* has been purified to homogeneity by a combination of affinity and hydroxyapatite chromatography.[313] Sodium dodecyl sulphate–gel electrophoresis indicated that the oligomeric enzyme contains two different subunits (mol. wts. of 4.8×10^4 and 5.8×10^4). Cross-linking studies using dimethyl suberimidate have demonstrated that the native enzyme consists of dimers. Seven isoenzymes of the amylase have been identified after polyacrylamide gel electrophoresis and amylose-digestion zymograms. The amylase of *P. stutzeri* is known to produce maltotetraose from linear and branched α-D-glucans by an *exo* mechanism. The relatively high conversion rate of starch (75% hydrolysis), and the hydrolysis of cross-linked blue starch by this amylase indicate that the enzyme can cleave its substrates also by an *endo* mechanism. Further strong evidence for an *endo*

[313] J. Schmidt and M. John, *Biochim. Biophys. Acta*, 1979, **566**, 88.

mechanism was obtained from the action of the amylase on maltotetraose units which are located within the pullulan molecule. Dextran, pullulan, and maltotetraose are competitive inhibitors. H_4Edta caused reversible inactivation, but amylase activity could be restored by addition of Ca^{2+}; heavy-metal ions were inhibitory.

31 *endo*-(1 → 4)-β-D-Glucanases

An extracellular *endo*-(1→4)-β-D-glucanase has been isolated and purified *via* gel filtrations and ion-exchange chromatographies, from the culture fluid of *Lenzites trabea* grown on D-glucose and cellulose.[314] The enzyme (mol. wt. 2.9×10^4 by gel filtration, pH optimum 4.4, temperature optimum 70 °C) was also found to hydrolyse xylan.

Extracellular polysaccharides from *Rhizobium trifolii*, U226, Coryn and Bart A; *Rhizobium phaseoli*, U453, *Rhizobium leguminosarum*, U331; and *Rhizobium meliloti*, U27, after chemical modification, became substrates for certain β-D-glucanases.[311] The *Streptomyces endo*-(1→4)-β-D-glucanase hydrolyses reduced and deacetylated rhizobial polysaccharides, both before and after removal of carboxyethylidene substituents, to produce a series of oligosaccharides. The *Rhizopus arrhizus endo*-(1 → 3)-β-D-glucanase hydrolyses only fully modified polysaccharides to yield, in the case of *R. meliloti* U27, laminarabiose, and, in all other instances, a disaccharide identified as 3-*O*-β-D-galactopyranosyl-D-glucose. The same disaccharides were released by the *Rhizopus* enzyme from oligosaccharides produced by the action of the *Streptomyces* enzyme on fully modified polysaccharides. The results are discussed in relation to the available data for the structure of the polysaccharides and the specificity of the enzyme.

The contribution of *endo*-(1 → 4)-β-D-glucanases to the cellulolytic function of *Sporotrichum pulverulentum* has been discussed in a review of cellulose degradation by this organism.[163]

A (1 → 4)-β-D-glucanase was isolated from culture filtrates of the *Trichoderma viride* QM 9414 by gel filtration, ion-exchange chromatography and isoelectric focusing in a density gradient.[315] Polyacrylamide gel electrophoresis at two different pH values, analytical isoelectric focusing in a polyacrylamide gel slab and molecular-sieve chromatography of the reduced and alkylated enzyme in a denaturing medium indicated a homogeneous protein. The enzyme (mol. wt. of 1.10×10^4, pI 4.66) is not a glycoprotein. An immunoadsorbent made from activated agarose and antiserum afforded a rapid and highly specific purification of the enzyme.

32 *exo*-(1 → 4)-β-D-Glucanase

The contribution of *exo*-(1 → 4)-β-D-glucanases to the cellulolytic function of *Sporotrichum pulverulentum* has been discussed in a review of cellulose degradation by this organism.[163]

[314] D. Herr, F. Baumer, and H. Dellweg, *Arch. Mikrobiol.*, 1978, **117**, 287.

[315] U. Hakansson, L. G. Fagerstam, L. G. Pettersson, and L. Andersson, *Biochem. J.*, 1979, **179**, 141.

33 *exo*-(1 → 6)-α-D-Glucanases

An *exo*-(1 → 6)-α-D-glucanase (EC 3.2.1.70) produced extracellularly by *Arthobacter globiformis* was found to invert the anomeric configuration of D-glucose released from dextran, and to require calcium for protection against warming.[316] Among isomaltodextrins used as substrates for this enzyme, the rate of hydrolysis for isomaltose was the lowest and increased with the degree of polymerization of the oligosaccharides up to DP = 7. The minor activities accompanying purified *exo*-(1 → 6)-α-D-glucanase preparations (release of D-glucose from starch, splitting of maltose, nigerose, and kojibiose) were ascribed to the *exo*-(1 → 6)-α-D-glucanase itself. Fourteen native dextrans and soluble potato starch were subjected to digestion by this enzyme and the rate, process, and extent of hydrolysis of these substrates were studied relative to the composition of non-(1 → 6)-α-D-linkages of these polysaccharides.

A 4-nitrophenyl α-D-glucopyranoside-hydrolysing α-D-glucosidase of a thermophile, *Bacillus thermoglucosidius* KP 1006, has been purified to an electrophoretically-homogeneous state.[147] Its molecular weight was estimated as 6.0×10^4 by gel electrophoresis. The molecular activity (k_0) and the K_m values at 60 °C and pH 6.8 for 4-nitrophenyl α-D-glucopyranoside were 233 s^{-1} and 0.24 mM, respectively. The enzyme cleaved the non-reducing terminal (1 → 6)-α-D-glucosidic bonds of isomaltose, panose, isomaltotriose, isomaltotetraose, and isomaltopentaose. The k_0 values were 72.4, 194, 208, 233, and 167 s^{-1}, and the K_m values were 3.3, 9.5, 11, 13, and 21 mM, respectively. Each isomalto-oligosaccharide was hydrolysed to D-glucose by the cleavage of single D-glucose units from its non-reducing end. The study suggested that the enzyme is an oligo-1,6-D-glucosidase and an *exo*-(1 → 6)-α-D-glucanase.

34 *endo*-(1 → 6)-β-D-Glucanases

A kind of *endo*-(1 → 6)-β-D-glucanase has been purified from the culture filtrate of an *Acinetobacter* species grown in the medium containing baker's yeast cells as a carbon source.[317] A 100-fold purified preparation was obtained by ion-exchange chromatography. The enzyme hydrolysed pustulan, giving a series of gentio-oligosaccharides and D-glucose. Gentiotriose and gentiotetraose were hydrolysed by this enzyme yielding D-glucose and gentiobiose, and D-glucose, gentiobiose and gentiotriose, respectively. Gentiobiose was not hydrolysed. Baker's yeast D-glucans obtained from the isolated cell walls were also hydrolysed by this enzyme giving a series of oligosaccharides and D-glucose. From the action patterns on these carbohydrates, it was concluded that the enzyme was an *endo*-(1 → 6)-β-D-glucanase.

Bacillus circulans was grown on cells of six species of yeasts as inducer substrates, and the courses of progress of β-D-glucanase production were compared.[307] Also, the isoelectric focusing patterns of (1 → 3)-β-D- and (1 → 6)-β-D-glucanases were determined (see p. 501).

[316] T. Ohya, T. Sawai, S. Uemura, and K. Abe, *Agric. Biol. Chem.*, 1978, **42**, 571.
[317] S. Katohda, F. Suzuki, S. Katsuki, and T. Sato, *Agric. Biol. Chem.*, 1979, **43**, 2029.

D-Glucose repressed the biosynthesis of (1→3)-β-D-glucanase in *Neurospora crassa*.[309] The production of enzyme during growth in D-glucose-supplemented medium was negligible, but if deprived of carbon source the fungus actively synthesized high levels of enzyme when growth ceased. Similar results were obtained for (1→6)-β-D-glucanase but less enzyme was produced (see p. 502).

The filamentous fungus *Penicillium italicum*, when grown in a synthetic medium, produced and secreted (1→6)-β-D-glucanase into the culture medium.[318] This enzyme has been partially purified by gel filtration. After this step the active fractions were free from (1→3)-β-D-glucanase, α-amylase, and β-D-glucosidase activities. Only four proteins, one associated with the enzyme, were found by polyacrylamide gel electrophoresis under non-denaturing conditions. The enzyme behaved as an acidic protein (pI 4.65, pH optimum 5) and an *endo*-hydrolytic mode of action. The activity was lost at pH's greater than 7. The enzyme was also found associated with the mycelium. Its synthesis was repressed by D-glucose or growth-promoting sugars. Derepression in low D-glucose-containing medium required protein synthesis. 8-Hydroxyquinoline, an RNA synthesis inhibitor, added during the derepression period did permit some increase in the enzyme specific activity but prevented it when added at the beginning of that period.

35 D-Glucanases (Miscellaneous)

The action patterns have been studied of a purified, intracellular dextranase and three intracellular α-D-glucosidases from *Pseudomonas* UQM733 on pure isomalto-oligosaccharides.[158] The D-glucosidases had optimal activity on isomaltotetraose and were therefore classified as 'oligo-D-glucanases' [two of them as '(1→6)-α-D-oligoglucan glucohydrolases' and one as an 'α-D-oligoglucan glucohydrolase']. They have been used to determine the structure of two branched isomalto-oligosaccharides obtained by enzymic degradation of dextran.

Repetitive action of purified β-D-glucanase from *Penicillium notatum* on amyloids provided a means of establishing the structure of these polysaccharides.[319] There is a central chain of (1→4)-linked β-D-glucose moieties [sometimes (1→3)-linked] which is branched with nearly equivalent proportions of 6-*O*-α-D-xylopyranosyl and β-D-galactosyl-(1→2)-α-D-xylopyranosyl moieties. The region substituted by disaccharide is inaccessible to the action of the enzymic preparation, thus confirming the irregularity of these substituents on the central β-D-glucan chain.

The β-D-glucanases which occur in the fission yeast *Schizosaccharomyces pombe* have been reported.[320]

In a study of the production of α-D-glucans by yeasts and yeast-like organisms, most of a total of 177 strains were found able to utilize (1→4)-α-D-glucans by means of enzymes acting on the reducing ends of the outer D-glucan chains.[257]

[318] T. Santos, C. Nombela, J. R. Villanueva, and G. Larriba, *Arch. Mikrobiol.*, 1979, **121**, 265.
[319] A. Dali-Yousef, P. Le Dizet, and J.-E. Courtois, *Phytochemistry*, 1979, **18**, 1949.
[320] B. Y. Reichet and G. H. Fleet, *Proc. Austral. Biochem. Soc.*, 1979, **12**, 7.

36 Glucoamylases

The affinity chromatography of glucoamylase on (4-mercuribenzoyl)cellulose has been examined.[239]

The difference between the substrate specificities of glucoamylase and α-D-glucosidase has been interpreted by the relative value of subsite affinities of the first and third sub-sites counting from the terminal (that first subsite) to which the non-reducing end of substrate is bound.[137]

A large fluorescence enhancement of 2-(4-toluidinylnaphthalene)-6-sulphonate caused by amylose decreases as the substrate is degraded by amylase.[220] This property was used to follow the enzymatic hydrolysis of amylase and to analyse the action pattern of six kinds of amylases, porcine pancreatic, Taka-amylase A, two bacterial α-amylases, soybean β-amylase, and *Rhizopus niveus* glucoamylase (see p. 477).

The amino-acid and carbohydrate (D-glucose, D-galactose, D-xylose, L-fucose, and 2-amino-2-deoxyhexose) compositions of rabbit intestinal glucoamylase have been determined.[321] The molecular weight of the enzyme, as determined by the Archibald's approach to equilibrium method in the analytical ultracentrifuge is 2.0×10^5, but gel filtration of the enzyme on Biogel® P-300 and Sepharose® 6B columns gave anomalous results, suggesting a molecular weight higher than 9.0×10^5, an anomaly which remains unexplained. Several attempts made to dissociate the protein into subunits were unsuccessful.

Evidence has been presented for a similar distribution of glucoamylase (α-D-glucosidase) in subcellular fractions of rat and trout livers.[130]

Pure rat intestinal 'maltase'/glucoamylase was partially inactivated in 1% sodium dodecyl sulphate by heating at 40–70 °C for 5 min at pH 7.5, or by lowering the pH to 5.4–6.6 at 24 °C (see p. 451).[128]

The activity of glucoamylase in livers of carp (*Cyprinus carpins*) and goldfish (*Carassius auratus*) has been shown comparable.[322] The activity of the hepatic enzyme is approximately one-half that of glycogen phosphorylase in carp, whereas in goldfish the activity of glucoamylase is less than one-sixth that of the phosphorylase. Hepatic glucoamylase was proposed as an important glycogenolytic enzyme in carp but as making an insignificant contribution to glycogenolysis in goldfish.

Aspergillus awamori produced a glucoamylase which was separated from the α-D-glucosidases by ion-exchange chromatography (see p. 456).[144]

Glucoamylase I (mol. wt. 9×10^4; *N*-terminal amino-acid L-alanine) was selectively produced by a submerged culture of *Aspergillus awamori* var. *kawachi* as an enzyme having the ability to digest even raw starches, and was converted into a modified active glucoamylase decreasing in molecular weight during the digestion with the acid protease of the same mould strain.[323] The modified glucoamylase (mol. wt. 8.3×10^4; *N*-terminal amino-acid L-alanine plus L-isoleucine) resembled an intermediate type of glucoamylase I, and exhibited hydrolysis curves for gelatinized potato starch and maltose in the same manner

[321] S. Sivakami and A. N. Radhakrishnan, *Indian J. Biochem. Biophys.*, 1978, **15**, 354.
[322] I. Picukans and B. L. Umminger, *Comp. Biochem. Physiol. B.*, 1979, **62**, 455.
[323] S. Hayashida and E. Yoshino, *Agric. Biol. Chem.*, 1978, **42**, 927.

as that of glucoamylase I′; but it did not digest raw starches and was not adsorbed on to corn starch, and showed the lower hydrolysis limit of 80% for glycogen. The carbohydrate contents of glucoamylase I decreased 59% during the digestion with fungal α-D-mannosidase. The carbohydrate-split glucoamylase (mol. wt. 8.6×10^4; *N*-terminal amino-acid L-alanine) was unstable at pH 9 or at 65 °C to the same extent as glucoamylase I′, but was identical with glucoamylase I in the hydrolysis curves for various substrates, the digestibility for raw starches and the adsorbability on to corn starch. Multiple forms of glucoamylase were observed when native glucoamylase I was degraded in a stepwise fashion with fungal acid protease and α-D-mannosidase.

Glucoamylase from *Aspergillus niger* has been used in a study of the hydrolysis of starch in a membrane reactor in which an Amicon ultrafiltration cell was employed.[324] It was shown that the enzyme did reduce the formation of a gel layer and gave higher and more stable performances of the membrane reactor than the ones obtained in previous studies.

Three forms of glucoamylase (mol. wts. 7.6×10^4, 3.8×10^4, and 3.8×10^4) of *Aspergillus oryzae* had optimum pH ranges of 4.5–5.0 at 40 °C and optimum temperature ranges of 40–60 °C.[325] They were most stable over the pH range 4.0–7.0 and temperatures up to 40 °C. Hydrolysis activities of the three glucoamylases on different substrates were studied. They differed in their rate of attack on many substrates. Glucoamylase I had strong debranching activity and was highly active in raw corn-starch digestion. Glucoamylase II and III had weak debranching activities and could hardly digest raw starch.

A glucoamylase has been obtained from *Cephalosporium eichhorniae*; a study of its properties showed it to be thermophilic.[326]

In a study of the glucoamylase activity produced by *Endomycopsis bispora* the dependence of secretion of the enzyme upon morphology was examined.[327]

A glucoamylase has been isolated from fruit bodies of *Lentinus edodes* by a procedure including fractionation with ammonium sulphate, ion-exchange chromatography, and preparative gel electrofocusing.[151] The preparation was homogeneous on gel electrofocusing and ultracentrifugation. The glucoamylase (mol. wt. 5.5×10^4) hydrolysed maltose, maltotriose, phenyl α-maltoside, soluble starch, amylose, amylopectin, and glycogen, β-D-glucose being the sole product formed in the digests of these substrates.

Both of the two forms of glucoamylase (glucoamylases I and II) from the wheat bran culture of *Mucor rouxianus* hydrolysed amylopectin, amylose, glycogen, soluble starch, maltotriose, and maltose, but did not act on isomaltose and isomaltotriose.[328] Phenyl α-maltoside was hydrolysed into D-glucose and phenyl α-D-glucopyranoside by both glucoamylases. Maltose was hydrolysed about one-fifth as rapidly as amylopectin. Both enzymes produced D-glucose from amylopectin, amylose, glycogen, soluble starch in the yields of almost complete hydrolysis. They hydrolysed amylose with the inversion of configura-

[324] A. M. Madgaukar, Y. T. Shah, and J. T. Cobb, *Biotechnol. Bioeng.*, 1977, **19**, 1719.
[325] M. N. N. Miah and S. Ueda, *Stärke*, 1977, **29**, 235.
[326] D. F. Day, *Curr. Microbiol.*, 1978, **1**, 181.
[327] H. Ruttloff, K. Winkler, and F. Zickler, *Z. Allg. Mikrobiol.*, 1979, **19**, 195.
[328] Y. Yamasaki, A. Tsuboi, and Y. Suzuki, *Agric. Biol. Chem.*, 1977, **41**, 2139.

tion, producing β-D-glucopyranose. Glucoamylase II hydrolysed raw starch at three-fold higher rate than glucoamylase I. The former hydrolysed rice starch almost completely into D-glucose, whereas the latter hydrolysed it incompletely (nearly 50%).

Penicillium oxalicum has been shown to produce two forms of glucoamylase and an α-D-glucosidase.[154]

The substrate specificity of the two forms of glucoamylase (glucoamylases I and II) from the mycelia of *Penicillium oxalicum* has been investigated.[329] Both glucoamylases hydrolysed amylopectin, amylose, glycogen, soluble starch, maltotriose, and maltose, but not isomaltose and isomaltotriose. D-Glucose was the sole hydrolysis product found in the digests of these substrates. Both enzymes hydrolysed phenyl α-maltoside to D-glucose and phenyl α-D-glucopyranoside. Maltose was hydrolysed about one-sixth as rapidly as amylopectin. Both enzymes produced D-glucose from amylopectin, amylose, glycogen, and rice starch in the yields of almost complete hydrolysis, and also hydrolysed amylose with the inversion of configuration, producing β-D-glucopyranose. Glucoamylases I and II were glycoproteins containing 3.93% and 3.22% neutral sugar, respectively. Glucoamylase II contained D-mannose, D-galactose, and D-glucose as the neutral sugar constituent.

Piricularia oryzae, the pathogenic fungus of rice blast disease, has been shown to produce a glucoamylase as a sole amylolytic enzyme with little concomitant activity of other related enzymes.[330] The purified enzyme preparation showed a single band on electrophoresis. The enzyme (mol. wt. 9.4×10^4, pH optimum 6.5, temperature optimum 50–55 °C) was completely inactivated by heating for 15 min at 60 °C. The enzyme hydrolysed various kinds of amylaceous substrates and some malto-oligosaccharides into D-glucose. D-Glucobioses such as isomaltose, nigerose, kojibiose, αα-trehalose, ββ-trehalose as well as phenyl α-glucopyranoside were also hydrolysed.

The Michaelis constant and maximal velocity of α-amylase-free glucoamylase from *Rhizopus delemar* have been shown to decrease with increasing periodate oxidation of amylose.[331] These kinetic features have been explained on the basis of competitive inhibition by the oxidized non-reducing end of the (1 → 4)-α-D-glucan chain with the active centres of the enzyme. A kinetic model was proposed to demonstrate this special kind of inhibition where the concentration of inhibitor is directly proportional to the substrate concentration. The experimental data fitted this model, and the plots of K_m^{-1} and V^{-1} against the ratio of oxidized/unoxidized non-reducing end-groups were straight lines.

Glucoamylase from *Rhizopus niveus* has been shown to have an *exo*-action on (1 → 3)-α-D- and (1 → 6)-α-D-glucan linkages as well as on malto-oligosaccharides.[229]

In a model experiment, glucoamylase (*ex Rhizopus niveus*) was shown to inhibit α-D-glucan synthesis as catalysed by potato phosphorylase (see p. 498).[297]

[329] Y. Yamasaki, Y. Suzuki, and J. Ozawa, *Agric. Biol. Chem.*, 1977, **41**, 1443.
[330] A. Yuhki, T. Watanabe, and K. Matsuda, *Stärke*, 1977, **29**, 265.
[331] L. B. Carvalho, *Carbohydr. Res.*, 1979, **75**, 257.

The hydrolysis of maltose by glucoamylase from *Rhizopus niveus* was carried out in the presence and absence of dextran sulphate, which are the components of supports of immobilized enzymes.[332] The interaction between dextran and the enzyme was observed by fluorescence spectrophotometry. The kinetic and fluorescence experiments indicated that dextran became bound to glucoamylase and was apparently a non-competitive inhibitor of the enzyme. The dissociation constant of the enzyme–dextran complex was estimated to be 34%. The reaction rate was hardly affected at pH 4.0 and 4.5 by addition of dextran sulphate, whereas the kinetic parameters depended considerably on the concentration of dextran sulphate at pH 3.5. These findings indicated that there might exist some interactions between the enzyme and dextran sulphate.

Thermal stability of glucoamylase from *Rhizopus niveus* has been investigated in detail mainly at 60 ± 0.1 °C (pH 4.5) in relation to kinetics both in the presence and in the absence of various ligands.[333] With substrate analogues, such as D-glucose, lactose, and D-glucono-1,4-lactone, and with glycerol, thermostability of the enzyme greatly increased, whereas it decreased with dioxan, ethanol, and glycol. To elucidate such phenomena clearly, several model equations were propounded on the assumption that there were two main factors which would play an essential role in heat stability of the enzyme; binding of ligands and the dielectric constants of solvents. From the model equations, binding constants of the ligands were estimated in order to confirm the validity of the assumption; *e.g.*, the inhibitor constant for D-glucose (at 59.5 °C) corresponding to the reciprocal of binding constant was evaluated to be 0.25 M as was expected.

The properties of a thermostable glucoamylase from a thermophilic fungus *Thermomyces lanuginosus* have been examined.[334]

Glucoamylase has been immobilized with retention of enzymic activity by water-soluble carbodi-imide-aided reaction with chitosan;[335] by reaction on glass involving diazotized 5-aminosalicylic acid bonded to a deposited imperfectly crystallized film of titanium dioxide;[336] by reaction on phenol–formaldehyde resin Duolite 5761 and on silanized Spherosil porous silica beads;[244] by interaction with and incorporation into polyelectrolyte complexes such as chitosan–dextran sulphate, chitosan-poly(vinylsulphate), and chitosan–heparin;[337] by adsorption on to SP-Sephadex® C-25 and C-50 by ionic linkage[338] by coupling to a variety of cellulose derivatives prepared by bonding aliphatic and aromatic diamines and amino-acids on to cellulose *via* epichlorohydrin;[239] by reaction with periodate, diamines, and borohydride, followed by reaction of the product with amino-derivatives of agarose; by adsorption to immobilized concanavalin A

[332] S. Adachi, K. Nakanishi, R. Matsuno, and T. Kamikubo, *Agric. Biol. Chem.*, 1977, **41**, 1673.
[333] S. Moriyama, R. Matsuno, and T. Kamikubo, *Agric. Biol. Chem.*, 1977, **41**, 1985.
[334] V. B. Rao, R. Maheshwari, N. V. S. Sastry, and P. V. S. Rao, *Curr. Sci.*, 1979, **48**, 113.
[335] T. Kasumi, M. Tsuji, K. Hayashi, and N. Tsumura, *Agric. Biol. Chem.*, 1977, **41**, 1865.
[336] J. F. Kennedy and M. F. Chaplin, *Enzyme Microb. Technol.*, 1979, **1**, 197.
[337] R. Yamaguchi, Y. Arai, S. Hirano, and T. Ito, *Agric. Biol. Chem.*, 1978, **42**, 1297.
[338] S. Adachi, Y. Kawamura, K. Nakanishi, R. Matsuno, and T. Kamikubo, *Agric. Biol. Chem.*, 1978, **42**, 1707.

followed by crosslinking with glutaraldehyde;[339] by adsorption on to/reaction with a range of new polyphenolic resins[169] and by reaction on to inorganic (glass) supports activated by the surface deposition of an imperfectly crystallized film of titanium dioxide (the enzyme may be linked to the activated support using 5-aminosalicylic acid, with or without diazotization, tannic acid, or tin(II) chloride, and by the silane–glutaraldehyde method on the same glass support).[170]

37 Glycanases (Miscellaneous)

The D-mannose 6-phosphate of human β-D-glucuronidase was released from the enzyme by treatment with *endo*-glycanase H with concomitant loss of susceptibility to adsorptive endocytosis.[177] Thus, these studies provide direct evidence that D-mannose 6-phosphate is present on high-uptake enzyme forms, that it is present in the recognition marker for uptake, and that it is present on oligosaccharide that is released by *endo*-glycanase H.

In connection with the structural study of D-galacto-L-fucan sulphate (sulphated polysaccharide of *Undaria pinnatifida*), D-galacto-L-fucanase, α-L-fucosidase, and β-D-galactosidase existing in hepato-pancreas of abalone (*Haliotis* sp.) were investigated.[78] From the crude enzyme mixture, two partially purified fractions (FS-1 and FS-2) were obtained by ion-exchange chromatography followed by gel filtration. FS-1 showed the α-L-fucosidase activity and was free from the activity of D-galacto-L-fucanase and D-galacto-L-fucan sulphatase. FS-2 showed four activities of D-galacto-L-fucanase, D-galacto-L-fucan sulphatase, arylsulphatase, and fucoidanase and was free from the α-L-fucosidase activity. These results indicate that the D-galacto-L-fucanase may be *endo*-type enzyme and the same one as fucoidanase. FS-1, however, showed an activity on the partially degraded D-galacto-L-fucan sulphate, and contained two types (optimum pH 3 and 5) of α-L-fucosidase: one with a pH optimum of 3 was inhibited with D-galacto-L-fucan sulphate and the other with a pH optimum of 5 was not inhibited.

The filter paper disintegrating activity (FD) and the plant tissue macerating activity (MA) in the enzyme preparation (Driselase) produced by *Irpex lacteus* have been studied.[288] The optimum pH value of FD was 3.5–5.0 and that of MA was 4.0–6.0. Optimum temperature of FD was 45 °C and that of MA was 40–45 °C. Both FD and MA were stable in a wide pH range investigated and they were thermostable up to 45 °C. FD was remarkably inhibited by Ag^+, Hg^{2+}, and Sn^{2+}; on the other hand, MA was not affected by any of the metal ions tested. Driselase exhibited high cellulase, hemicellulase, and pectinase activities, but its excellent points were characterized by multiple enzyme systems that were able to be represented by, for example, MA and soybean meal disintegrating activity. Using various grains, it was examined with a microscope how tissues were macerated and cell walls were disintegrated with Driselase. The use of the enzyme in the modification of animal feeding stuffs was investigated (see p. 492).[281]

[339] M.-Y. Hsiao and G. P. Royer, *Arch. Biochem. Biophys.*, 1979, **198**, 379.

38 Heparin Hydrolases

Homogenates of rat small intestine can depolymerize rat-skin macromolecular heparin to products similar in size to commercial heparin.[340] This activity is attributed to an enzyme provisionally named 'macromolecular heparin depolymerase'. An assay for macromolecular heparin depolymerase activity in rat small intestine has been developed, based on the action of the enzyme on ^{35}S-labelled macromolecular rat-skin heparin. The depolymerized products are separated into two peaks by gel chromatography and the amount of label in the second peak, expressed as a percentage of the total radioactivity, is the index of enzyme activity. The enzyme (pH optimum 6.0, temperature optimum 45 °C) was shown to be most stable in 50 mM-Tris/maleate buffer containing 1 mM-H_4edta. Macromolecular heparin depolymerase activity measured as a function of time and substrate concentration produced curves typical of an enzymic reaction. Evidence was obtained demonstrating that the activity did not originate from bacteria in the intestine. Macromolecular heparin depolymerase activity was increased by dilution and storage at 7 °C for 24 hr, suggesting that homogenates of rat small intestine contain an unstable inhibitor of the enzyme.

39 Hyaluronate Lyases

Hyaluronate lyase purified from *Streptomyces hyalurolyticus* has been used to provide a specific assay for hyaluronic acid, its action resulting in the generation of β-D-gluco-4-ene-pyranosyluronic acid groups.[341] The assay is based on the long established periodate oxidation of such groups to *inter alia* β-formylpyruvic acid which specifically gives a chromophore with thiobarbituric acid.

40 Hyaluronidases

The activity of bull-sperm hyaluronidase is increased by the inclusion of polycations in the assay mixture.[342] At pH 3.8, bovine serum albumin and histone give the greatest stimulation, whereas protamine sulphate, spermidine, and hyamine 2389 stimulate to a lesser extent. Enzyme activity increases with serum albumin concentration to a nearly constant, high level at serum albumin concentrations greater than 1 mg ml^{-1}. Other stimulatory compounds show a similar concentration dependence except that inhibition of enzyme activity occurs at high concentrations of stimulator. The degree of stimulation depends on the pH, sample concentration, and substrate concentration. Enzyme preparations with a low protein content give the greatest stimulation, while preparations with a high protein content show little stimulation. The concentration of serum albumin required for maximum stimulation increases with increased hyaluronic acid concentration. The results suggest that the stimulation of sperm hyaluronidase is non-specific and results from an interaction of the polycation with hyaluronic acid. Since protein in the enzyme preparation substitutes for exogenous stimulator to a varying degree, serum albumin should be included in the assay

[340] E. Young and A. A. Horner, *Biochem. J.*, 1979, **180**, 587.
[341] G. W. Jourdian, M. Wolfman, R. Sarber, and J. Distler, *Anal. Biochem.*, 1979, **96**, 474.
[342] G. A. Doak and W. L. Zahler, *Biochim. Biophys. Acta*, 1979, **570**, 303.

mixture for sperm and testicular hyaluronidase to ensure measurement of maximum enzyme activity.

Bull seminal-plasma hyaluronidase has been purified 180-fold by affinity chromatography on concanavalin A-Sepharose®, heparin-Sepharose®, and chromatography on Sephadex® G-200 and Sephacryl® S-200.[343] With hyaluronic acid as the substrate, the specific activity and turnover number of purified hyaluronidase were 3.63 μmol min^{-1} per mg (10400 National Formulary units mg^{-1} of protein) and 214 min^{-1} (mol of product formed per mol of enzyme per min), respectively. Polyacrylamide gel electrophoresis indicated that the purified enzyme migrated as a single band at pH 4.3 and 5.3. Bull seminal-plasma hyaluronidase was markedly inhibited by hydroxylamine, phenylhydrazine, and semicarbazide.

41 Inulinases

General properties and the mode of action have been investigated for purified extracellular inulinase from the culture filtrate of *Aspergillus niger*.[344] Optimal pH and temperature of the enzyme reaction were 5.0 and 55 °C, respectively. The enzyme was stable from pH 4 to 7 (30 °C, 24 hr) and completely inactivated when heated for 30 min at 80 °C (pH 5.0). The enzyme was activated by divalent metal ions such as Mn^{2+}, whereas it was markedly inactivated by Hg^{2+}, Ag^{+}, and 4-chloromercuribenzoate. The enzyme catalysed the hydrolysis of inulin, sucrose, and raffinose by the splitting of terminal D-fructosyl units, but not of bacterial levan and melezitose. The enzyme seemed to act on inulin, liberating D-fructose from the D-fructose end of inulin (K_m 1.87 mM) and producing one molecule of D-glucose finally. This enzyme was therefore considered to be an *exo*-inulinase.

42 Isoamylases

The use of amylose gel cross-linked by epichlorohydrin for affinity chromatography of extracellular isoamylase of *Pseudomonas amyloderamosa* has been studied.[345] The isoamylase was adsorbed well on the amylose gel and was eluted specifically with maltodextrin. The eluted enzyme was precipitated with ammonium sulphate to remove maltodextrin, and then the solution of the precipitate was dialysed and concentrated by vacuum filtration. By this procedure 96 mg of the enzyme were purified to homogeneity from 20 l of culture broth in about 70% yield.

43 Laminaranases

Saccharomyces cerevisiae produced two laminaranases, an *endo*-(1 → 3)-β-D-glucanase and an *exo*-(1 → 3)-β-D-glucanase (see pp. 501 and 503).[306]

[343] P. N. Srivastava and A. A. Farooqui, *Biochem. J.*, 1979, **183**, 531.
[344] T. Nakamura, S. Maruki, S. Nakatsu, and S. Ueda, *J. Agric. Chem. Soc. Jpn.*, 1978, **52**, 581.
[345] K. Kato, Y. Konishi, A. Amemura, and T. Harada, *Agric. Biol. Chem.*, 1977, **41**, 2077.

44 Lysozymes

Lysozymes from animal and plant sources (human milk, hen egg whites, turnips) have been purified by affinity chromatography on agarose derivatives containing a phenylacetyl ligand.[346] While this absorbent was also able to bind chymotrypsin, the use of imidazole permitted separation of lysozymes from the protease. The phenylacetyl-agarose was a general affinity sorbent bearing a ligand of non-biochemical origin in contrast to those, for example, which use nucleotide co-factors. By careful selection of desorption conditions, different enzymes can be purified with the same sorbent. The results also suggested that binding of lysozymes to an affinity ligand does not require a spacer arm structure between ligand and support matrix.

An evaluation of 3,4-dinitrophenyl tetra-*N*-acetyl-β-chitotetraoside as a substrate for the measurement of lysozyme in normal and pathological sera has been reported.[347]

Human lysozyme has been determined in human cerebrospinal fluid by using a specific antiserum with nephelometric end point assay (2 hr, 50 μl sample in 225 μl reaction mixture with 35 g l^{-1} polyethylene glycol) or the specific degradation of bacterial cell wall (test-combination Properzym, from Behringwerke, Marburg/Lahn). The range lay between 0.005 to 0.008 g l^{-1} for both methods; recovery was 103 ± 6%.[348] In 17 samples there was only a partial agreement between the two methods, because 50% of the samples appeared to contain some unknown interference. Elevated lysozyme levels were found in bacterial and abacterial meningitis, mental subnormality, polyradiculitis, cerebral tumours, and lumbar syndromes.

For the isolation of human lysozyme from the urine of leukemia patients, a simple method has been established which involves precipitation of urinary proteins by 60% saturation with ammonium sulphate, fractionation of crude lysozyme by gel filtration, and purification by ion-exchange chromatography.[349] By this method approximately 60% of the lysozyme in the urine was isolated in a pure state in ten days.

Enzymatic activity of murine macrophage lysozyme in gels under normal physiological conditions of ionic strength, osmolarity, temperature, and pH is negligible.[350] Under isotonic conditions macrophage lysozyme can attain maximum activity at acid pH ('acid' lysozyme). At neutral and alkaline pH values, macrophage lysozyme can be fully activated by lowering the osmolarity to 155 imOsm (alkaline lysozyme). Such modulation of enzymatic activity is not seen when macrophage lysozyme is assayed in solution. The different behaviour of macrophage lysozyme in solution and in gel was briefly discussed in terms of lysozyme function in intact cells.

The changes occurring in endogenous lysozyme during hen egg white thinning and the possible effect of lysozyme on egg white thinning have been investi-

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[347] G. A. Turner, H. Ghneim, and J. Freeman, *Anal. Clin. Biochem.*, 1979, **16**, 51.
[348] T. O. Kleine, L. Chang, and G. Rohner, *J. Clin. Chem. Clin. Biochem.*, 1979, **17**, 506.
[349] M. Masuda, S. Kobayashi, K. Mizuno, and F. Sakiyama, *J. Biochem. (Tokyo)*, 1978, **84**, 971.
[350] M. Artman and R. J. Seeley, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 1217.

gated.[351] Calculated from the electrophoretic patterns in sodium dodecyl sulphate, the relative concentration of lysozyme of the gel fraction was 1.4 times that of the liquid fraction in the fresh thick white. After storage for five days, there were little differences in lysozyme contents between the gel and liquid fraction in the thick white. This was also confirmed from the changes in lysozyme content, estimated from the activity, of the gel and liquid fraction in the thick white during storage. It is suggested from these results that the lysozyme has a strong affinity to the gel in the fresh thick white, but dissociates from the gel into the liquid fraction in the thick white during an earlier phase of storage. The lysozyme activity in the thick white correspondingly decreased, at a rate depending on the temperature at which the thick white was kept. The lower the storing temperature, the smaller the decrease of lysozyme activity. The lysozyme activity of the thick white was also affected by the pH of the white. The pH of thick white was lowered in an atmosphere of carbon dioxide. The lysozyme activity of the thick white stored in pH 6.5 was little lowered. It was noted that the lysozyme activity of the thick white was remarkably affected by the storing temperature and pH, corresponding to the main causal factors of the egg white thinning. This suggested that the denaturation of lysozyme in the thick white during storage may be at least partly responsible for the egg white thinning. The gel structure in the fresh thick white is probably supported in a regularly ordered manner in which ovomucin fibres are surrounded by such proteins as lysozymes having a strong affinity to ovomucin. Therefore, the weakening of the gel structure of thick white during egg white thinning may be caused not only by the changes in ovomucin but also by the denaturation of such proteins as lysozymes supporting the gel structure of thick white.

Affinity chromatography of hen egg white lysozyme on chitosan without any intermediate reagent has proved to be a suitable means of purification of the enzyme, subsequently elution being achieved with 1-aminopropane.[352]

The peptide map of a trypsin hydrolysate of hen egg white lysozyme oxidized at the S—S bonds was obtained and characterized. A comparative study of it with the map of the same hydrolysate of lysozyme reduced at the S—S bonds was conducted.[353] Comparison of the (structural) signal regions of hen egg white lysozyme and of ovine α-lactalbumin have been reported.[354]

By a combined gravimetric and i.r. technique, spectra of lysozyme protein films have been recorded during sorption isotherms at constant water content h (mg ^2H_2O per mg dry protein) in the range $0 \leqslant h \leqslant 0.35$ at 27 and 38 °C.[355] Computer-aided differential analysis shows the effect of progressive hydration on some significant sites of the protein such as the ionizable acidic side-chains and the backbone amide carbonyls, as well as the spectrum of the absorbed water itself. In order to derive thermodynamic properties of these sites, the

[351] A. Kato, T. Wakinaga, N. Matsudomi, and K. Kobayashi, *Agric. Biol. Chem.*, 1978, **42**, 175.
[352] R. A. A. Muzzarelli, G. Barontini, and R. Rocchetti, *Biotechnol. Bioeng.*, 1978, **20**, 87.
[353] T. V. Chentsova and N. A. Kravchenko, *Biochemistry (Eng. Transl.)*, 1978, **43**, 1554.
[354] J. C. Mercier, G. Haze, P. Gaye, G. Petrissant, D. Hue, and M. Boisnard, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 662.
[355] G. Careri, A. Giansanti, and E. Gratton, *Biopolymers*, 1979, **18**, 1187.

measured sorption isotherm is decomposed in terms of a model which postulates the existence of two classes of primary sorption sites only, and these two contributions are independently checked by their data. The free energy of binding of the strong and weak binding sites is found to be 2.0 ± 0.2 and 0.40 ± 0.1 kcal mol^{-1}, respectively. A water-induced transition region is clearly detected in all the observed properties at $0.06 < h < 0.10$ at 38 °C and is shown to be due to changes involving both the structure of the absorbed water and the coverage of the absorption sites. A detailed picture of the hydration events is offered, and the relevance of these findings to protein dynamics is discussed.

The far-i.r. absorption spectrum of lysozyme was measured at room and liquid-nitrogen temperatures.[356] Dried layers of single crystals of tetragonal lysozyme chloride with a diameter of 100–300 μm were grown on a silicon plate. Such single-crystalline samples were considered to have the following advantages in obtaining far-i.r. spectra: (i), surface scattering is reduced; (ii) the protein molecules are closely packed; and (iii), air-drying of the crystals reduces the number of water molecules without considerably changing the original configuration. The spectrum obtained consisted of a strong background absorption and a number of absorption peaks that were not clearly observed with the sample in the form of lyophilized powder. The peaks were ascribed to various delocalized vibrations of the main and side chains in the molecule. The peaks were also compared with the positions of Raman lines. The uniform background was assigned to the water molecules remaining in the crystals.

A comparative study on the secondary structure of hen egg white, turtle (*Testudo grorea*) egg white, and fruit fly (*Ceratitis capitata*) egg lysozymes has been carried out using c.d. spectroscopy.[357] Differences in the near-u.v. spectra of these enzymes agreed with the different contents of L-tryptophan residues. The far-u.v. c.d. spectra showed the existence of a high content of unordered structure in the insect lysozyme in comparison with the high participation of α-helical conformation in the hen egg white enzyme. Differences in the dichroic behaviour between both enzymes were also remarkable under denaturing conditions.

The difference absorption spectra of hen and turkey lysozymes in the alkaline pH region had three maxima at around 245, 292, and 300 nm and had no isosbestic points.[358] The ratio of the extinction difference at 245 nm to that at 295 nm changed with pH. These spectral features are quite different from those observed when only L-tyrosyl residues are ionized, and it was impossible to determine precisely the p*K* values of the L-tyrosyl residues in lysozyme by spectrophotometric titration. A time-dependent spectral change was observed above about pH 12. This is not due to exposure of a buried L-tyrosyl residue on alkali denaturation. The disulphide bonds and the peptide bonds in the lysozyme molecule were cleaved by alkali above about pH 11. The intrinsic p*K* value of L-tyrosine-23 of hen lysozyme was determined to be 10.24 (apparent p*K* 9.8) at 0.1 ionic strength and 25 °C from the c.d. titration data. Comparison of the c.d.

[356] M. Ataka and S. Tanaka, *Biopolymers*, 1979, **18**, 507.
[357] J. M. Fernandes-Sousa, J. G. Gavilanes, A. M. Mucicio, and R. Rodriguez, *Comp. Biochem. Physiol.*, 1979, **62**, 389.
[358] S. Kuramitsu and K. Hamaguchi, *J. Biochem. (Tokyo)*, 1979, **85**, 443.

titration of turkey lysozyme with that of hen lysozyme suggested that L-tyrosine-3 and L-tyrosine-23 in turkey lysozyme have apparent pK values of 11.9 and 9.8, respectively.

A preliminary analysis is presented of whether the isotropic temperature factors derived from refinement of the tetragonal crystal structure of hen egg white lysozyme at 2 Å resolution can be interpreted in terms of molecular motion.[359] If the contributions to the temperature factors from experimental errors, crystal disorder, and imperfections of the molecular model are neglected, the apparent thermal motion is found to be compatible with the pair of molecules that have the strongest interactions in the crystal either moving as a rigid-body with libration about a common axis, or vibrating in an intramolecular mode with the principal amplitudes radial to this axis. There is only weak evidence for hinge-bending, *i.e.* that the two lobes of lysozyme vibrate so that their separation varies. Side-chains that are exposed to the solvent and some segments of the main polypeptide chain (including residues 70–73, 82–84, 101–103) have greater apparent thermal motion than the remainder of the molecule. Although this analysis at a single temperature cannot establish whether thermal motion or static disorder (including conformational variability) underlies the observed effects, it suggests that the accurate determination of temperature factors will be useful in detailed studies of the dynamic properties of macromolecules.

Analysis at 0.25 nm resolution of the crystal structures of hen egg white lysozyme-Gd^{III} and lysozyme-Gd^{III}-2-acetamido-2-deoxy-D-glucose, prepared by diffusion methods, show that there are two main binding positions for Gd^{III}, one of which is close to L-glutamic acid-35 and the other close to L-aspartic acid-52.[360] The two sites are 0.36 nm apart. There is no evidence for the weak binding of Gd^{III} to any of the eight other carboxy-groups of lysozyme. In the presence of Gd^{III}, the binding of 2-acetamido-2-deoxy-D-glucose is similar to that observed for the binding of the β-anomer in subsite C. There are numerous small conformational changes in the protein on binding Gd^{III} and the sugar, and these have been quantified to a first approximation by real-space refinement. These changes are similar in both structures, and involve, among other small movements, shifts of one of the disulphide bridges by up to 0.05 nm. The movement of residues 70–74 previously observed in the binary complex of lysozyme-2-acetamido-2-deoxy-D-glucose is not observed in the ternary complex of lysozyme-Gd^{III}-2-acetamido-2-deoxy-D-glucose. The nature of the lysozyme-Gd^{III} complex is discussed in the light of evidence from other crystallographic studies and n.m.r. solution studies. Preliminary findings for a lysozyme-Gd^{III} complex prepared by co-crystallization methods were reported.

Using kinetic data on recombination macroradicals in freezed water solutions of lysozyme, superslow molecular motions of the biopolymer have been studied.[361] Recombination of macroradicals is observed at 160–240 K and it has a stepwise character. The kinetic data are described quantitatively by the model,

[359] M. J. E. Sternberg, D. E. P. Grace, and D. C. Phillips, *J. Mol. Biol.*, 1979, **130**, 231.
[360] S. J. Perkins, L. N. Johnson, P. A. Machin, and D. C. Phillips, *Biochem. J.*, 1979, **181**, 21.
[361] V. I. Devkin and A. I. Mikhailov, *Biofizika*, 1978, **23**, 775.

in which the set of processes with different rate constants is considered (the so-called model of polychromatic kinetics). It has been shown that polychromatic character of the process is conditioned by energy activation distribution, which is changed from $E_{min} = 8 \pm 1$ kcal mol^{-1} to $E_{max} = 13 \pm 1$ kcal mol^{-1} with 'rectangular' distribution function $f(E) \cong m(E_{max} - E_{min})^{-1}$. Pre-exponential factor $K_0 = 2 \times 10^{-9}$ cm^3 s^{-1}. The recombination-kinetic method used permits estimation of frequencies (inverse correlation time) of translation mobility macroradicals of protein up to the frequency order 10^{-2} s^{-1} (or velocities of motions ~ 1 Å s^{-1}).

A number of modification products were isolated by ion-exchange chromatography for a mixture of *N*-carboxymethylated derivatives (CM derivatives) of lysozyme, produced by treatment of the protein with mono-iodoacetic acid.[362] In each CM derivative the position of the modified amino-acid residues was established. Possible causes of such selectivity of the process of carboxymethylation are discussed. It was also shown that salt activation of CM derivatives of lysozyme proceeds more readily, the higher the degree of their modification. As a result of this, the enzymatic activity of CM derivatives of lysozyme is higher than that of the native enzyme, which is correlated with their increased affinity for the substrate (chitin).

Natural abundance ^{13}C n.m.r. spectroscopy has been used to study the carboxymethylation of L-histidine-15 of hen egg white lysozyme upon treatment with iodoacetate.[363] A comparison of the spectrum of chromatographically pure (N^{ϵ_2}-carboxymethyl-L-histidine) lysozyme with that of the intact protein indicated that the chemical modification did not significantly affect the conformation of the protein.

A novel method for the preparation of Kyn 62-lysozyme, in which L-tryptophan-62 of hen egg white lysozyme is replaced by kynurenine, has been reported.[364] Crude Kyn 62-lysozyme purified by affinity and Bio Rex® 70 chromatography, successively retained affinity for chitin and was essentially an active enzyme with a slightly weakened but distinct catalytic activity. After this modification, the enzyme activity was changed differently depending on the kind of substrate. At the individual optimum pH's, lytic activity was largely retained (80% active), but the catalytic efficiency for hydrolysing glycol chitin was relatively low (30% active). Lysis of *Micrococcus lysodeikticus* cell suspensions was optimally catalysed by Kyn 62-lysozyme at pH 6.2 and at 0.088 ionic strength. These values are lower by 1.3 pH unit and 0.04 ionic strength, respectively, than those of intact lysozyme. The optimum pH and ionic strength for the hydrolysis of neutral substrates were scarcely affected. These results suggest that significance of electrostatic interaction in the lysis of lysozyme.

Conditions for the selective cleavage of the kyneurenyl bond in ozone-oxidized hen egg white lysozyme in aqueous hydrazine solution have been

[362] L. A. Chistyakova, I. A. Cherkasov, and N. A. Kravchenko, *Biochemistry (Engl. Transl.)*, 1978, **43**, 919.

[363] W. J. Goux and A. Allerhand, *J. Biol. Chem.*, 1979, **254**, 2210.

[364] N. Yamasaki, T. Tsujita, T. Eto, S. Masuda, K. Mizuno, and F. Sakiyama, *J. Biochem. (Tokyo)*, 1979, **86**, 1291.

established.[365] Further derivation and peptide analysis showed that the *N'*-formylkynurenine is present at position 62 in place of L-tryptophan in the native protein.

The interaction of sodium dodecyl sulphate with recrystallized hen egg white lysozyme was analysed by following enzyme activity and with ^{1}H n.m.r. fluorescence, and u.v. difference spectroscopies using various alkyl sulphates and variously modified lysozymes (acetylated, carbamylated, succinylated, and guanidinated lysozymes).[366] Sodium dodecyl sulphate formed a stable complex with lysozyme without causing a gross conformational change in the enzyme molecule. Some sodium dodecyl sulphate molecules bound to the active site cleft of lysozyme and therefore strongly inhibited the activity of lysozyme. Hydrophobic regions and positive charges for the protein side, and a hydrophobic tail (possibly more than eight carbons in alkyl chain) and a negative charge for the detergent side were required for the formation of the complex.

Some mucus glycoproteins form soluble complexes with hen egg white lysozyme at neutral pH values.[367] The extent of complex formation was determined, by an ultracentrifugal difference method, for a range of glycoproteins covering the common blood-group specificities. Interaction was strongest with those glycoproteins of blood-group Le^a specificity; these were also richest in neuraminic acid. Interaction diminished with increase of ionic strength, and was not detectable at $I = 0.50$; however, an asialoglycoprotein was found to retain some activity. The interaction is accordingly primarily, but probably not exclusively, coulombic in origin. The buoyant density of lysozyme in CsCl, CsBr, CsI, and Cs_2SO_4 was determined; the values in the last three salts were anomalously high. This finding accounts for the previously noted difficulty of separating free protein from glycoproteins by single-stage centrifugation in CsBr. Conditions for effective separation of glycoproteins from secretions containing lysozyme by density-gradient centrifugation were reported.

It was found that a water-insoluble complex can be formed between purified lysozyme and flavoprotein, both of which are constituents of hen egg white, and that the optimal pH of the complex formation was about 7.[368] When the mixing ratio of lysozyme and flavoprotein in molar concentration was 2:1 or above 3:1, the molar ratio of lysozyme and flavoprotein of the complex was 2:1 or 3:1, respectively. However, the changes of molar ratio of the complex were observed in the presence of other egg white proteins. The complex obtained directly from egg white by treatment with cold acetone and by dialysis consisted of lysozyme, flavoprotein, conalbumin and ovomucin-like substance, and the molar ratio of the former three components in the complex was calculated from the gel-filtration pattern to be 16:4:1.

Lysozymes of larvae of *Galleria mellonella* and *Bombyx mori* have been isolated by GC-50 Amberlite chromatography from heated haemolymphs.[369]

[365] F. Sakiyama, *J. Biochem. (Tokyo)*, 1977, **82**, 365.
[366] T. Imoto, S. Sumi, M. Tsuru, and K. Yagishta, *Agric. Biol. Chem.*, 1979, **43**, 1809.
[367] J. M. Creeth, J. L. Bridge, and J. R. Horton, *Biochem. J.*, 1979, **181**, 717.
[368] Y. Hidaka and Y. Matsuoka, *J. Agric. Chem. Soc. Jpn.*, 1977, **51**, 159.
[369] G. Croizier and L. Croizier, *C. R. Hebd. Seances Acad. Sci., Ser. D.*, 1978, **286**, 469.

It was observed by Laurell electrocyneresis, immunodiffusion, and immunoelectrophoresis techniques, that lysozymes of these insects are antigenically distinct. Neutralization of the lytic activity of the enzyme by antisera reveals an antigenic relationship between the lysozymes of both insects.

The role of carboxy-groups in the catalytic activity of lysozyme from a fungus (*Chalaropsis* species) has been investigated with a carbodi-imide–nucleophile procedure.[370] A search for a suitable competitive inhibitor to protect the active site of the enzyme during modification led to the examination of the oligosaccharides of chitin and chitosan. In contrast to hen egg white lysozyme, chitin oligosaccharides were neither substrates nor inhibitors of the fungal lysozyme. All twelve carboxy-groups in the enzyme, theoretically capable of reaction with a nucleophile in the presence of a water-soluble carbodi-imide, reacted with either glycinamide or taurine in either the presence or absence of guanidinium chloride. The operable carboxy-groups were recognized as L-aspartic acid-6 and L-glutamic acid-33. Since there are no sequence homologies between the fungal lysozyme and hen lysozyme, which has L-aspartic acid-52 and L-glutamic acid-35 as active site amino-acids, it is clear that the two amino-acid residues are utilized for the same catalytic function in two totally dissimilar proteins. C.d. spectroscopy has been used to investigate the secondary structure of the *Chalaropsis* species enzyme and a comparison was made with hen egg white lysozyme: α-helix 21 and 22%, β-pleated sheet 63 and 18%, random coil 16 and 60%, respectively.[371] The spectra were completely different from one another, and the fungal lysozyme has one of the highest contents of β-structure thus far reported for globular proteins. The enzyme was stable over the pH range 2.70–7.03 over which there was no c.d. spectral change, but above pH 8.0 the spectrum changed significantly and resembled that obtained by guanidinium chloride-induced denaturation.

Preliminary crystallographic data for lysozyme produced by *Streptomyces erythraeus* shows that crystals of the enzyme are orthorhombic, space group $P2_12_12_1$ with unit cell dimensions: $a = 50.01$ Å, $b = 61.98$ Å, and $c = 67.85$ Å; and one molecule (mol. wt. approx. 1.85×10^4) per asymmetric unit.[372]

The autolytic enzyme lysozyme, ('*endo*-β-1,4-*N*-acetylmuramoylhydrolase') of *Streptococcus faecium* was released in a soluble form from insoluble cell wall–autolytic enzyme complexes by treatment with dilute NaOH at 0 °C.[373] Treatment of cell wall–enzyme complexes, obtained from either exponential- or stationary-phase cells, with 0.008 to 0.01 N NaOH gave maximum yields of autolytic enzyme activity. At a fixed concentration of NaOH, the yield of autolysin increased with increasing wall densities and was accompanied by the release of methylpentose and phosphorus in amounts proportional to the autolysin. Since extraction of wall–enzyme complexes with 4.5 M LiCl at 0 °C also removed methylpentose and phosphorus, release of enzyme with NaOH did not appear to result from hydrolysis of covalent linkages. The autolytic enzyme activity released from intact cells, or cell walls, was predominantly in the latent

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[372] R. Sarma, M. Kakudo, S. Hara, and T. Ikenaka, *J. Mol. Biol.*, 1979, **131**, 409.
[373] J. B. Cornett, C. A. Johnson, and G. D. Shockman, *J. Bacteriol.*, 1979, **138**, 699.

(proteinase activable) form which could be activated by trypsin or a proteinase present in commercial bovine plasma albumin.

45 D-Mannanases (Miscellaneous)

β-D-Mannanase (β-D-mannosidases) purified from the germinated seeds of *Trifolium repens* by a procedure that included chromatography on hydroxyapatite, gel filtration on acrylamide/agarose (Ultragel® 5/4) and preparative polyacrylamide gel electrophoresis.[185] The final purification has been completely resolved into two β-D-mannanases (I and II) with distinct specificities (see p. 468).

Proteins of a crude enzyme preparation obtained from the cultivation medium of the basidiomycete *Phellinus abietis* were separated by gel filtration and ion-exchange chromatography.[189] The preparation contained a minimum of three enzymes capable of splitting α-D-mannosidic bonds: α-D-mannosidase, *exo*-D-mannanase, and *endo*-D-mannanase, which were separated. Some properties of the D-mannanase complex of the crude enzyme preparation were examined. The D-mannanase complex exhibited two pH optima, its temperature optimum being at 45 °C.

β-D-Mannanase activities in the commercial enzyme preparations Driselase and cellulase, in culture populations of *Bacillus subtilis*, in commercial snail gut (*Helix pomatia*) preparations and in germinated seeds of lucerne, *Leucaena leucocephala* and honey locust, have been purified by substrate affinity chromatography on D-gluco-D-mannan-AH-Sepharose®.[374] On isoelectric focusing, multiple protein bands were found, all of which had β-D-mannanase activity. Each preparation appeared as a single major band on sodium dodecyl sulphate–polyacrylamide gel electrophoresis. The enzymes varied in their final specific activities, K_m values, optimal pH, isoelectric points, and pH and temperature stabilities, but had similar molecular weights. The enzymes have different abilities to hydrolyse D-galacto-D-mannans which are highly substituted with D-galactose. The preparations Driselase and Cellulase contain β-D-mannanases which can attack highly substituted D-galacto-D-mannans at points of single unsubstituted D-mannosyl residues if the D-galactose residues in the vicinity of the bond to be hydrolysed are all on one side only of the main chain.

Eight oligosaccharides were isolated from an enzymatic hydrolysate of cell walls of *Rhodotorula glutinis* with red yeast cell wall lytic enzyme of *Penicillium lilacinum*.[375] Two of these oligosaccharides were composed of D-mannose only and the others were composed of D-mannose and D-glucose. Linkage type between β-D-mannose residues in the oligosaccharides was mainly (1 → 4)-. All of the linkages between β-D-glucose and β-D-mannose residues were (1 → 6)-. The red yeast cell wall lytic enzyme hydrolysed (1 → 3)-β-D-mannoside bonds. From these results, it might be concluded that the lytic enzyme from *P. lilacinum* was a D-gluco-D-mannanase.

The phytopathogen *Xanthomonas campestris* when grown on several polysaccharide substrates produced β-D-mannanases (see p. 496).[290]

[374] B. V. McCleary, *Phytochemistry*, 1979, **18**, 757.
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46 Oligo-1,6-D-Glucosidases

The concurrent action of the 'sucrase' and 'isomaltase' *i.e.* sucrose α-D-glucohydrolase and oligo-1,6-D-glucosidase, active-sites of the hybrid sucrase–isomaltase from rat intestine on an α-limit dextrin was studied by use of 6^3-maltotriosylmaltotriose isolated from controlled action of pullulanase on pullulan.[376] Hydrolysis of the α-dextrin hexasaccharide occurred by sequential removal of D-glucose residues from its non-reducing end. The initial pentasaccharide product was shown to be 6^3-maltosylmaltotriose since reaction of its sodium borotritide derivative with pullulanase product maltose and [^{3}H]maltotriitol. Further hydrolysis by sucrase–isomaltase yielded 6^3-glucosylmaltotriose → maltotriose → maltose → D-glucose. The free isomaltase monomer was capable of hydrolysing the initial substrate and all intermediate products; it displayed maximal specificity for removal of the (1 → 6)-linked α-D-glucose stub from 6^3-glucosylmaltotriose but little specificity for maltose. The free sucrase monomer was incapable of hydrolysing the (1 → 6)-linked α-D-glucose stub but possessed appreciable activity against all (1 → 4) α-D-linkages and was particularly active against maltotriose and maltose. Based on calculation of the relative contribution of free sucrase and free isomaltase to the hydrolytic rate constants (K_{cat}) for the parent and intermediate substrates, either active site can initiate α-dextrin hydrolysis by removing the non-reducing (1 → 4)-linked α-D-glucosyl residue, but the isomaltase site is essential for cleavage of the (1 → 6)-linked α-D-glucose stub. The sucrase site is primarily responsible for the final cleavage of the released malto-oligosaccharides to D-glucose. Instead of acting synergistically, the two α-D-glucosidase sites appear to cleave α-limit dextrins by virtue of their complementary specificity.

Oligo-1,6-D-glucosidase–sucrose α-D-glucohydrolase ('isomaltase–sucrase') is an intestinal membrane enzyme consisting of two active moieties each with its hydrolytic site available for nutrient digestion at the luminal-cell interface (see p. 451).[129]

The enzyme complex oligo-1,6-D-glucosidase–sucrose α-D-glucohydrolase ('sucrase–isomaltase'), an intrinsic dimeric glycoprotein (mol. wt. 2.2×10^5) has been shown to be located in the AB intestinal brush border membrane by a hydrophobic segment, amounting to less than 10% of the total protein mass, which is located near the *N*-terminus of the oligo-1,6-D-glucosidase subunit.[377] Papain cleaved exclusively the polypeptide chain of the isomaltase subunit and did not modify the sucrase subunit. As the cleavage by papain at the *N*-terminal region of the oligo-1,6-D-glucosidase resulted in irreversible solubilization of oligo-1,6-D-glucosidase–sucrose α-D-glucohydrolase, it was concluded that the *N*-terminal polypeptide split off must be responsible for most, if not all, of the interactions anchoring the enzyme complex to the membrane, and that such interactions are partly dependent upon the hydrophobic character of the polypeptide.

[376] G. M. Gray, B. C. Lally, and K. A. Conklin, *J. Biol. Chem.*, 1979, **254**, 6038.
[377] J. Brunner. H. Hauser, H. Braun, K. J. Wilson, H. Wacher, B. O'Neill, and G. Semenza, *J. Biol. Chem.*, 1979, **254**, 1821.

Oligo-1,6-D-glucosidase together with α-D-glucosidase has been shown to be produced by *Bacillus brevis* in the presence of maltose, maltotriose, *etc.* (see p. 457).[146]

A 4-nitrophenyl α-D-glucopyranoside-hydrolysing α-D-glucosidase of a thermophile, *Bacillus thermoglucosidius*, has been purified to an electrophoretically homogeneous state (see p. 458).[147]

47 Pectate, Pectin, and Poly-D-Galacturonate Lyases

The stability of pectate and pectin depolymerizing enzymes produced by *Mucor puriformis, Rhizopus sexualis, R. stolonifer, Botrytis cinerea, Aureobasidium pullulans, Trichosporon pullulans,* and *Cryptococcus albidus* var. *albidus* in sulphite liquor has been studied in relation to the breakdown of sulphited strawberries.[378] Marked breakdown of fruit occurred only when pectolytic activity could be detected in the liquor for more than two weeks using a viscometric assay. Of the fungi tested, *Rhizopus* species produced enzymes that were the most stable in sulphite liquor. For each of the *Mucor* and *Rhizopus* species tested, the stability of poly-D-galacturonases in sulphite liquor was very similar for extracts of infected fruit and culture filtrates. It was suggested that sulphite labile (= acid labile) and sulphite stable (= acid stable) forms of the poly-D-galacturonases are present.

Bacillus species RK9 has been isolated from soil and was shown to produce a constitutive poly-D-galacturonate lyase. Production of the enzyme required the presence of complex nitrogen (peptone and yeast extract). Highest activity was obtained with an initial pH of 9.7.[379] The organism was alkalophilic, no growth occurring below pH 7.5. The enzyme was purified by salt precipitation and diethylaminoethyl-cellulose ion-exchange chromatography. Calcium alone, or divalent cations, activated the enzyme (pH optimum 10.0) 2.9-fold. Complete inhibition of enzyme activity was effected by H_4edta. Hydrolysis of substrate occurred in a random fashion and the enzyme was 50% more active towards acid-soluble pectic acid than towards sodium polypectate.

A pectinolytic bacterium isolated from a mixed microbial population by means of a chemostat enrichment procedure was identified as *Erwinia carotoyora*.[380] It grew only on highly methylated pectin and produced a pectin lysase which released unsaturated monomer and dimer from 71% esterified citrus pectin. The pectin lyase was inducible only by pectins having a high methyl ester content.

Various enterobacteria have been shown to exhibit several patterns of biosynthesis and excretion of poly-D-galacturonate lyase.[381] In a strain of *Erwinia chrysanthemi*, poly-D-galacturonate lyase is an extracellular enzyme and is almost totally excreted. In strains of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* poly-D-galacturonate lyase is a periplasmic and cytoplasmic enzyme.

[378] J. E. Harris and C. Dennis, *J. Sci. Food Agric.*, 1979, **30**, 704.
[379] C. T. Kelly and W. M. Fogarty, *Can. J. Microbiol.*, 1978, **24**, 1164.
[380] M. L. Almengor-Hecht and A. T. Bull, *Arch. Mikrobiol.*, 1978, **119**, 163.
[381] A. K. Chatterjee, G. E. Buchanan, M. K. Behrens, and M. P. Starr, *Can. J. Microbiol.*, 1979, **25**, 94.

In a strain of *Erwinia carotovora* poly-D-galacturonate lyase activity is extracellular, periplasmic, and cytoplasmic. In an 'oxytocum' strain of *Klebsiella pneumoniae* poly-D-galacturonate lyase is entirely cytoplasmic: none is found in the periplasm and almost none in culture supernatants. Cells (but not supernatants, because poly-D-galacturonate lyase is not excreted) of *K. pneumoniae* show much higher levels of poly-D-galacturonate lyase activity when grown on poly-D-galacturonate than when grown on other carbon sources. A clinical strain of *Y. enterocolitica* synthesizes high levels of poly-D-galacturonate lyase activity (almost all cell-bound) when grown in the presence of D-gluconate, glycerol, or poly-D-galacturonate, and considerably less activity when grown on D-glucose. A clinical strain of *Y. pseudotuberculosis* synthesizes barely detectable quantities of cell-bound poly-D-galacturonate lyase regardless of the carbon source. The phytopathogenic *Erwinia* strains synthesize and extrete high levels of poly-D-galacturonate lyase activity when grown on poly-D-galacturonate, D-galacturonate, D-gluconate, or glycerol. Growth on D-glucose represses poly-D-galacturonate lyase activity in the *Erwinia* strains: the extent of repression differs between the two strains. The different patterns of synthesis and excretion are discussed in the light of the possible catabolic and (or) cytolytic (plant tissue-macerating) functions and the related ecological significance of poly-D-galacturonate lyase in these enterobacteria.

The poly-D-galacturonate lyase associated with healthy and *Gloeosporium papayae* rot infected papaya (*Carieca papaya*) fruit, and secreted in papaya pulp medium by the pathogen has been investigated.[278]

Production of enzymes degrading plant cell walls has been studied using media containing cellobiose or ammonium ions as limiting nutrients.[273] Pectin lyase was primarily cell-associated during exponential growth in batch culture but accumulated in the supernatant during the stationary phase.

Enzyme preparations active against crystalline cellulose, marble-milled filter paper, carboxymethylcellulose, hemicellulose, and xylan were obtained from cultures of *Ruminococcus flavefaciens* (see p. 461).[161]

Large spiral organisms (*Treponemes*) isolated from the rumen of cattle produced and released into the external environment a complex of pectinolytic enzymes, consisting mainly of poly-D-galacturonate lyase most active at pH 8.0–9.0 and another enzyme acting at pH below 7.0, probably a poly-D-galacturonase.[382] The mixture of enzymes degraded poly-D-galacturonate to 'saturated and unsaturated mono-D-galacturonates' as the end products.

48 Poly-D-Galacturonases

The application of a newly designed flow-through viscometer for use in the automated determination of hydrolytic enzyme activities (as described on p. 475 for α-amylase) has been applied to the assay of pectinase.[210]

Both pectate-based culture filtrates and ripe strawberries, artificially inoculated with *Rhizopus stolonifer, R. sexualis, Mucor piriformis,* or *Aureobasidium pullulans*, contained *endo*-poly-D-galacturonase and pectinesterase.[383] Uninfected

[382] M. Wojciechowicz and A. Ziolecki, *Appl. Environ. Microbiol.*, 1979, **37**, 136.
[383] S. A. Archer, *J. Sci. Food Agric.*, 1979, **30**, 692.

strawberries contained traces of *exo*-poly-D-galacturonase together with pectinesterase. No other pectin-degrading enzymes were detected in either sound or infected fruit. Naturally infected strawberries from commercial sources contained pectinesterase and variable amounts of poly-D-galacturonase, the greatest amounts being associated with fruit carrying the heaviest microbial contamination. Disintegration of strawberries in sulphite liquor was associated with pectin degradation as judged by loss of liquor viscosity, reduction in pectin substance degree of polymerization, and the appearance in liquors of D-galacturonic acid. Poly-D-galacturonase was detected in liquors during the early stages of distintegration but activity subsequently disappeared due to instability of the enzyme at low pH. Addition of culture filtrates to sound sulphited fruit to give poly-D-galacturonase levels similar to those detected in some commercial liquors reproduced the fruit breakdown symptoms. Disintegration could be substantially reduced if poly-D-galacturonase was inhibited either by lowering the pH, or by addition of anionic surfactant. It was concluded that poly-D-galacturonase secreted by various decay fungi and present in fruit at the time of sulphitolysis is primarily responsible for breakdown. The possible involvement of non-enzymic synergists is discussed.

The stability of pectin-depolymerizing enzymes (poly-D-galacturonases) produced by *Mucor piriformis*, *Rhizopus sexualis*, *R. stolonifer*, *Botrytis cinerea*, *Aureobasidium pullulans*, *Trichosporon pullulans*, and *Cryptococcus albidus* var. *albidus* in sulphite liquor was studied in relation to the breakdown of sulphited strawberries. Marked breakdown of fruit occurred only when pectolytic activity could be detected in the liquor for more than two weeks using a viscometric assay. Of the fungi tested, *Rhizopus* species produced enzymes which were most stable in sulphite liquor. For each of the *Mucor* and *Rhizopus* species tested, the stability of poly-D-galacturonases in sulphite liquor was very similar for extracts of infected fruit and culture filtrates. It was suggested that sulphite labile (= acid labile) and sulphite stable (= acid stable) forms of the poly-D-galacturonases are present.

The poly-D-galacturonases of four fungi, *Rhizopus stolonifer, R. sexualis, Mucor piriformis* and *Aureobasidium pullulans*, suspected of involvement in the breakdown of sulphited strawberries, have been resolved into a number of distinct enzyme forms.[384] These isoenzymes differ from each other with respect to isoelectric points, molecular weights, resistance to extremes of pH and temperature, and susceptibility to inhibitors. The isoenzymes produced by any one organism in culture were identical to those produced in inoculated strawberries, although the relative proportions of the different forms was altered. Ten separate samples of microbially contaminated fruit from commercial sources, all of which subsequently suffered disintegration during storage in sulphite liquor, were analysed for poly-D-galacturonase activity. The main isoenzyme present in each batch had an isoelectric point close to 7.1. Comparisons between the properties of poly-D-galacturonases from field sources and those of known origin revealed that *R. sexualis* was the most likely source organism for nine of the ten enzymes, *R. stolinifer* probably being responsible for the tenth.

[384] S. A. Archer and A. H. Fielding, *J. Sci. Food Agric.*, 1979, **30**, 711.

The poly-D-galacturonase activity excreted by an agressive strain of *Colletrichum lagenarium* consists of two proteins which differ in their molecular weights (1.2×10^5 and 6×10^4) and their mode of hydrolysis of poly-D-galacturonic acid.[385]

The poly-D-galacturonase associated with healthy and *Gloeosporium papayae* rot infected papaya (*Carica papaya*) fruit, and secreted in papaya pulp medium by the pathogen has been investigated.[278]

The filter paper disintegrating activity (FD) and the plant tissue macerating activity (MA) in the enzyme preparation (Driselase) produced by *Irpex lacteus* have been studied,[280] and the use of the enzyme in the modification of animal feeding stuffs was investigated (see p. 492).

Seven of thirty yeast stock cultures, covering nine genera, and thirteen of thirty-nine yeasts isolated from groups gave positive reactions when screened for pectinolytic activity on pectin gel plates.[386] The extracellular pectinolytic activity from *Saccharomyces fragilis* Y49 was partially constitutive and was repressed by high oxygen tensions.

In a study of macerating activity of *endo*-poly-D-galacturonase produced by *Saccharomyces fragilis*, it was found that pulping properties and strength characteristics of paper therefrom, in early stage of defibration, were indirectly related to the activity of the enzyme.[387]

Large spiral organisms (*Treponemes*) isolated from the rumen of cattle produced and released into the external environment a complex of pectinolytic enzymes, consisting mainly of poly-D-galacturonate lyase, most active at pH 8.0–9.0, and another enzyme acting at pHs below 7.0, probably a poly-D-galacturonase.[382] The mixture of enzymes degraded poly-D-galacturonate to saturated and unsaturated 'mono-D-galacturonates' as the end products.

The rate of hydrolysis of oligomers by the (*endo*-)poly-D-galacturonase of yeast was in the order: heptamer > hexamer > pentamer > tetramer; this suggested that the active site accommodates at least seven units.[388] Since the heptamer disappeared concurrently with the bulk of larger oligomers, the maximum number of units appeared to be seven. The release of labelled (unsaturated, or ^3H labelled and reduced) end units from larger substrate was interpreted to indicate that the enzyme interacts with three saccharide units toward the reducing end from the bond to be broken, and with four units toward the non-reducing end. The relative affinities for the enzyme of saccharide units in various positions are unequal, as indicated by the very low relative rate of monomer production from the hydrolysis of hexamer and pentamer, and the apparently unequal probability of two other modes of hexamer hydrolysis [(tetramer + dimer) = 2.5 (trimer + trimer)].

[385] J.-P. Barthe and A.-M. Rème-Delga, *C. R. Hebd. Seances Acad. Sci., Ser. D.*, 1979, **288**, 63.
[386] M. P. Wimborne and P. A. D. Rickard, *Biotechnol. Bioeng.*, 1978, **20**, 231.
[387] Y. Kobayashi and R. Matsuo, *Agric. Biol. Chem.*, 1979, **43**, 1369.
[388] J. M. McClendon, *Phytochemistry*, 1979, **18**, 765.

49 *exo*-Poly-D-Galacturonases

Both pectate-based culture filtrates and ripe strawberries, artificially inoculated with *Rhizopus stolonifer, R. sexualis, Mucor piriformis* or *Aureobasidium pullulans*, contained *endo*-poly-D-galacturonase and pectinesterase (see p. 524).[383]

50 *exo*-Poly-D-Galacturonate Lyases

A pectate lyase has been purified from the culture filtrate of *Streptomyces nitrosporeus* by ion-exchange chromatography and gel filtration. The purified enzyme was demonstrated to be homogeneous by disc electrophoresis.[389] The optimum pH for the activity was 9.3–9.5 and the enzyme required calcium ions for maximum activity. The initial reaction rate was higher on partially esterified pectin than on poly-D-galacturonic acid. The enzyme was more specific for tetra-, penta-, and hexa-D-galacturonic acids than for poly-D-galacturonic acid. But di- and tri-D-galacturonic acids were not good substrates for the enzyme. The K_m values of the enzyme for tri-, tetra-, penta-, hexa-D-galacturonic acids and acid soluble poly-D-galacturonic acid at 1.0 mM calcium ion concentration were 1.5, 0.19, 0.23, 0.38, and 0.051% respectively.

When poly-D-galacturonic acid was degraded by the purified pectate lyase of *Streptomyces nitrosporeus*, unsaturated tri-D-galacturonic acid was formed as the main end-reaction product.[390] Paper chromatographic analysis of reaction products showed that the enzyme cleaved poly-D-galacturonate by a terminal mechanism. The attack sites of oligo-D-galacturonates by the enzyme were only third bonds from the reducing ends of the molecule. Reduced poly- and penta-D-galacturonates with sodium borohydride were resistant to the action of the enzyme. From these results it has been shown that the pectate lyase of *S. nitrosporeus* is a new type *exo*-poly-galacturonate lyase removing terminally units of unsaturated tri-D-galacturonic acid from the reducing ends of poly-D-galacturonate chains.

51 Pullulanases

A debranching enzyme has been purified about 100-fold over the crude enzyme solution from non-glutinous rice seeds at the milky stage by using ion-exchange chromatography and gel filtration.[391] This disc-electrophoretically homogeneous enzyme showed pullulanase activity (pH optimum 5.6). The enzyme debranched the $(1 \rightarrow 6)$-α D-glucosidic linkages in pullulan or an α-amylolysis product from starch most favourably, several β-limit dextrins from starch or from amylopectin rapidly, and phytoglycogen β-limit dextrin and amylopectin moderately, while it was unable to debranch phytoglycogen. These substrate specificities were similar to those of the so-called 'limit dextrinases' already reported with respect to several plant sources.

[389] J. Yamada and M. Izawa, *Agric. Biol. Chem.*, 1979, **43**, 37.
[390] H. Hustedt, K. H. Kroner, W. Stach, and M. R. Kula, *Biotechnol. Bioeng.*, 1978, **20**, 1989.
[391] M. Sato and A. Kaji, *Agric. Biol. Chem.*, 1977, **41**, 2193.

In a procedure described for the simultaneous large-scale isolation of pullulanase and (1→4)-α-D-glucan phosphorylase from *Klebsiella pneumoniae*, the pullulanase is solubilized from the cell wall by cholate treatment; cells and cell debris are removed by partition in a poly(ethyleneglycol)–dextran two-phase system and from the upper phase of this system the pullulanase is isolated by ultrafiltration and precipitation with *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide to 80% purity with 70% yield and in an α-amylase-free state.[392]

52 Sucrose α-D-Glucohydrolases

Studies on the enzyme complex oligo-1,6-D-glucosidase–sucrose α-D-glucohydrolase ('sucrase–isomaltase') of the AB intestinal brush border membrane have shown that a hydrophobic section of the oligo-1,6-D-glucosidase is responsible for binding of the complex to the membrane (see p. 522).[377]

Oligo-1,6-D-glucosidase–sucrose α-D-glucohydrolase ('sucrase–isomaltase') is an intestinal membrane enzyme consisting of two active moieties each with its hydrolytic site available for nutrient digestion at the luminal-cell interface.[129] At least 90% of the hydrolytic activity can be localized to the brush border membrane and the remainder in the cytoplasm has been considered to originate from brush border contamination (see p. 451).

The concurrent action of sucrose α-D-glucohydrolase and oligo-1,6-D-glucosidase active sites of the hybrid oligo-1,6-D-glucosidase–sucrose α-D-glucohydrolase ('sucrose–isomaltase') from rat intestine on an α-limit dextrin was studied by use of 6^3-maltotriosylmaltotriose isolated from the products of controlled action of pullulanase on pullulan (see p. 522).[376]

53 αα- and ββ-Trehalases

A continuous coupled polarographic assay, which couples αα-trehalase hydrolysis to oxygen consumption using D-glucose oxidase and catalase as ancillary enzymes has been developed for the measurement of αα-trehalase activity.[393] With this procedure, oxygen consumption was a linear function of time and the coupled reaction rate was directly proportional to the amount of protein assayed. The validity of this assay was confirmed by comparative studies with a discontinuous colorimetric assay for the quantitation of D-glucose. In addition, the applicability of this assay was appraised by determining the K_m of the enzyme for αα-trehalase (polarographic assay 1.3 mM, discontinuous colorimetric assay 1.2 mM).

αα-Trehalase from cryptobiotic brine shrimp (*Artemia salina*) embryos has been purified and characterized.[394] Most of the enzyme (mol. wt. 7.5×10^4, pI 6.2, pH optimum 5.6, K_m 4.3×10^{-3} M for αα-trehalose) was present in an insoluble form and could be solubilized by deoxycholate treatment at high

[392] M. Sato and A. Kaji, *Agric. Biol. Chem.*, 1977, **41**, 2199.
[393] K. A. Killick, *Anal. Biochem.*, 1979, **94**, 360.
[394] P. Ballario, M. Bergami, M. G. Cacace, F. Scala, and L. Silvestri, *Comp. Biochem. Physiol.*, 1978, **B61**, 265.

ionic strength and with sonication. The enzyme was highly specific for αα-trehalase, but lactose and cellobiose were hydrolysed by it to a limited extent.

The appearance of reducing power during incubation of silk gland homogenates of the silk worm, *Bombyx mori*, was shown to be due to the same process as observed with an enzyme–substrate system.[395] The silk gland cells play the role of an enzyme (αα-trehalase) and the liquid silk serves as a substrate. The appearance of reducing power from silk gland homogenates was due not to the specific physiological properties of the liquid silk, but to those of the silk gland cells of the silk glands during metamorphosis of the silkworm. A material with reducing power appearing during incubation of the silk gland homogenates was identified as D-glucose.

Piricularia oryzae, the pathogenic fungus of rice blast disease, has been shown to produce a glucoamylase which hydrolysed *inter alia* αα-trehalase and ββ-trehalase (see p. 509).[330]

A simple adsorption and elution of the trehalase of *Trichoderma reesei* on bentonite increased the specific activity 70–80 times, with a recovery of 90%.[396] This αα-trehalase had an optimum pH of 4.4, a pI of 5.7, a K_m of 3.1×10^{-3} M, and a specific activity of 50 μmol mg^{-1} min^{-1}.

54 *endo*-(1 → 4)-β-D-Xylanases

The enzymatic degradation of (1 → 4)-β-D-xylan single crystals with β-D-xylanases has been investigated by electron microscopy and electron diffraction.[397] The enzyme attack takes place at the edge of the crystals and progresses towards their centres. This is consistent with an *endo*-enzyme mechanism, where the enzyme interacts essentially with the accessible D-xylan chains located at the crystal periphery.

Some properties of purified *endo*-(1→4)-β-D-xylanase from the ligniperdous fungus *Trametes hirsuta* have been investigated.[398] The enzyme was stable between pH 4.0 and 8.0 with optimum activity at pH 5.0–5.5. The temperature optimum was 50 °C and the enzyme was stable for up to 30 min at 45 °C; however, it was denatured at higher temperatures. The K_m for 4-*O*-methyl-D-glucurono-D-xylan was 6.36×10^{-3} equivalents of D-xylose per litre, the activation energy was 28 kJ mol^{-1}. The enzyme (mol. wt. $2.2–2.4 \times 10^4$ by gel chromatography) was activated by Ca^{2+} and inhibited by Ag^+ and Hg^{2+}. On the basis of the effects of 2-hydroxy-5-nitrobenzyl bromide, *N*-bromosuccinimide, and *N*-acetylimidazole it was assumed that L-tryptophan and possibly L-tyrosine residues influence the enzyme catalysts.

A good proportion of *Streptomyces* isolates from natural sources produced extracellular D-xylanase.[399] Nineteen isolates showing high activity were able to degrade completely or partially wheat bran in the growth medium. Chromato-

[395] S. Shimada, *Agric. Biol. Chem.*, 1979, **43**, 203.
[396] P. Vijayakumar, W. Ross, and E. T. Reese, *Can. J. Microbiol.*, 1978, **24**, 1280.
[397] H. Chanzy, J. Comtat, M. Dube, and R. H. Marchessault, *Biopolymers*, 1979, **18**, 2459.
[398] M. Kubačková, S. Karácsonyi, L. Bilisics, and R. Thoman, *Folia Microbiol.*, 1978, **23**, 202.
[399] H. K. Sreenath, R. Joseph, and V. S. Murthy, *Folia Microbiol.*, 1978, **23**, 299.

graphic analysis of commercial D-xylan degradation products suggested that the isolates produced either *endo-* or *exo*-(1 → 4)-β-D-xylanases or their mixtures. Mixed additions of culture fluids showed a highly synergistic effect, up to an increase by 200%. In a few cases antagonism was seen which, however, could be removed by dialysis of the culture fluid.

55 *exo*-(1 → 4)-β-D-Xylanases

Enzyme preparations active against crystalline cellulose, marble-milled filter paper, carboxymethylcellulose (CM-cellulose), hemicellulose, and D-xylan have been obtained from cultures of *Ruminococcus flavefaciens*.[161] 'Carboxymethyl cellulase' and D-xylanase activities were present in a high molecular weight complex (see p. 461).

A good proportion of *Streptomyces* isolates from natural sources produced extracellular D-xylanase.[399] Nineteen isolates showing high activity were able to degrade completely or partially wheat bran in the growth medium. Chromatographic analysis of commercial xylan degradation products suggested that the isolates produced either *endo-* or *exo*-(1 → 4)-β-D-xylanases or their mixtures. Mixed additions of culture fluids showed a highly synergistic effect, up to an increase by 200%. In a few cases antagonism was seen which, however, could be removed by dialysis of the culture fluid.

56 Xylanases (Miscellaneous)

Extra- and intracellular glycan hydrolases were isolated from *Aspergillus flavus* and partially characterized; both preparations exhibited β-D-galactanase activity (see p. 499).[302] Gel chromatography of the extracellular enzyme preparation revealed one protein fraction containing β-D-galactanase activity and a second one exhibiting mainly β-D-xylanase activity.

An *endo*-(1 → 4)-β-D-glucanase isolated and purified from the culture fluid of *Lenzites trabea* was also found to hydrolyse xylan.[314]

Production of enzymes degrading plant cell walls has been studied using media containing cellobiose or ammonium ions as limiting nutrients. Cellulase ('carboxymethylcellulase'), D-xylanase, and pectin lyase were primarily cell-associated during exponential growth in batch culture but accumulated in the supernatant during the stationary phase.[373] Activities of cellulase and D-xylanase were higher in cellobiose-limited than in NH_4^+-limited continuous cultures, were inversely related to the growth rate and became progressively more cell-associated as the growth rate increased. The proportion of fermentation products in cellobiose-limited continuous cultures was dependent on the growth rate and the calculated cell yields per mole of adenosine varied between 11.92 and 16.39. These results were discussed in relation to the growth and metabolism of *Ruminococcus flavefaciens in vivo*.

D-Xylanase from *Streptomyces* species E.86 has been purified by a procedure including ion-exchange chromatography, and gel filtration.[400] The purified

[400] I. Kusarabe, M. Kawaguchi, T. Yasui, and T. Kobayashi, *J. Agric. Chem. Soc. Jpn.*, 1977, **51**, 429.

enzyme (pH optimum 5.5–6.2, temperature optimum 55–60 °C) was homogeneous on both disc-electrophoretical and ultracentrifugal analyses. Heating the enzyme solution for 30 min at 55 °C did not influence the stability, but the same treatment at 70 °C resulted in the loss of 100% of original activity. The enzyme was stable in the range pH 4.5–10.5, but became inactivated at pH values such as pH 2.8 or 11.5. None of the metal ions accelerated the enzyme activity, and Cd^{2+}, Sn^{2+}, Pb^{2+}, and Cu^{2+} partially inhibited the reaction. The D-xylanase was not inactivated by H_2Na_2edta, iodoacetic acid, and 4-chloromercuribenzoate. When various D-xylo-oligosaccharides and their reduced derivatives were used as substrates, the higher oligomer was hydrolysed much faster than the lower. Activation energy on hydrolysis of D-xylotetraose and inactivation energy of the enzyme were 1.3×10^4 cal and 1.2×10^5 cal, respectively. The enzyme (pI 7.3) had a sedimentation coefficient of 4.1 S at 0.9% protein concentration, and the molecular weight was determined to be 4.05×10^4 from the Archibald method using ultracentrifugal analysis.

57 Carbohydrate Isomerases

D-Glucose Isomerases. – A description of the commercial use of commercial D-glucose isomerase (D-xylose isomerase), including immobilized forms of the enzyme in conversion of corn syrups to high D-fructose syrups, has been given.[16]

D-Glucose isomerase has been immobilized with retention of enzymic activity by water soluble carbodi-imide aided reaction with chitosan.[335] By reaction with/adsorption on to a range of new polyphenolic resins,[169] and as *Streptomyces phaeochromogenes* cells in fine-particle form using radiation-induced polymerization at low temperatures with previously salted out hydrophilic monomers such as 2-hydroxyethylacrylate and 2-hydroxyethylmethacrylate.[401]

58 Carbohydrate Oxidases

Cellobiose Oxidases. – A recently discovered oxidative enzyme of importance in *in vitro* cellulose degradation seems to be a cellobiose oxidase.[163] The contribution of this enzyme to the degradation has been discussed in a review of cellulose enzymolysis.

D-Galactose Oxidases. – D-Galactose oxidase oxidizes the primary hydroxy-group of D-galactose and terminal non-reducing D-galactose units of oligosaccharides, polysaccharides, glycoproteins, and glycolipids.[402] The enzyme is also capable of oxidizing other six-carbon compounds structurally related to D-galactose, including 2-acetamido-2-deoxy-D-galactose, D-talose, 2-deoxy-D-galactose, and 2-*O*-methyl- and 3-*O*-methyl-D-galactose. It has now been found that D-galactose oxidase is capable of oxidizing three- and four-carbon compounds, specifically D-glyceraldehyde, glycerol, and D-threitol. The enzyme oxidizes 1,5-anhydro-D-galactitol and methyl α-D-galactopyranoside but not

[401] M. Kumakura, M. Yoshida, and I. Kaetsu, *Appl. Environ. Microbiol.*, 1979, **37**, 310.
[402] J. H. Pazur, H. R. Knull, and G. E. Chevalier, *J. Carbohydr. Nucleosides, Nucleotides*, 1977, **4**, 129.

D-galactitol, D-lyxitol, ethyl β-D-galactofuranoside, D-lyxose, or D-threose. Two micro-techniques, a galactostix test-strip, and a chromatography-spray method, have been valuable for determining which compounds are oxidized by D-galactose oxidase. By use of the test-strip method it was possible to determine whether D-galactose and 2-acetamido-2-deoxy-D-galactose are terminal or internal residues in several types of carbohydrate and glycoprotein antigens. By use of the chromatography-spray technique it has been found that the D-galactose and 2-acetamido-2-deoxy-D-galactose residues of sugar nucleotides are oxidized by D-galactose oxidase, a finding at variance with an earlier report.

D-Glucose Oxidases. – Preliminary investigations have indicated that D-glucose 6-sulphate and D-glucose 1-sulphate are neither substrates nor inhibitors of D-glucose oxidase.[90]

D-Glucose oxidase has been immobilized with retention of enzymic activity by water soluble carbodi-imide-assisted reaction with chitosan,[335] by entrapment of *Actinoplanes missouriensis* whole cell D-glucose isomerase Maxazyme® in α-cellulose beads,[244] by glutaraldehyde linking with ovalbumin on to mycelium pellets of an *Aspergillus* species,[403] by reaction with periodate, diamines, and borohydride, followed by reaction of the product with amino-derivatives of agarose, by adsorption to immobilized concanavalin A followed by cross-linking with glutaraldehyde,[339] by attachment on to the interior surface of activated polyamide tubing by alkylation of the surface, attachment of a diamine spacer, reactivation of the now modified surface with bifunctional reagents, and coupling of the enzyme,[404] and by chelation of/reaction with hydrous titanium(IV) oxide.[405]

Pyranose Oxidases. – Submerged cultures of the basidiomycete *Oudemansiella mucida*, strain III, were shown to accumulate D-arabino-2-hexosulose.[406] The maximum yields during cultivations in shaker flasks or in a laboratory fermenter represented 20–50% conversion of substrate D-glucose. The accumulation is transient, the aldoketose being again utilized after D-glucose exhaustion. Its production was stimulated by fluoride ions. The enzyme responsible for the $C_{(2)}$-specific oxidation of D-glucose acts as an intracellular oxidase with a maximum activity in the exponential phase of growth and was considered to be a pyranose oxidase. D-*Arabino*-2-hexosylose was also detected in the cultivation medium of the wood-rotting fungi *Pleurotus ostreatus, Laetiporus sulphureus,* and *Phellinus abietis.*

59 Carbohydrate Transferases

Cyclomaltodextrin D-Glucanotransferases. – Intermolecular transglycosylation of cyclomaltodextrin glycosyltransferase from *Bacillus megaterium* has been exam-

[403] I. Karube, K.-I. Kirano, and S. Suzuki, *Biotechnol. Bioeng.*, 1977, **19**, 1233.
[404] M. Werner, R. J. Mohrbacher, C. J. Riendeau, E. Murador, and S. Cambiaghi, *Clin. Chem.*, 1979, **25**, 20.
[405] J. F. Kennedy and I. M. Kay, *J. Chem. Soc., Perkin Trans. 1*, 1976, 329.
[406] J. Volc, P. Sedmera, and V. Musilek, *Folia Microbiol.*, 1978, **23**, 292.

ined in a reaction system containing D-galactose and soluble starch.[407] The yield of transfer products to D-galactose were two to three per cent of those to D-glucose. Four kinds of transfer products to D-galactose as the acceptor were obtained and were identified as 1-*O*-α-D-glucosyl-D-galactose, 3-*O*-α-D-glucosyl-D-galactose, 2-*O*-α-D-glucosyl-D-galactose, and 4-*O*-α-D-glucosyl-D-galactose. The transglycosylation to D-galactose was shown to occur at various hydroxy-groups of the acceptor: at C-1, C-3, and C-2(C-4) hydroxy-group in the proportion of 26 : 10 : 1.

60 Glycopeptide Linkage Hydrolases

4-L-Aspartyl-β-D-glucosylamine Amidohydrolases. – A new type of glycopeptidase hydrolysing N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl) hydrogen L-asparaginate linkages has been partially purified from almond emulsion by gel filtration and ion-exchange chromatographies.[408] The enzyme degraded pineapple stem bromelain glycopeptide (16) to yield equimolar amounts of intact oligosaccharide, peptide (17) and ammonia. The K_m value for the stem bromelain glyocpeptide was 4 mM, and the optimum pH was 5.2. The enzyme was markedly inhibited by Cu^{2+}, Fe^{3+}, and Zn^{2+}. Thiol inhibitors and actinomycete protease inhibitors had no effect. The glycopeptides used as substrates were prepared from stem bromelain, ovalbumin, or ovotransferrin. The enzyme hydrolysed glycopeptides with 3–11 amino-acid residues, whereas it did not hydrolyse glycopeptides with 1–2 amino-acid residues. Furthermore, L-asparagine oligosaccharide was not inhibitory to the enzyme.

L-Asn-L-Asn-(D-Man_3, D-Xyl_1, L-Fuc_1, D-Glc*N*Ac_2)-L-Glu-L-Ser-L-Ser

(16)

L-Asn-L-Asp-L-Glu-L-Ser-L-Ser)

(17)

61 Sulphatases

2-Acetamido-2-deoxy-D-galactose 4-Sulphate Sulphatases. – Chondroitin sulphates, dermatan sulphate, heparan sulphate, heparin, keratan sulphate, and oligosaccharides derived therefrom have been used for the measurement of sulphatase activity of rat skin extracts.[409] Chromatographic fractionation of the extracts followed by specificity studies revealed the existence of five different sulphatases, specific for (a) the non-reducing 2-acetamido-2-deoxy-D-gluco-

[407] S. Kitahata, S. Okada, and A. Misaki, *Agric. Biol. Chem.*, 1979, **43**, 151.
[408] N. Takahashi and H. Nishibe, *J. Biochem. (Tokyo)*, 1978, **84**, 1467.

pyranosyl 6-sulphate end groups of heparan sulphate and keratan sulphate, (b) the non-reducing 2-acetamido-2-deoxy-D-galactopyranosyl 6-sulphate or D-galactopyranosyl 6-sulphate end groups of chondroitin 6-sulphate or keratan sulphate, respectively, (c) the non-reducing 2-acetamido-2-deoxy-D-galacto-pyranosyl 4-sulphate end groups of chondroitin 4-sulphate and dermatan sulphate, (d) certain suitably located *N*-sulphated 2-amino-2-deoxy-D-glucose units of heparin and heparan sulphate, or (e) certain suitably located L-iduronic acid sulphate units of heparan sulphate and dermatan sulphate. Two arylsulphatases, one of which was identical in its chromatographic behaviours with (c), were also demonstrated to be present. It was concluded that normal skin fibroblasts contain at least five specific sulphatases, and diminished activity of any one may result in a specific storage disease.

2-Acetamido-2-deoxy-D-galactose 6-Sulphate Sulphatases. — 2-Acetamido-2-deoxy-D-galactose 6-sulphate sulphatases have been purified about 2×10^4-fold from the soluble extract of human placenta with 2-acetamido-2-deoxy-D-galacto-pyranosyl 6-sulphate-D-glucopyranosyluronic acid-2-acetamido-2-deoxy-D-[1-^{3}H]galactitol 6-sulphate as substrate in the activity assay.[410] The enzyme appears to be a glycoprotein (mol. wt. 1.0×10^5 by gel filtration). On gel electrophoresis in the presence of sodium dodecyl sulphate the major protein band had a mol. wt. of 7.8×10^4. Variable charge heterogeneity was observed in several enzyme preparations. The purified enzyme released up to one sulphate molecule from the disulphated trisaccharide. It was active towards 2-acetamido-2-deoxy-D-galactose 6-sulphate and exhibited no measurable 2-acetamido-2-deoxy-D glucose 6-sulphate sulphatase or any other known lysosomal sulphatase activity. Hydrolysis of [1-^{3}H]galactitol 6-sulphate was achieved by incubation neither with a crude nor with a purified enzyme preparation. Chondroitin 6-sulphate and a keratan sulphate, as well as heparin and heparan sulphate, served as competitive inhibitors of the enzyme. Purified 2-acetamido-2-deoxy-D-galactose 6-sulphate sulphatase activity was optimal at pHs 4.9 and 4.4 when assayed in 0.02 M sodium acetate buffer and at pHs 4.2 and 5.2 in 0.1 M sodium acetate buffer. A single pH optimum at pH 4.8 was observed for the crude enzyme and for the purified enzyme after mild periodate treatment. The sulphatase activity was inhibited by a variety of anions and cations and activated by thiol-specific and thiol reagents.

2-Acetamido-2-deoxy-D-galactose 6-sulphate sulphatase activity has been identified in rat skin (see p. 533).[409]

A sulphatase, which liberates sulphate from UDP-2-acetamido-2-deoxy-D-galactose 6-sulphate (the nucleotide occurring in quail egg white at high concentration), has been isolated from quail oviduct.[411] The tissue also contained sulphatase activities for UDP-2-acetamido-2-deoxy-D-galactose 4-sulphate and nitrocatechol sulphate but these activities were removed from the 6-sulphatase fraction during purification. The UDP-2-acetamido-2-deoxy-D-galactose 6-

[409] H. Habuchi, M. Tsuji, Y. Nakanishi, and S. Suzuki, *J. Biol. Chem.*, 1979, **254**, 7570.
[410] J. Glössl, W. Truppe, and H. Kresse, *Biochem. J.*, 1979, **181**, 37.
[411] Y. Nakanishi, M. Tsuji, H. Habuchi, and S. Suzuki, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 863.

sulphate appears to be very closely related to a sulphatase activity for the non-reducing 2-acetamido-2-deoxy-D-galactose 6-sulphate end group in chondroitin sulphate, *i.e.* the two activities could not be separated from each other by various fractionation procedures and were affected in a parallel fashion by mild heating. The results, coupled with those of earlier studies on UDP-2-acetamido-2-deoxy-D-galactose 4-sulphate in hen oviduct, suggested that in avian oviducts a sulphation/desulphation system may exist wherein sulphated sugar nucleotides and sulphated glycosaminoglycans are involved as alternative or competitive substrates.

A 6-sulphatase specific for sugars of the D-*galacto* configuration has been purified from a crude extract of *Actinobacillus* species.[412] The preparation contained activity towards both 2-acetamido-2-deoxy-D-galactose 6-sulphate and D-galactose 6-sulphate (relative activity, 2.4:1). The enzyme also released inorganic sulphate from the non-reducing D-galactose 6-sulphate end group of a trisaccharide disulphate from keratan sulphate by degradation with *endo*-β-D-galactanase, 2-acetamido-2-deoxy-D-glucose 6-sulphate sulphatase and *exo*-β-D-2-acetamido-2-deoxyglucosidase. In addition, a tetrasaccharide trisulphate bearing the non-reducing 2-acetamido-2-deoxy-D-glucose 6-sulphate end group, also enzymatically prepared from ketatan sulphate, was degraded to give rise to inorganic sulphate, 2-acetamido-2-deoxy-D-glucose and D-galactose by the sequential action of this enzyme, 6-sulphate sulphatase, *exo*-β-D-2-acetamido-2-deoxyglucosidase and β-D-galactosidase.

2-Acetamido-2-deoxy-D-glucose 6-Sulphate Sulphatases. – 2-Acetamido-2-deoxy-D-glucose 6-sulphate sulphatase, which liberates sulphate from the 2-acetamido-2-deoxy-D-glucopyranosyl 6-sulphate group at the nonreducing terminus of an ^{3}H-labelled trisaccharide prepared from heparan sulphate, has been purified from human urine to homogeneity on polyacrylamide gel electrophoresis.[413] The enzyme appeared to be a glycoprotein (mol. wt. 9.7×10^4) displaying charge heterogeneity (pI 5.4–8.3) and was active against the trisaccharide, and D-glucose and 2-acetamido-2-deoxy-D-glucose 6-sulphates (K_m values 0.15, 7.7, and 1.5 mM, respectively). Although predominantly an *exo*-sulphatase, the enzyme catalysed the hydrolysis of sulphate from internal 2-acetamido-2-deoxy-D-glucose 6-sulphate residues at a low rate. The enzyme which was inhibited by aluminium, Hg^{2+}, and phosphate, sulphate, and cyanide ions, displayed highest activity in kidney and cultured fibroblasts, but was present in all human tissues tested.

2-Acetamido-2-deoxy-D-glucose 6-sulphate sulphatase activity has been recognized in rat skin (see pp. 533–534).[409]

Arylsulphatases. – A pool of acid hydrolases exists within the acellular lining material of the alveoli and distal airways of the lungs.[25] These extracellular hydrolases, obtained using pulmonary lavage procedures, appear to be of a selected variety insofar as some hydrolases (β-D-2-acetamido-2-deoxyglucosidase

[412] K. Nakazawa and K. Kagabe, *Biochim. Biophys. Acta*, 1978, **527**, 391.
[413] R. Basner, H. Kresse, and K. van Figura, *J. Biol. Chem.*, 1979, **254**, 1151.

and α-D-mannosidase) are highly active whereas others (β-D-glucuronidase and arylsulphatase) are barely detectable (see p. 421).

L-Tyrosine *O*-sulphate has been shown to be hydrolysed by pure human arylsulphatase A at 5% of the rate for nitrocatechol sulphate, but at a comparable rate to that for cerebroside sulphate.[414] The reaction (K_m value 0.35 mM for L-tyrosine *O*-sulphate) was optimal at pH 5.3–5.5 and displayed zero order kinetics with time and enzyme concentration.

The enzyme showed no stereospecificity and hydrolysed D-tyrosine *O*-sulphate with K_m and V_{max} values similar to those for the L-isomer. Arylsulphatase B was less than 5% as effective as arylsulphatase A in catalysing the hydrolysis of the tyrosine sulphates. The biological relevance of the D-tyrosine sulphatase activity of arylsulphatase A was considered to remain uncertain.

Arylsulphatase ('C') from sheep brain acetone powder was solubilized with the chaotropic agent, potassium thiocyanate.[415] Antichaotropes such as ammonium sulphate or sodium citrate significantly enhanced the activity of the solubilized enzyme indicating that hydrophobicity was an important factor influencing the enzyme activity. Dialysis or gel filtration of the solubilized enzyme resulted in a marked loss of activity. A dialysable activator could reconstitute the activity in the presence of the antichaotropes. The activator was purified partially and preliminary studies indicated it to be a low molecular weight peptide. Kinetic studies showed that elevation of enzyme activity by the antichaotropes and activator of arylsulphatase ('C') was due to an increase in V_{max} with a decrease in K_m.

Antibodies against homogeneous rabbit liver arylsulphatase A have been produced in a goat and the effects of these antibodies on the kinetic parameters of the enzyme have been studied.[416] The results indicate that the binding of antibody to the enzyme does not alter the enzyme active site, since K_m and K_i values are unaffected. However, a small reduction in the enzyme activity was observed as the result of a reduction of V_{max} in the enzyme–antibody complex. The binding of antibodies led to a change in the pH-rate profile, giving one broad pH optimum shifted toward higher pH value. The enzyme–antibody complex still showed the characteristic arylsulphatase A anomalous kinetics at pH 5.5, but the inactivation was significantly slower than for the native enzyme. As calculated from quantitative immunoprecipitation data, the native enzyme bound 5–7 molecules of immunoglobulin G. The number of IgG molecules which bound to the turnover-modified enzyme was reduced to 3–4. The loss of antigenic determinants from the turnover-modified enzyme indicated either that significant conformational changes occur during the turnover-induced modification, or that a covalent modification of residues present at the antigenic sites has occurred, or both.

Arylsulphatase activity has been demonstrated in rat skin (see pp. 533–535).[409]

Fairly high activities of arylsulphatase have been found in the sperm and mature testes of sea urchins (*Strogylocentrotus intermedius, S. nudus, Hemi-*

[414] A. L. Fluharty, R. L. Stevens, E. B. Goldstein, and H. Kihara, *Biochim. Biophys. Acta*, 1979, **566**, 321.
[415] S. Lakshimi and A. S. Balasubramanian, *Biochim. Biophys. Acta*, 1979, **567**, 184.
[416] J. Rybarska-Stylinska and R. L. van Etten, *Biochim. Biophys. Acta*, 1979, **570**, 107.

centrotus pulcherimus, and *Anthrocidaris crassispina*) whereas the activities in the ovaries and eggs of these animals are low.[417] Neither the sand dollar (*Clypeaster japonicus*) nor the starfishes (*Asterias amurensis* and *Asteria pectinfera*) have significant activities of the enzyme in their gonads and gametes. Most of the arylsulphatase in the sperm of *S. intermedius* lies in the seminal plasma, but that which is spermotazoa borne probably exists on the surface and in the acrosome. The ubiquitous distribution of sperm arylsulphatase in sea urchins on the contrary to its absence in starfish or sand dollar is discussed in connection with the penetration of sperm through egg investments.

D-Galacto-L-fucan Sulphatases. – In connection with the structural study of D-galacto-L-fucan (sulphated polysaccharide of *Undaria pinnatifida*) carbohydrate sulphatases existing in hepatopancreas of abalone (*Haliotis* species) were investigated.[78] In a crude mixture of enzymes prepared from hepatopancreas D-galacto-L-fucan sulphatase was detected. From the crude mixture a fraction was obtained by ion-exchange chromatography followed by gel filtration and showed four activities: D-galacto-L-fucanase, D-galacto-L-fucan sulphatase, arylsulphatase, and fucoidanase and was free from α-L-fucosidase activity.

D-Galactose 6-Sulphate Sulphatase. – D-Galactose 6-sulphate sulphatase activity has been found in rat skin.[409]

L-Iduronic Acid 2-Sulphate Sulphatases. – L-Iduronic acid sulphate sulphatase activity has been recognized in rat skin (see pp. 533–536).[409]

62 Miscellaneous Enzymes

Dextransucrases. – In a model experiment, glucoamylase was shown to inhibit α-D-glucan biosynthesis as catalysed by potato phosphorylase (p. 498).[297] Both glucoamylase (*ex Rhizopus niveus*) and *endo*-dextranase (*ex Chaetomium gracile*) inhibited dextran biosynthesis with dextransucrases of *Leuconostoc mesenteroides*. The inhibition was ascribed to competition between glucoamylase and dextransucrase for the D-glucosyl groups at the non-reducing end of dextran. The measurement of initial velocity suggested that the ping-pong Bi-Bi mechanism proposed for the levansucrase of *Bacillus subtilis* is also applicable to dextransucrase.

α-Lactalbumins. – The proposed similarity of conformation between bovine α-lactalbumin and hen egg-white lysozyme was tested by the comparison of the thermodynamic parameters obtained from the temperature dependence of denaturation.[418] For the denaturing reaction by guanidinium chloride, the value of ΔCp for α-lactalbumin is almost identical with that for lysozyme, which suggests that the amount of the hydrophobic side chains buried in the interior of the molecule is the same in the nature state; the value of ΔH^0 and ΔS^0 for α-lactalbumins are also close to those for lysozyme, and the small differences are

[417] T. Moriya and M. Hoshi, *Comp. Biochem. Physiol. B*, 1979, **64**, 293.
[418] K. Takase, R. Niki, and S. Arima, *Agric. Biol. Chem.*, 1976, **40**, 1273.

explicable by the proposed molecular model of α-lactalbumin, which implies that the somewhat large difference in G^0 observed previously between the two proteins does not originate from large conformational differences. These results support the premise of the conformational similarity between α-lactalbumin and lysozyme as represented by the molecular model. The heat-denatured state of α-lactalbumin was also characterized by the parameters and discussed.

Ovine mammary gland mRNAs were translated in a wheat-germ cell-free system in the presence of radioactive amino-acids.[354] Automated Edman degradation performed on α-lactalbumin isolated by immunoprecipitation from the mixture of radiolabelled lactoproteins showed the occurrence of a hydrophobic 19 residue long *N*-terminal extension. The pre-protein represents the primary translation product since the *N*-terminal methionyl residue was found to be donated by initiator Met-$tRNA_i^{Met}$. Comparison of the signal regions of ovine α-lactalbumin and hen egg white lysozyme were reported.

63 Levansucrases

Actinomyces viscosus produces both a soluble extracellular levansucrase and a cell wall-associated levansucrase.[419] The enzyme from cell walls has been solubilized by lysozyme digestion. The soluble extracellular and cell wall-associated forms of the enzyme were compared and appeared to be identical, based on molecular weight estimations, kinetic parameters, and reactions with antisera. The product of both forms of the enzyme was a high molecular weight, branched levan, as shown by its reactivity with myeloma proteins specific for $(2 \rightarrow 1)$ and for $(2 \rightarrow 6)$ linkages in β-D-fructosans. Although levansucrase remained tightly bound to the levan which it biosynthesized, the enzyme did not bind to exogeneously added levan.

64 Pectinesterases

Sodium pectate preparation, suspended in an alkaline mixture of ethanol and water, has been cross-linked with epichlorohydrin. By varying the amount of epichlorhydrin in the reaction mixture preparations which possessed different degrees of cross-linking were obtained and were used as chromatographic media for the separation and purification of two orange pectinesterase isoenzymes.[420] The binding of the isoenzymes was found to depend on the degrees of cross-linking of the pectate. On pectate with an intermediate degree of cross-linking (0.46) both isoenzymes were retained and subsequently eluted, with a sodium chloride gradient, as separated peaks with a seven to eight-fold increase in specific activity. Both pectinesterase isoenzymes saponified 93% esterified pectin with a high degree of cross-linking. It is thought that the binding of these enzymes to cross-linked pectate involves biospecific affinity.

Both pectate-based culture filtrates and ripe strawberries, artificially inoculated with *Rhizopus stolonifer, R. sexualis, Micor piriformis* or *Aureobasidium pullulans*, contained *endo*-poly-D-galacturonase together with pectinesterase.[383]

[419] M. J. Pabst, J. O. Cisar, and C. L. Trummel, *Biochem. Biophys. Acta*, 1979, **566**, 274.
[420] F. M. Rombouts, A. K. Wissenburg, and W. Pilnik, *J. Chromatogr.*, 1979, **168**, 151.

No other pectin-degrading enzymes were detected in either sound or infected fruit. Naturally infected strawberries from commercial sources contained pectinesterase and variable amounts of poly-D-galacturonase, the greatest amounts being associated with fruit carrying the heaviest microbial contamination. Disintegration of strawberries in sulphite liquor was associated with pectin degradation as judged by loss of liquor viscosity, reduction in pectin substance degree of polymerization and the appearance in liquors of D-galacturonic acid.

An 'acid' pectinesterase has been purified from the culture of *Corticium rolfsii* IFO 6146 by a combination of salting out, gel filtration, and ion-exchange chromatographies.[421] The purified enzyme was found by ultracentrifugal analysis to be homogeneous protein (mol. wt. 3.7×10^4 by gel filtration). The enzyme was stable over the pH range of 1.1–10.0 and retained 90% of its activity when stored at pH 1.1 at 5 °C for 72 hr. The maximum activity occurred at pH 2.5–4.5 at 45 °C. The enzyme was fairly active at a very low pH (1.1) or low temperature (10 °C). Hg^{2+} was a potent inhibitor. The enzyme was active not only on high molecular pectin methyl esters but also on low molecular esters. The high enzymatic activity at very low pH values and the unusual tolerance to hydrogen ions are characteristics of pectinesterase from *C. rolfsii*.

The pectinesterase associated with healthy and *Gloeosporium papayae* rot infected papaya (*Carcia papaya*) fruit, and secreted in papaya pulp medium by the pathogen has been investigated.[278]

Enzyme preparations active against crystalline cellulose, marble-milled filter paper, carboxymethylcellulose, hemicellulose, and D-xylan were obtained from cultures of *Ruminococcus flavefaciens*.[161] These preparations also contained swelling factor, pectin methylesterase, pectin lyase, and low levels of β-D-glucosidase and β-D-xylosidase. 'Carboxymethylcellulase' and D-xylanase activities were present in a high molecular weight complex, but substrate competition studies showed that different active sites were probably responsible for each activity. Analysis of products and viscosity changes during enzymic hydrolysis of carboxymethylcellulose and D-xylan indicated that the most active enzymes were of the *exo*-(1 → 4)-β-D-glycanase type. A variety of reducing sugars were released from cell walls of *Lolium perenne* (perennial ryegrass) by enzyme preparations.

Together with poly-D-galacturonase and poly-D-galacturonate lyase, pectinesterase has been found in cultures of large spiral organisms (*Treponemes*) from the serum of cattle.[382]

65 Sulphamidases

Sulphamidase active against certain suitably located *N*-sulphated 2-amino-2-deoxy-D-glucose units of heparin and heparan sulphate has been discovered in rat skin (see p. 533).[409]

[421] O. Yoshihara, T. Matsuo, and A. Kaji, *Agric. Biol. Chem.*, 1977, **41**, 2335.

Index of Enzymes Referred to in Chapter 6[a]

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
endo-β-D-2-Acetamido-2-deoxy galactanase	α-D-Galactoside D-galactosyl-*N*-acetamidodeoxy-galacto-hydrolase		473
2-Acetamido-2-deoxy-D-galactose 4-sulphate sulphatase			533
2-Acetamido-2-deoxy-D-galactose 6-sulphate sulphatase			534
2-Acetamido-2-deoxy-D-glucose 6-sulphate sulphatase			535
β-D-2-Acetamido-2-deoxy-galactosidase	2-Acetamido-2-deoxy-β-D-galactoside acetamidodeoxy-galactohydrolase	3.2.1.53	420
endo-β-D-2-Acetamido-2-deoxyglucanase	D-Mannosyl-glycoprotein-(1→4)-*N*-acetamidodeoxy-β-D-glycohydrolase (see also Chitinase, E.C. 3.2.1.14)	3.2.1.96	474
β-D-2-Acetamido-2-deoxy-glucosidase	2-Acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.30	420
β-D-2-Acetamido-2-deoxy-hexosidase	2-Acetamido-2-deoxy-β-D-hexoside acetamidodeoxy-hexohydrolase	3.2.1.52	474
Agarase	Agarose 3-glycanohydrolase	3.6.1.3	474
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Oligo-(1 → 6)-D-glucosidase	Dextrin 6-α-D-glucanohydrolase	3.2.1.10	522
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Pullulanase	Pullulan 6-glucanohydrolase	3.2.1.41	527
Sialidase[b]			469
Sucrose α-D-glucohydrolase	Sucrose α-D-glucohydrolase	3.2.1.48	528
Sulphamidase			539
Sulphatase			533
αα-Trehalase	αα-Trehalose glucohydrolase	3.2.1.28	528
ββ-Trehalase			528
endo-(1 → 4)-β-D-Xylanase	1,4-β-D-Xylan xylanohydrolase	3.2.1.8	529
exo-(1 → 4)-β-D-Xylanase	1,4-β-D-Xylan xylohydrolase	3.2.1.37	530
Xylanases (miscellaneous)			530
D-Xylose isomerase	D-Xylose ketol isomerase	5.3.1.5	531
β-D-Xylosidase		[3.2.1.37]	472

[a] See Introduction (Part II, Chapter 1, p. 233).

[b] Sialidase is a term generally used to include neuraminidase and unspecified acylneuraminyl hydrolases. Since there is little evidence for the existence of acylneuraminyl hydrolases that do not cleave 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonyl residues, use of the term neuraminidase is preferred.

7

Glycolipids and Gangliosides

BY I. M. MORRISON

1 Introduction

Glycolipid biosynthesis and glycolipid catabolism have been reviewed in separate chapters of a book on glycoconjugates.[1] Another book on cell-surface carbohydrate chemistry contains chapters on the role of the Golgi Apparatus in the biosynthesis of glycolipids, membrane glycolipids as receptors for glycoprotein hormones, bacterial toxins and interferon, and glycosphingolipids of bovine erythrocyte membranes.[2] The location and function of gangliosides as well as their biosynthesis and catabolism have been reviewed in a book on the neurochemistry of amino-sugars.[3] Another article on glycolipids has appeared in a historical volume of Comprehensive Biochemistry in which the unravelling of biosynthetic pathways is explained.[4]

2 Animal Gangliosides

Gangliosides from as little as one milligram of dry weight of brain tissue have been isolated by a simple, rapid method involving chloroform–methanol extraction and a single-step silicic acid column separation. The gangliosides were separated from the bulk of the non-ganglioside lipid and could be analysed by t.l.c.[5] A simplified procedure for the quantitative extraction of lipids from brain tissue involved a pre-extraction with dilute aqueous acetic acid. Using pig brain tissue as a model, complete extraction of lipid, including gangliosides, could be obtained with chloroform–methanol mixtures.[6] The ganglioside distribution in fat tissues of various animals has been compared by two-dimensional t.l.c. Of the tissues examined (human, rabbit, rat, mouse, chicken, pig, and frog), the ganglioside G_{M_3} was most abundant in all but the frog which was very different in containing three unusual monosialogangliosides. The gangliosides from human, rat, and chick tissue only contained *N*-acetylneuraminic acid residues, whereas the others contained both *N*-acetyl and *N*-glycolyl derivatives.[7]

[1] 'Glycoconjugates, Vol. 2, Mammalian Glycoproteins, Glycolipids, and Proteoglycans', ed. M. I. Horowitz and W. Pigman, Academic Press, New York, 1978.
[2] 'Cell Surface Carbohydrate Chemistry', ed. R. E. Harmon, Academic Press, New York, 1978.
[3] 'Neurochemistry of Aminosugars. Neurochemistry and Neuropathology of the Complex Carbohydrates', ed. E. G. Brunngraber, C. C. Thomas, Springfield, 1979.
[4] 'Comprehensive Biochemistry, Vol. 33, Pt. A, A History of Biochemistry, Pt. 5', M. Florkin, Elsevier, Amsterdam, 1979.
[5] C. C. Irwin and L. N. Irwin, *Anal. Biochem.*, 1979, **94**, 335.
[6] F. Phillips and O. S. Privett, *Lipids*, 1979, **14**, 590.
[7] M. Ohashi, *Lipids*, 1979, **14**, 52.

The orientation of gangliosides in bilayer vesicular membranes has been studied using liposomes containing increasing proportions of gangliosides (from bovine brain) and correspondingly decreasing proportions of egg yolk lecithin.[8] The results, using ^{1}H n.m.r. spectroscopy and viscosity measurements, indicated that the gangliosides were primarily or entirely localized in the outer layer of the vesicles.

The interactions among different gangliosides and chemically related glycosphingolipids and their behaviour in mixed monolayers with different phospholipids have been investigated.[9] The mixed monolayers of any of the different gangliosides showed an immiscible behaviour at high surface pressures, which was probably a consequence of both steric hindrance and electrostatic repulsion between their polar head groups. Di- and tri-sialogangliosides could be differentiated from neutral sphingolipids and monosialogangliosides on the basis of their interaction with phospholipids, which were correlated with the perpendicular electric field at the interface contributed by the carbohydrate residues. The possible significance of these observations was discussed in relation to the participation of gangliosides in the organization of membranes and to their capability of inducing membrane fusion.

The micellar properties of purified ganglioside (1) and of a mixed bovine ganglioside fraction have been studied by gel filtration, equilibrium dialysis, and centrifugation in sucrose gradients.[10] Since the dissociation of micelles in water is very slow, equilibrium was reached by association of monomers rather than dissociation of micelles. The mixed gangliosides showed two micellar sizes (10 and 4.5S), the slower sedimenting species being increasingly formed from the larger one with time. The critical micelle concentration of the mixed gangliosides was approximately 10^{-8} M by all methods.

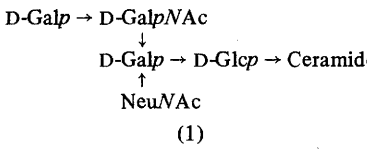
The rate of hydrolysis of the ganglioside G_{M_1}, tritiated in the ceramide moiety, by the action of the neuraminidase from *Clostridium perfringens*, was determined by measuring the radioactivity of the neutral glycolipid product.[11] The critical micelle concentration of the ganglioside was also determined using the formation of tri-iodide anion in aqueous iodine solution as an indicator as well as by ultra-centrifugation experiments. These determinations were required to identify the physical state of the substrate in the conditions used for the neuraminidase treatment. Both methods gave the same critical micelle concentration of 28–29 μM ganglioside. The reaction rate of the hydrolysis of G_{M_1} as a function of substrate concentration levelled off at 25–28 μM ganglioside. The velocity was decreased immediately above the critical micelle concentration and finally became constant at higher ganglioside concentrations. *N*-Acetylneuraminyl-lactosylceramide, lactosylceramide, and asialo-G_{M_1} ganglioside all had an inhibitory effect on the reaction, but *N*-acetylneuraminyl-lactose, lactose, D-glucose, D-galactose, and *N*-acetylneuraminic acid were not inhibitory. Factors

[8] N. E. Chupriyanova, V. I. Volkova, A. V. Viktorov, L. I. Barkusov, A. Y. Veinberg, and G. I. Samokhvalov, *Biofizika*, 1978, **23**, 817.

[9] B. Maggio, F. A. Cumar, and R. Caputto, *Biochem. J.*, 1978, **175**, 1113.

[10] S. Formisano, M. L. Johnson, G. Lee, S. M. Aloj, and H. Edelhoch, *Biochemistry*, 1979, **18**, 1119.

[11] H. Rauvala, *Eur. J. Biochem.*, 1979, **97**, 555.



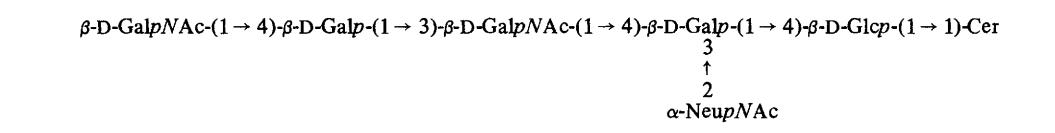
(1)

other than the saccharide structure must be important in such circumstances. The inhibition effected by the glycolipids may be due to an increase in the micellar state of the G_{M_1} ganglioside.

A specific interaction was observed between the ganglioside (1), which had been incorporated in glycerol monoleate planar bilayer membranes, and follitropin.[12] A five-fold increase in conductance was measured in the presence of (1) while neither di- or tri-sialogangliosides showed any effect. The increase was suppressed by the presence of an equimolar mixture of D-galactose, 2-acetamido-2-deoxy-D-galactose, and *N*-acetylneuraminic acid. The results favour the hypothesis that gangliosides may contribute to the formation of functional glycoprotein receptors.

Polysialogangliosides, but not monosialogangliosides or neutral glycosphingolipids, induced the release of dopamine from synaptosomes in the presence of Ca^{2+} ions.[13] The effect was discussed in relation to the ability of polysialogangliosides to induce membrane fusion in chicken erythrocytes and to their behaviour in lipid monolayers. It was suggested that characteristic interactions with phosphatidylcholine involving a decrease in the surface potential might participate in the polysialoganglioside-induced release of the neurotransmitter.

The interaction of cholera toxin with G_{M_1} vesicles is strong enough to permit perturbation in the lipid membrane, but it does not affect the dissociation of the active α-chain from the vesicle-bound toxin.[14]



(2)

A novel ganglioside, neuraminosylpentahexaosylceramide, (2) has been isolated from human brain.[15] The ganglioside was resistant to attack by the neuraminidase from *Vibrio cholerae*, but its structure was confirmed by treatment with β-D-2-acetamido-2-deoxyhexosidase, and by the results of methylation, chromium(VI) oxide oxidation, and partial acid hydrolysis analyses. Hydrolysis with formic acid suggested that the glycosidic bond of the non-reducing terminal 2-acetamido-2-deoxy-D-galactose residue was more labile than that of the penultimate D-galactose residue. The major lipids in brain tissue of patients with four

[12] M. Deelers, P. Chatelain, A. Poss, and J. M. Ruysschaert, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 1102.
[13] F. A. Cumar, B. Maggio, and R. Caputto, *Biochem. Biophys. Res. Commun.*, 1978, **84**, 65.
[14] N. Tomasi, C. Ausiello, A. Battistini, and G. D'Agnole, *FEBS Lett.*, 1979, **106**, 309.
[15] M. Iwamori and Y. Nagai, *J. Biochem. (Tokyo)*, 1978, **84**, 1601.

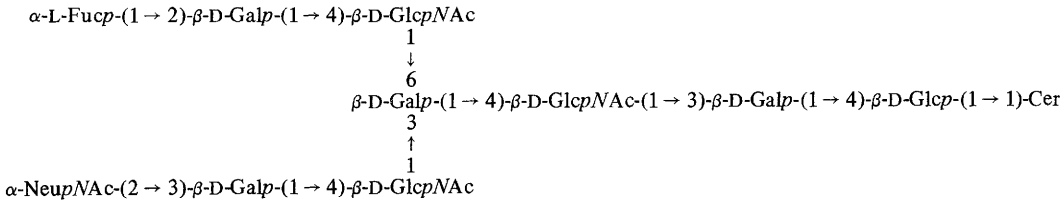
types of genetic hyperglycosaminoglycanuria (mucopolysaccharidosis), I (Hurler), II (Hunter), IIIA (Sanfilippo), and V (Scheie) have been determined.[16] The gangliosides G_{M_2} and G_{M_3}, which are normally minor components, had elevated levels in all patients except that with Type V. Ceramide dihexoside levels were also raised in Type I, II, and III patients. The accumulation of sphingolipids could be responsible for the brain damage and mental retardation of patients with Types I, II, and IIIA. The gangliosides G_{M_2} and G_{A_2}, from the brain of a patient with Sandhoff's disease, were radiolabelled in the non-reducing terminal 2-acetamido-2-deoxy-D-galactosyl group.[17] This residue was oxidized with D-galactose oxidase and subsequently reduced with sodium borotritide. The addition of catalase and peroxidase was recommended at the tritiation step to give products of higher specific activity.

To determine whether alterations of the profiles of glycosphingolipids occur consistently in human tumours, the neutral glycosphingolipids and gangliosides of lung tumours and adjacent lung tissue were analysed.[18] D-Glucosyl-, lactosyl-, globotriaosyl-, and globotetraosyl-ceramides were found in the neutral fraction from healthy tissue. These were also found in tumour tissue, but the majority of tumour tissues also contained lactotriaosyl- and neolactotriaosyl-ceramides. In addition, the lactosylceramide levels were elevated. The ganglioside patterns were equally as complex, in contrast to results showing that the patterns in tumour cells were simplified when grown *in vitro*.

The neutral glycosphingolipid contents of human heart (12–16 h after death) were 90–160 nmol g^{-1} net weight and trihexosylceramide and globoside were the most abundant components.[19] The total ganglioside content was much lower (*ca.* 50 nmol g^{-1} net weight). G_{M_3} and G_{M_1} were the most abundant components, but mono-, di- and tri-sialogangliosides were also found. There were differences between heart tissue and other human tissue.

The novel fucoganglioside (3) has been isolated from human erythrocyte membranes and has been characterized by the action of *endo-* and *exo*-glycosidases as well as m.s.[20] The ganglioside possessed blood-group H activity. Eight mononeuraminosylgangliosides have been isolated from human erythrocyte membranes.[21] Four of these, (4)–(7), had either new neuraminosyl linkages or new core structures. A ganglioside with a unique *N*-acetylneuraminosyl (2 → 3)-2-acetamido-2-deoxy-D-galactose partial structure has also been isolated by the same team from human erythrocyte membrane.[22] It was novel in containing both 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose residues. Its structure, (8), was characterized by successive *exo*-glycosidase treatments, methylation analysis, and direct probe m.s. of the permethylated intact and desialylized glycolipid. The ganglioside was further characterized by the presence of myristic acid as the major component of the ceramide portion.

[16] G. Constantopoulos and A. S. Dekaban, *J. Neurochem.*, 1978, **30**, 965.
[17] A. Novak, J. A. Lowden, Y. L. Gravel, and L. S. Wolfe, *J. Lipid Res.*, 1979, **20**, 678.
[18] R. Narasimhan and R. K. Murray, *Biochem. J.*, 1979, **179**, 199.
[19] G. M. Levis, J. N. Karli, and S. D. Moulopoulos, *Lipids*, 1979, **14**, 9.
[20] K. Watanabe, M. Powell, and S. I. Hakomori, *J. Biol. Chem.*, 1978, **253**, 8962.
[21] K. Watanabe, M. E. Powell, and S. I. Hakomori, *J. Biol. Chem.*, 1979, **254**, 8223.
[22] K. Watanabe and S. I. Hakomori, *Biochemistry*, 1979, **18**, 5502.



(3)

α-NeupNAc-(2 → 6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 3)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-Cer

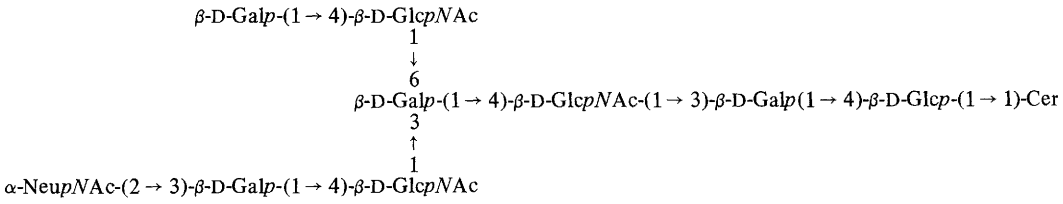
(4)

α-NeupNAc-(2 → 3)-D-Galp-(1 → 3)-D-GalpNAc-(1 → 4)-D-Galp-(1 → 4)-D-Glcp-(1 → 1)-Cer

(5)

α-NeupNAc-(2 → 6)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 3)-β-D-Galp-(1 → 4)-β-D-GlcpNAc-(1 → 3)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 → 1)-Cer

(6)



(7)

A blood-group I- and B-active branched ganglioside with two different carbohydrate chains was isolated and characterized as (9) by the same methods as (8).[23] The action of various combinations of *exo*-glycosidases produced eight glycolipids from the parent compound and their inhibitory activities were tested against anti-I and anti-i cold agglutinins. The results indicated that various I specificities were located on various domains within the structure, but that the majority of i specificities were determined by the straight lacto-*N*-norhexaosyl chain. The weak B activity was enhanced by the removal of the neuraminic acid residue suggesting that the B antigenicity is suppressed by a neuraminic acid residue on a neighbouring chain.

In humans with melanoma tumours, the levels of lipid-bound neuraminic acid were significantly higher in both plasma and erythrocytes compared to healthy humans.[24] Dineuraminosyl-lactosylceramide in plasma and neuraminosyl-lactosylceramide in red cells were the gangliosides mainly responsible for this increase. In patients who had been treated surgically the levels stayed high in plasma but fell in the red cells. The increase in dineuraminosyl-lactosylceramide suggests that this ganglioside is a major component of the neuraminic acid-containing glycolipid fraction of malignant melanocytes. The antigen of 'serum-sickness' type of heterophile antibodies in pathological human sera were identified as gangliosides containing *N*-glycolylneuraminic acid.[25] The equine erythrocyte antigen was identified as haematoside (10) and the bovine erythrocyte antigen was the paragloboside (11). These results indicate that 'serum-sickness' antibodies react with a common disaccharide moiety at the non-reducing terminus of both glycosphingolipids.

Fibroblasts, cultured from the skin of patients with the genetic diseases mucolipidosis I and mucolipidosis II, were found to be deficient in a neuraminidase specific for both $(2 \rightarrow 3)$ and $(2 \rightarrow 6)$ linkages of α-neuraminosyl groups in gangliosides.[26] Obligate heterozygotes showed intermediate activity in mucolipidosis I but a normal activity in mucolipidosis II. The neuraminidase activity of mucolipidosis I fibroblasts was normal with G_{M_3} and G_{D_3}, but diminished with a bovine brain ganglioside preparation. With mucolipidosis II fibroblasts, the neuraminidase activity was decreased towards the bovine brain ganglioside preparation and G_{M_3} and partially decreased with G_{D_3}. The data suggested that the simultaneous deficiency in mucolipidosis I of both an α-$(2 \rightarrow 3)$ and an α-$(2 \rightarrow 6)$ oligosaccharide neuraminidase strongly suggests that the two activities are genetically related and are either the properties of a single enzyme or of oligomeric isoenzymes sharing a common polypeptide sub-unit. The normal G_{M_3} and G_{D_3} ganglioside neuraminidase activities in mucolipidosis I, on the other hand, indicate that oligosaccharide ganglioside neuraminidases are genetically distinct.

[23] K. Watanabe, S. I. Hakomori, R. A. Childs, and T. Feisi, *J. Biol. Chem.*, 1979, **254**, 3221.
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[25] H. Higashi, M. Naiki, S. Matuo, and K. Okouchi, *Biochem. Biophys. Res. Commun.*, 1977, **79**, 388.
[26] M. Cantz and H. Messer, *Eur. J. Biochem.*, 1979, **97**, 113.

α-Neu*N*Ac-(2 → 3)-β-D-Gal*p*N*A*c-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*N*A*c-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-Cer

(8)

α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*N*A*c
1
↓
6
β-D-Gal*p*-(1 → 4)-β-D-Glc*p*N*A*c-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-Cer
3
↑
1
α-Neu*N*Ac-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*N*A*c

(9)

α-Neu*pN*Gl-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-Cer

(10)

α-Neu*pN*Gl-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*N*A*c-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-Cer

(11)

A reverse correlation was found between the malignant melanocytes in human tissue, as a percentage of total cells, and their neutral lipid content.[27] The ganglioside patterns in all samples analysed were similar with G_{M_3}, G_{M_2}, and G_{D_3} as major components, but there was no correlation between the ganglioside content of the tumours and the level of malignant cells. The two major gangliosides, isolated from the cell line AH7974F, were a neuraminosyl-paragloboside and the other G_{M_1b}.[28] In the neuraminosyl-paragloboside, the neuraminic acid residue was linked to the non-reducing terminal D-galactose residue. The ganglioside compositions of human neural tumours were determined and the neuraminic acid content was found to be between that of grey and white matter.[29] All tumours contained more of the less complex gangliosides than normal brain and this difference was more marked in the rapidly growing tumours. The cultured cells had a lower ganglioside content than the parent tissue. In a neuronal cell line (B104), the gangliosides seemed to act as differentiators and survival factors.[30] The kinetics of the uptake of labelled gangliosides supported the idea of the stable incorporation of exogenously added gangliosides into the cells. The neuraminic acid content of the ganglioside fraction of bromodeoxyuridine-differentiated Astroglial cells in culture remained at the same level but the distribution was modified.[31] Complex gangliosides, G_{M_1} and $G_{D_{1a}}$, appeared while the concentration of the simpler ones, like G_{M_3} and G_{D_3}, decreased. There was no G_{T_1} or G_{Q_1} present.

When calf brain was treated to give the cytosol of neuronal bodies and glial cells, this contained 29 μg of ganglioside-bound neuraminic acid per gram, whereas the cytosol from nerve endings only contained 3.1 μg of bound neuraminic acid.[32] The gangliosides were precipitated on addition of ammonium sulphate, although pure gangliosides are soluble, and the fraction had a constant ganglioside : phosphorus ratio that was similar for both cytosol preparations. These results suggested that the gangliosides were present in a ganglioside–protein complex.

A new bacterial neuraminidase (from *Arthrobacter ureafaciens*) was characterized by its action on bovine brain gangliosides.[33] Detergents markedly enhanced the enzymatic activity which had, particularly, a highly specific action on neuraminidase-resistant ganglioside G_{M_1}. The activity was 100-fold higher than that of the only other enzyme reported so far to cleave the bond in G_{M_1}. It was suggested that the hydrophobic ceramide moiety increased affinity of the lipid substrate to the enzyme, but inhibited hydrolysis of the substrate, possibly owing to its hydrophobic interaction with hydrophobic portions of the enzyme molecule. The inhibition may be overcome in detergent solution owing to the formation of mixed micelles of neuraminosyl glycolipid and detergent molecules.

[27] J. Portoukalian, G. Zwingelstein, and J. F. Doré, *Eur. J. Biochem.*, 1979, **94**, 19.
[28] Y. Hirabayashi, T. Taki, and M. Matsumoto, *FEBS Lett.*, 1979, **100**, 253.
[29] A. J. Yates, D. K. Thompson, C. P. Boesel, C. Albrightson, and R. W. Hart, *J. Lipid Res.*, 1979, **20**, 428.
[30] J. I. Morgan and W. Seifert, *J. Supramol. Struct.*, 1979, **10**, 111.
[31] J. Robert, P. Mandel, and G. Rebel, *Lipids*, 1979, **14**, 852.
[32] S. Sonnino, R. Ghidoni, S. Marchesini, and G. Tettamanti, *J. Neurochem.*, 1979, **33**, 117.
[33] M. Saito, K. Sugano, and Y. Nagai, *J. Biol. Chem.*, 1979, **254**, 7845.

Fibronectin mediates the adhesion of cells to collagen by first binding to the collagen substrate followed by attachment of the cells to the fibronectin–collagen complex. Bovine brain gangliosides prevented the adhesion of cells to the fibronectin–collagen complex, but had no effect on the initial formation of the fibronectin–collagen complex.[34] The order of efficiency was G_{T_1} and $G_{D_{1a}} > G_{D_{1b}} \gg G_{M_1} \gg G_{M_2}$. G_{M_3} was not an inhibitor. Only the carbohydrate portions of the molecules were effective inhibitors and the oxidized ganglioside was not an inhibitor. The authors suggested that specific gangliosides or related neuraminic acid-containing glycoconjugates on the cell surface may act as receptors for fibronectin.

Bovine brain gangliosides were oxidized by periodate ion and N^4-2,4-dinitrophenyl-L-2,4-diaminobutyric acid hydrazide was covalently attached to the aldehyde groups.[35] The dinitrophenyl-labelled gangliosides were incorporated into mature thymocytes and these were incubated with rabbit anti-dinitrophenol and fluorescein-labelled anti-rabbit immunoglobulin G, causing uniform rings on the cell surface. Gangliosides may play a primary role in the lymphocyte activation process.

Three fractions of complex gangliosides were isolated from bovine erythrocytes.[36] Each contained branched oligosaccharide chains and comprised 11–13 monosaccharide residues per molecule and included 1–2 units of *N*-acetylneuraminic acid.

The structures of the major gangliosides from bovine thyroid were determined by methylation analysis and enzymatic degradation studies.[37] These structures, (12)–(15) were confirmed by m.s. on the permethylated gangliosides

Neu*p*NAc-(2 → 3)-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-Cer

(12)

Neu*p*NGl-(2 → 3)-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-Cer

(13)

D-Gal*p*-(1 → 3)-D-Gal*p*NAc-(1 → 4)-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-Cer
3
↑
2
Neu*p*NAc

(14)

L-Fuc*p*-(1 → 2)-D-Gal*p*-(1 → 3)-D-Gal*p*NAc-(1 → 4)-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-Cer
3
↑
2
Neu*p*NAc

(15)

[34] H. K. Kleinman, G. R. Martin, and P. H. Fishman, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 3367.

[35] S. Spiegel, A. Ravid, and M. Wilchek, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 5277.

[36] H. Miller-Podraza, *Biochim. Biophys. Acta*, 1979, **586**, 209.

[37] G. A. F. Van Dessel, A. R. Lagrou, H. J. J. Hilderson, W. S. H. Dierick, and W. F. J. Lauwers, *J. Biol. Chem.*, 1979, **254**, 9305.

and the corresponding asialo derivative. One of these gangliosides, (15), was also identified by another group from a similar source and characterized by g.c.–m.s.[38]

Undernourishment of rats before birth and up to 21 days old had no effect on the ganglioside content of either forebrain, brain stem, or cerebellum, but extension to 121 days old showed significant reductions in each brain area.[39] Different fractions were affected in different ways. $G_{D_{1a}}$ was most affected in forebrain, $G_{D_{1b}}$ in brain stem, and G_{T_1} in cerebellum: the concentration of each was increased at 121 days.

To elucidate the differences in ganglioside metabolism between adhesive (AH7974) and non-adhesive (AH7974F) rat ascites hepatoma lines, various glycosyltransferase activities were determined.[40] The results established that the ganglioside in AH7974F cells was synthesized *via* asialo-G_{M_2} and asialo-G_{M_1} but not *via* G_{M_3}, but in the AH7974 cell line, G_{M_3} was found to be an end-product of glycolipid metabolism.

Addition of mixed gangliosides abolished the stimulatory effect of cholera toxin on progesterone production in isolated rat ovarian cells, but had no influence on the effect of gonadotropin.[41] It was concluded that gangliosides were not involved in hormone–receptor interactions.

The concentration of G_{M_3} in rabbit brain, thymus, lung, liver, stomach, intestine, kidney, testis, muscle, and erythrocyte tissues was determined.[42] The neuraminic acid composition was also determined. *N*-Glycolylneuraminic acid-containing G_{M_3} was found in thymus, lung, kidney, and intestine as well as *N*-acetylneuraminic acid-containing G_{M_3}, but *N*-acetylneuraminic acid was the sole neuraminic acid of G_{M_3} from other tissue.

Axonally transplanted gangliosides from retina were more highly labelled in the optic tectum of chicken exposed to light compared to those maintained in the dark.[43] There was no difference in the labelling of retinal gangliosides between the two groups.

Gangliosides with short carbohydrate chains have been isolated from brain tissue of eight species of cartilaginous fish.[44] Sequential enzymatic hydrolysis showed that the structure (16) was the major elasmobranch brain ganglioside.

Three new gangliosides have been isolated and characterized as structures (17)–(19) from the starfish, *Asterina pectinifera*.[45,46] These gangliosides were novel in each containing an internal neuraminic acid residue.

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Neu*p*NAc → Neu*p*NAc-(2 → 3)-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-Cer

(16)

D-Ara-(1 → 6)-β-D-Gal*p*-(1 → 4)-Neu*p*NGl-(2 → 3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer
8
↑
1
β-D-Gal*p*

(17)

β-D-Ara*p*-(1 → 6)-β-D-Gal*p*-(1 → 4)-(8-O-Me)-Neu*p*NGl-(2 → 3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-1→1)-Cer

(18)

β-D-Ara*p*-(1 → 6)-β-D-Gal*p*-(1 → 4)-Neu*p*NGl-(2 → 3)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer

(19)

3 Animal Glycolipids

A review on cancer-specific neo-antigens includes sections on their chemical, enzymatic, and organizational aspects as well as on their relationship to abnormal blood-group substances in man.[47] Another review has considered various topics on sulphated glycolipids.[48]

A further review has compared the structural features of the carbohydrate chains of glycoproteins and glycolipids.[49] Carbohydrate chains of glycoproteins and glycolipids that only contain a few monosaccharide units are usually quite distinct. Medium-length oligosaccharide chains possess many common non-reducing terminal monosaccharide sequences, whereas the oligosaccharide units of glycoproteins and glycolipids that contain longer carbohydrate chains are usually very similar. The authors suggest that glycoproteins and glycolipids should not be considered as completely separate classes of compounds since some biological functions, such as cell-surface receptors, may be shared.

Neutral glycosphingolipids from animal tissues and cells can be separated on diethylaminoethyl-modified supports.[50] Diethylaminoethyl-silica gel was a better ion-exchanger than either diethylaminoethyl-Sephadex® or diethylaminoethyl-controlled-porous glass. An aminopropyl derivative of silica gel has been used as a solid support for the preparation of glycolipid immunoadsorbents and the purification of antibodies.[51] A carboxy-function was generated in the neutral glycosphingolipids by oxidation of the double bond of the sphingenine moiety and this group could bind to the derivatized silica gel. The new ion-exchange

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material had better flow rates, capacity, and handling characteristics than previously used materials as well as a lower susceptibility to microbial attack.

A series of papers has been published on the ^{1}H n.m.r. spectroscopic analysis of the anomeric structures of glycosphingolipids.[52–54] The solubility problem was overcome by using the methylated derivatives and only *ca.* 200 μg of sample was required per analysis. With the globo-series (*i.e.* 1–5 neutral monosaccharide residues), the shifts were characteristic of the monomer residues present, the type of glycosidic bond, and the position of the monomer in the oligosaccharide chain. A complex set of resonances were obtained from H-1 of the 2-acetamido-2-deoxy-hexoses due to the separate conformers obtained from the 2-*N*-methyl-acetamido-2-deoxy-groups on *N*-methylation. This difficulty was removed on reduction of the sample with lithium aluminium hydride. Similar analyses were carried out on a series of ABH-active blood-group substances. Reduction of the compounds resulted in a strong deshielding of H-1 of the D-galactosyl group in the sequence (20), which occurs in Type I chains, but not of H-1 of the D-galactose group in the sequence (21), which occurs in Type II chains. Type I and Type II chains could therefore be distinguished from each other. These studies were extended to the Lewis-active and Lewis-like glycosphingolipids where different types of chains could be distinguished in the same manner.

β-D-Gal*p*-(1 → 3)-D-Glc*pN*Ac
(20)

β-D-Gal*p*-(1 → 4)-D-Glc*pN*Ac
(21)

The first ^{13}C n.m.r. spectrum of a cerebroside was reported.[55] All the signals of D-glucosylceramide, from the liver of a patient with Gaucher's disease, were assigned by comparison with model compounds. It was concluded that the D-glucose residue exists in a pyranose ring form and is linked to the ceramide by a β-D-glycosidic bond. The method should be applicable to other glycoconjugates.

A sensitive but simple new procedure has been developed for the simultaneous determination of D-gluco- and D-galacto-cerebrosides, mono-D-galactosyldiglyceride, and sulphatide.[56] The method involves extraction of glycolipid, benzoylation, desulphation of sulphatides, and product analysis by h.p.l.c. The method could measure as little as 5 pmol of cerebroside and used a column of Spherisorb with a gradient elution of 0.5–10% propan-2-ol in hexane. Glycosphingolipids could be acetylated and the amino-sugars 4-nitrobenzoylated for

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[53] K.-E. Falk, K.-A. Karlsson, and B. E. Samuelsson, *Arch. Biochem. Biophys.*, 1979, **192**, 177.
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analysis by h.p.l.c.[57] The glycolipids were separated on a ZorbaxSil column using a gradient of 0.5–7.0% propan-2-ol in hexane : chloroform (2 : 1). As little as 20 μg of glycolipid could be analysed.

The thermotropic behaviour of multilamellar liposomes prepared from mixtures of D-glucocerebrosides and dipalmitoyl-phosphatidylcholine has been studied using scanning calorimetry.[58] The D-glucocerebroside has a marked effect on the gel–liquid crystalline transition of the dipalmitoylphosphatidylcholine. The change in enthalpy is decreased with increasing amounts of D-glucocerebroside but the change is not linear. It is suggested that the in-plane distribution of D-glucocerebroside is affected by both the physical state of the lipid bilayer and the ratio of D-glucocerebroside to phospholipid.

Using liver microsomes from various species, the D-galactose residue of D-galactosylmonophosphoryl dolichol was transferred to an endogenous receptor which had the properties of an oligosaccharide pyrophosphoryl dolichol.[59]

Light scattering measurements were used to determine the optimal ratio of antibody to antigen in the complex formation between the antibody and antigen.[60] Using this technique with a model system of egg albumin and a glycolipid in the form of a globoside-containing lecithin–cholesterol vesicle as the antigen, the optimal ratio could be determined in 1–2 hours as opposed to 1–2 days. The advantages of the technique were discussed with reference to the determination of antiglycolipid antibodies during immunization and the detection of cross-reactivity between different antigens and antisera.

The neutral glycolipids mono-, di, and tri-hexosylceramide, globoside, and the asialo gangliosides, G_{A_1} and G_{A_2}, were characterized from brain tissue of a patient with Tay-Sachs disease.[61] Assays of sphingolipid hydrolases *in vitro* generally require the presence of bile salts or other detergents. However, phosphatidyl-L-serine from bovine brain was found to be a relatively specific activator of human brain D-galactosylceramidase in the absence of sodium taurocholate.[62] The phosphatidylserine system was superior to the bile salt system in the linearity of the reaction with respect to the enzyme protein.

The cerebroside sulphotransferase activity of human lung adenocarcinoma tissue was higher than that of other carcinoma tissue or the normal tissue from which each carcinoma was derived.[63]

Analysis of the glycosphingolipids in a mucosal scraping of human small intestine showed a peculiar distribution pattern.[64] Glycolipids with one monosaccharide group (D-galactosyl- and D-glucosyl-ceramide) and those with more

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than four monosaccharide residues (blood-group glycolipids) are located on the epithelial wall, while the di, tri, and tetra-glycosylceramides are present in the nonmucosa. A higher proportion of hydroxylated ceramide moieities were present in the epithelial glycolipids than are normally found.

The D-galactosylceramide β-D-galactosidase from the liver of a case of Krabbe's disease has been purified.[65] The physical properties of this enzyme were very similar to those of a control from normal liver tissue but the catalytic properties and stability of the enzyme protein were severely affected in the mutant. The findings indicated that the mutation in Krabbe's disease leads to the synthesis of normal quantities of a catalytically and structurally altered protein.

Two livers, obtained at autopsy of patients with Gauchers disease, were examined for distribution of D-glucocerebroside by t.l.c. and g.l.c.[66] The results indicated that stored D-glucocerebrosides were evenly distributed throughout the livers since all levels were within ±1.95 standard deviations of the sample mean. L-Glucosylceramide is completely resistant to enzymatic hydrolysis by placental D-glucocerebrosidase and a similar enzyme present in whole tissue extracts.[67] The ceramide was used in the exploration of pathogenic processes in animal analogues of Gauchers disease. The residual D-glucosylceramidase activity in patients with Gauchers disease hydrolyses the D-glucose moiety of *N*-oleoyl-D-glucocerebroside at the same rate as that of *N*-stearoyl-D-glucocerebroside.[68] This observation indicates that a mutation affecting the substrate specificity of D-glucosylceramidase cannot account for the 10-fold lowering of the oleic acid-containing D-glucocerebrosides in patients with Gauchers disease. The hypothesis that the difference in fatty acid composition found in the D-glucocerebrosides is obtained as a result of a mutation affecting the specificity of the residual D-glucosylceramidase must be rejected. Several carriers have been evaluated for their use in the delivery of exogenous D-glucocerebrosidase to monocytes derived from patients with Gauchers disease.[69] Only γ-globulin-coated and resealed erythrocytes were effective in delivering the enzyme. D-Glucocerebrosidase, added in this way, normalized the intracellular enzyme level for at least 18 h. Soluble enzymes, enzymes in uncoated but resealed erythrocytes, and enzymes incorporated into liposomes were not effective.

A human serum containing a mono-clonal anti-(blood-group I) antibody has been used to investigate the distribution of blood-group I antigens on erythrocyte membrane components.[70] Different radioactive profiles were revealed by the two radiolabels used with solubilized stroma (^{3}H and ^{125}I). In the immunoprecipitates, the predominant ^{125}I-labelled band had molecular weight $0.9–1.0 \times 10^{5}$, whereas the ^{3}H label was associated with a diffuse band (molecular

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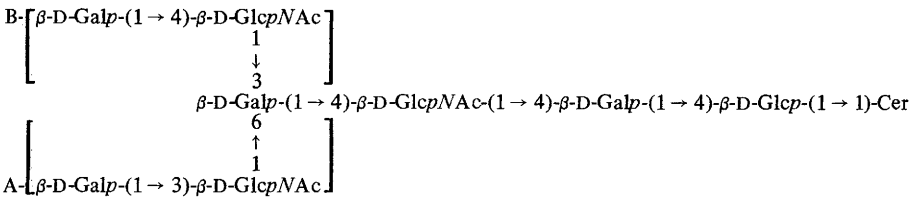
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weight $4–7 \times 10^4$) and the glycolipids. This diffuse band had a similar electrophoretic mobility to a sub-population of erythrocyte poly-(glycosyl) ceramides with blood-group I activity. The blood-group I activities of the purified glycosphingolipid (22) and eight of its analogues have been evaluated with various



(22)

antisera.[71] Three different types of I-specificity were distinguished. The first type required an intact chain (22-A), the second a chain with a branch point (22-B) and the third required both branches to be intact. Anti-I antibodies varied in their ability to react with their antigenic determinants in the presence of external substitutions with α-linked D-galactose or neuraminic acid residues. Fractions of complex glycosphingolipids have been prepared from adult, cord, and i-phenotype erythrocytes.[72] In contrast to the complex glycosphingolipids from adult erythrocytes which comprise about 30 glycosyl residues and five branch points per mole of D-glucose, the glycosphingolipids from cord and i erythrocytes comprise only six and fifteen glycosyl units, respectively, with an average of 0.7 branching points. The latter substances exhibit a high i activity which was not detected in the complex glycosphingolipids from adults.

Suppression of the synthesis of protein in HeLa cells either by viral infection or translation-inhibiting drugs induced alterations to the concentrations of cell glycolipids.[73] The mono- and di-glycosylceramide content increased while the triglycosylceramide and ganglioside content decreased. The presence of synthetic lactosylceramide in which the sphingosine moiety was replaced with dihydrosphingosine and palmitic acid was the fatty acid in preparations of neutral multilamellar liposomes enhanced their fusion interaction with cultured HeLa cells by 5.7 fold.[74] The effect appeared to be specific for carbohydrate and probably cannot be attributed to a change in the physical characteristics of the liposome bilayer owing to the presence of the cerebrosides. A chloroform–methanol extract from the cultures of *Shigella flexneri* increased the rate of phagocytosis in HeLa cells in a dose-dependent manner up to 62.5 $\mu g\ ml^{-1}$.[75] The involvement of surface receptors on the HeLa cells was discussed.

Four neutral glycolipids, as well as the gangliosides G_{M_2} and G_{M_3}, were identified in the lipid fraction from the human transformed cell lines RSa, RSb, and

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$1F^s$.[76] The glycolipids were considered to be components of the receptors for interferon in these cells. However, no inhibitory action on the growth-suppressing activity of interferon was shown by either a ganglioside mixture or G_{M_3} alone.

The renal cell lines, JTC-12 and MDCK, not only synthesize D-galactosylceramide 3-sulphate and lactosyl 3-sulphate *in vivo*, but competition studies indicate that both ceramides became sulphated by a single enzyme in both cell lines.[77]

Calorimetry and Raman spectroscopy were used to measured the gel state of cerebrosides from bovine brain.[78] The enthalpy of the gel–liquid crystalline phase transition for kerasin (15.8 kcal mol^{-1}) was markedly higher than that for phrenosin and unfractionated bovine brain cerebrosides (*ca.* 7 kcal mol^{-1}).

The production of the suspected trisaccharide lipid (previously reported by the same authors) by calf brain membranes was enhanced by the presence of GDP-D-mannose.[79] The intact trisaccharide lipid behaved like a dolichol-bound trisaccharide in which the glycosyl group is linked *via* a pyrophosphate bridge. Mild acid treatment released a product which co-chromatographed with authentic β-D-mannopyranosyl-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose and which released *NN'*-diacetylchitobiose on treatment with β-D-mannosidase. The calf brain mannosyltransferase involved in the synthesis of β-D-mannosyl-*NN'*-diacetylchitobiosylpyrophosphoryldolichol utilizes GDP-D-mannose, but not D-mannopyranosylpyrophosphoryldolichol, as the direct D-mannosyl donor. Both grey- and white-matter membranes from calf brain catalyse the transfer of the sugar residue from UDP-2-acetamido-2-deoxy-D-glucose to a 2-acetamido-2-deoxy-D-glucose-containing glycoprotein.[80] The glycoprotein from grey matter was not homogeneous in that the pronase digest contained L-arginine-*NN'*-diacetylchitobiose as well as the more commonly found L-arginine-2-acetamido-2-deoxy-D-glucose.

The heptaglycosylceramide (23) was isolated and characterized from bovine erythrocyte membranes.[81] A hexaglycosylceramide, identical to (23) except that the non-reducing terminal D-galactose residue was missing, was also characterized. These results confirm that the neutral glycosphingolipids of bovine erythrocyte membrane contain an oligosaccharide sequence similar to that in keratan-sulphate and which has already been found in gangliosides.

Bovine milk contains an enzyme, in soluble form, which catalyses the transfer of D-galactose from UDP-D-galactose to D-glucosylceramide.[82] The synthesis of the product, lactosylceramide, was inhibited by the presence of α-lactalbumin. Lactosylceramide was also an effective acceptor of D-galactose, but neutral glycosphingolipids or gangliosides were very poor receptors.

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α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*pN*Ac-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*pN*Ac-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-Cer

(23)

β-D-Gal*pN*Ac-(1 → 3)-α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-Cer

(24)

The antibiotics, tunicamycin, streptovirudin, and an as yet unnamed antibiotic, but with similar properties to the others, from *Bacillus cereus* all inhibited the formation of 2-acetamido-2-deoxy-D-glucosylpyrophosphoryldolichol in porcine aorta, but had no effect on the formation of D-mannopyranosylpyrophosphoryldolichol.[83] These antibiotics also inhibited the incorporation of D-glucose into D-glucosylpyrophosphoryldolichol and lipid-linked oligosaccharides. The antibiotic amphomycin inhibited the synthesis of D-glucosylpyrophosphoryldolichol and, to a lesser extent, lipid-linked oligosaccharides also in porcine aorta but had no effect on the synthesis of D-glucosylceramide except that more UDP-D-glucose was available for synthesis of the glycolipid.[84] More detailed studies on the action of tunicamycin have been carried out using a solubilized enzyme from a microsomal fraction of porcine aorta.[85] The enzyme which catalyses the transfer of 2-acetamido-2-deoxy-D-glucose 1-phosphate from UDP-2-acetamido-2-deoxy-D-glucose to dolichyl phosphate was severely inhibited (>50%) by only 0.05–0.1 μg ml^{-1} antibiotic at low enzyme concentrations. The inhibition was non-competitive with respect to the concentration of UDP-2-acetamido-2-deoxy-D-glucose, but was increased by preincubation with the antibiotic.

The major neutral glycolipids found in the fundic epithelium of guinea-pigs were characterized as D-glucosyl-D-galactosyl, di-D-galactosyl, and tri-hexosylceramide.[86] Differences were observed in the distribution of these glycolipids between different types of gastric cell. Both non-polar and polar carbohydrate-containing materials were isolated from the secondary granule and plasma-membrane fractions of polymorphonuclear leukocytes from guinea-pig.[87] The former contained at least two ceramide-like glycolipids and the latter comprised a single substance that contained D-glucose, 2-acetamido-2-deoxy-hexose, and a carboxylic acid derivative of a pentose but no fatty acid.

Glycosphingolipids with long carbohydrate chains have been isolated from ovine erythrocytes.[88] Their structures were confirmed by the usual techniques and the major component was the Forsman-active glycolipid. Lacto-*N*-neotetraosylceramide was another component and three highly polar glycolipids containing 10–12 sugar residues were also found.

The trihexosylceramide isolated from canine intestine was characterized as isoglobotriaosylceramide (24).[89]

An enzymic fraction from the microsomes of mouse brain was subfractionated by density-gradient centrifugation to identify ceramide-D-galactosyltransferase and cerebroside sulphotransferase activities.[90] Both enzymes were found to be located in the Golgi membranes. The Jimpy mouse shows a deficient myelination

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owing to the activity of cerebroside sulphotransferase in the microsomes of Jimpy brain.[91] The cerebroside sulphotransferase activity of Jimpy microsomes could be normalized by delipidation with acetone and adding an acetone-extract from normal microsomes. The lipid-extract from Jimpy microsomes had no effect on the cerebroside sulphotransferase activity of normal microsomes. An artificial cholesterol–phospholipid mixture had the same effect.

The concentrations of various glycolipids in the brain stem, cerebellum, cerebral hemisphere, and diencephalone of rats of different ages have been determined by h.p.l.c.[92] The concentrations of cerebrosides increased rapidly in all areas of the brain during the maturation period while that of the D-galactosyl diglyceride increased more slowly and had levelled off by 26 days. From studies of the microsomes of developing rat brain, it was concluded that hydroxyceramide is the precursor of hydroxycerebrosides and is formed by α-hydroxylation of lignoceryl-CoA and *N*-acetylation of sphingosine.[93] The concentrations of sulphated glycoglycerolipids in rat brain were found to decrease and eventually disappear after the developmental age.[94] Liposomes have been successfully used as acceptors in the assay for the lipid glycosyltransferases, UDP-D-galactose: ceramide D-galactosyltransferase, and UDP-D-glucose: ceramide D-glucosyltransferase, from rat brain.[95] The activities of the glycosyltransferases were dependent not only on the appropriate assay conditions, but also on the type and source of the phospholipids used to form the liposomes.

D-Glucosylceramide was isolated from rat intestine and the major sphingoid base was 4D-hydroxysphingamine.[96]

The incorporation of 2-acetamido-2-deoxy-D-glucose into endogenous lipid and protein acceptors has been investigated in heavy microsomes from rat liver by incubation with UDP-2-acetamido-2-deoxy-D-glucose and GDP-D-mannose.[97] The sugar was almost exclusively found in the dolichol monosaccharide-derivatives except when treated with pyrophosphate and subsequently incubated in the presence of GTP. The incorporation of amino sugar was then greatly enhanced and appeared as *NN'*-diacetylchitobiosylpyrophosphoryldolichol, oligosaccharide pyrophosphoryldolichol, and protein. It was concluded that the metabolic processes are hindered in the absence of GTP. D-Mannose, when incorporated into endogenous lipid- and protein-acceptors by the same system, only produced D-mannosylphosphoryldolichol; but when incubated in the presence of pyrophosphate, UDP-2-acetamido-2-deoxy-D-glucose, and GTP, incorporation into lipid and protein derivatives was enhanced.[98] GTP could not be substituted by other nucleotides. The kinetics of D-mannose incorporation

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into dolichol-linked oligosaccharides and into protein suggest that the protein acceptors are mainly D-mannosylated through transfer of preassembled oligosaccharide groups on dolichyldiphosphate.

The synthesis and turnover of sulpho-D-galactosylglycerolipid has been studied in the rat by measuring the loss from the testes and appearance in the vas deferens and epididymis.[99] These lipids are only synthesized in very early spermatocytes and, once made, they do not turn-over. The lipids are lost from the testes when geminal cells die or mature into spermatozoa.

A tetraglycosylceramide (24), which is similar to globoside 1 from human erythrocytes has been characterized from rat granuloma and macrophage tissue.[100] Another glycolipid (25) was characterized from the same source.

α-D-Gal*p*
1
↓
3
β-D-Gal*p*-(1 → 3)-β-D-Glc*pN*Ac-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-Cer
2
↑
1
α-L-Fuc*p*

(25)

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 3)-β-D-Glc*pN*Ac-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-Cer

(26)

α-L-Fucosyltransferase activity has been demonstrated in rat ascites hepatoma cells and this enzyme catalyses the addition of L-fucose residues to asialo-G_{M_1} to form an L-fucolipid, in the presence of a detergent with the structure (26).[101] The lipid co-chromatographed with authentic blood-group H-active L-fucolipid. The L-fucosyltransferase activity was highly specific for asialo-G_{M_1}.

When isolated brush borders from rabbit small intestine were incubated with $Na_2{}^{35}SO_4$ hydrophobic glycolipids were found to contain 24% of the incorporated ^{35}S and 73% was found in the glycoprotein fraction.[102] One principal, sulphated glycolipid that was present contained D-glucose, D-galactose, and 2-acetamido-2-deoxy-D-glucose (in the molar ratio 1:2:2) but was devoid of neuraminic acid residues.

Three neutral D-glucoglycerolipids have been isolated from the alveolar lavage of rabbits and their structures (27)–(29) were determined by mild alkaline hydrolysis, methylation, periodate and chromium(VI) oxide oxidation analyses.[103] A further sulphated D-glucoglycerolipid (30) was also isolated and characterized by the same procedures applied to the mature and desulphated lipid.[104]

[99] M. J. Kornblatt, *Can. J. Biochem.*, 1979, **57**, 255.
[100] E. Hanada, S. Handa, K. Konno, and T. Yamakawa, *J. Biochem. (Tokyo)*, 1978, **83**, 85.
[101] T. Taki, Y. Hirabayashi, M. Matsumoto, and K. Kojima, *Biochim. Biophys. Acta*, 1979, **572**, 105.
[102] J. R. Cooper and P. W. Kent, *Biochem. Biophys. Res. Commun.*, 1978, **83**, 622.
[103] B. L. Slomiany, F. B. Smith, and A. Slomiany, *Biochim. Biophys. Acta*, 1979, **574**, 480.
[104] A. Slomiany, F. B. Smith, and B. L. Slomiany, *Eur. J. Biochem.*, 1979, **98**, 47.

α-D-Glc*p*-(1 → 3)-(1-*O*-alkyl,2-*O*-acyl)-glycerol

(27)

α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 3)-[1-*O*-alkyl,2-*O*-acyl]-glycerol

(28)

α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 3)-[1-*O*-alkyl,2-*O*-acyl]-glycerol

(29)

(6-SO_3H)-α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 6)-α-D-Glc*p*-(1 → 3)-[1-*O*-alkyl,2-*O*-acyl]-glycerol

(30)

(4-*O*-Me)-β-D-Gal*p*-(1 → 3)-β-D-Glc*pN*Ac-(1 → 3)-α-L-Fuc*p*-(1 → 4)-β-D-Glc*pN*Ac-(1 → 2)-D-Man*p*-(1 → 3)[α-D-Xyl*p*-(1 → 2)]
6-*O*-2′-aminoethylphosphoryl-β-D-Man*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide

(31)

Antiserum against globotrihexosylceramide was raised in rabbits.[105] The partially purified antibody stimulated complement-dependent release of D-glucose from D-glucose-containing liposomes. The antibody cross-reacted with α-D-galactopyranosyl-(1 → 4)-β-D-galactopyranosyl-(1 → 4)-D-glucopyranose, but not with D-galactosyl-, lactosyl-, and globotetraosyl-ceramide, di-D-galactosyl-diglyceride or G_{M_1} ganglioside.

A combination of n.m.r. spectroscopy and m.s. along with degradation studies showed that the sea anemone, *Metridium senile*, contains a β-D-glucopyranosyl-ceramide with the novel base, D-*erythro*-1,3-dihydroxy-2-amino-9-methyloctadeca-*trans*-4-*trans*-8-diene.[106]

The shell of the sea urchin, *Anthocidaris crassispina*, contains a mixture of two sulphoglycolipids whose structures were confirmed by chemical and physicochemical methods.[107] The lipids were 1′-*O*-palmitoyl-3′-*O*-(α-D-quinovopyranosyl-6-sulphate)glycerol (96%) and its myristoyl counterpart (4%).

A novel ceramide octasaccharide (31) from the fresh-water bivalve, *Corbicula sandai*, which contained D-mannose-6-phosphate residues, is the first report of an internal L-fucose residue in a glycolipid.[108]

Two unusual cerebrosides have been isolated from the marine gastropod, *Monodonta labio*, which together comprise 1% of the total lipid.[109] The major cerebroside was identified as 1-*O*-[6′-*O*(*N*-methylaminoethylphosphonyl)-D-galactopyranosyl]-ceramide and the minor one had the same structure except that the amino-group did not contain a methyl substituent.

Glycolipids moving along an identified axon of the marine mollusc, *Aplysia californica*, were synthesized and incorporated into intracytoplasmic membranes solely in the perikarion.[110] Direct injection of tritiated sugar into the axon revealed no localized synthesis or exchange. There was no indication of any transfer into the axoplasm from the glia. The insertions of glycolipids into nascent membranes occurs co-ordinately with the insertion of protein components in the cell body.

4 Plant Glycolipids

The synthetic compound, 1,2-di-*O*-9′-octadecenyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol, which is an ether analogue of mono-D-galactosyldiacylglycerol, can be converted by plant enzymes into the 6-*O*-acyl derivative.[111] The availability of the 9′-octadecenyl compound can provide a substrate for studies on the direct desaturation of lipid-linked acyl or alkyl chains.

[105] J. T. R. Clarke and J. A. Embil, *Biochim. Biophys. Acta*, 1979, **582**, 283.
[106] K.-A. Karlsson, M. Leffler, and B. E. Samuelsson, *Biochim. Biophys. Acta*, 1979, **574**, 79.
[107] I. Kitagawa, Y. Hamamoto, and M. Kobayashi, *Chem. Pharm. Bull. (Jpn.)*, 1979, **27**, 1934.
[108] O. Itasaka and T. Hori, *J. Biochemistry*, 1979, **85**, 1469.
[109] F. Matsuura, *Biochemistry*, 1979, **85**, 433.
[110] A. A. Sherbany, R. T. Ambron, and J. H. Schwartz, *Science*, 1979, **203**, 78.
[111] E. Heinz, H. P. Siebertz, and M. Linscheid, *Chem. Phys. Lipids*, 1979, **24**, 265.

The major neutral glycolipids and phospholipids from the envelopes of spinach chloroplasts have been analysed with respect to proportion, positional distribution, and pairing of the fatty acids.[112] The diacylglycerol- and D-galacto-lipids share a common diacylglycerol portion. The only exception is di-D-galactosyldiacylglycerol, which contains the pairing 18:3/16:0 fatty acids as the diacyl substituents but lacks the 18:3/16:3 pairing thus reversing the distribution in other D-galactolipids. There was no evidence for a bioenergetic relationship between phosphatidylcholine and D-galactolipid at the level of the chloroplast envelope. It was observed in the envelopes of *Euglena* chloroplasts that an enzyme complex was present, which catalysed the reaction of two moles of mono-D-galactosyldiacylglycerol to yield one mole each of di-D-galactosyldiacylglycerol and di-acylglycerol.[113]

The lipid components of oats (*Avena sativa*) were separated by silicic acid column chromatography and by t.l.c.[114] The nine glycolipids identified were mainly D-galactoglycerides.

In studies on the glycoglycerolipids of rice bran, the mono-, di-, and triglycosyldiglyceride and (quinovosylsulphate)diglyceride fractions were isolated and characterized.[115] The ratio of D-galactose to D-glucose residues in the first three molecular species were 92:8, 85:15, and 63:37, respectively. Monoglycosylmonoacylglycerides and mono-, di-, tri-, and tetra-glycosyldiacylglycerides have been isolated and their structures (32)–(36) have been eluci-

$$\left.\begin{matrix}\beta\text{-D-Gal}p\\ \text{or}\\ \beta\text{-D-Glc}p\end{matrix}\right\}\text{-}(1\rightarrow 3)\text{-monoacylglycerol}$$

(32)

$$\left.\begin{matrix}\beta\text{-D-Gal}p\\ \text{or}\\ \beta\text{-D-Glc}p\end{matrix}\right\}\text{-}(1\rightarrow 3)\text{-diacylglycerol}$$

(33)

$$\alpha\text{-D-Gal}p\text{-}(1\rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 3),1,2\text{-di-}O\text{-acylglycerol}$$

(34)

$$\alpha\text{-D-Gal}p\text{-}(1\rightarrow 6)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 3),1,2\text{-di-}O\text{-acylglycerol}$$

(35)

$$\alpha\text{-D-Gal}p\text{-}(1\rightarrow 6)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 6)\text{-}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 3),1,2\text{-di-}O\text{-acylglycerol}$$

(36)

[112] H. P. Siebertz, E. Heinz, M. Linscheid, J. Joyard, and R. Douce, *Eur. J. Biochem.*, 1979, **101**, 429.
[113] A. Van Besouw and J. F. G. M. Wintermans, *FEBS Lett.*, 1979, **102**, 33.
[114] M. R. Sahasrabudhe, *J. Am. Oil Chem. Soc.*, 1979, **56**, 80.
[115] T. Miyazawa and Y. Fujino, *J. Agric. Chem. Soc. Jpn.*, 1979, **52**, 37.

dated.[116] This is the first report of the occurrence of the tetra-D-galactosyldiacylglycerol (36).[117] Novel oligoglycosylsterols were also isolated from the glycolipid fraction of rice bran.[118] D-Glucose was the only sugar residue present but sitosterol, campestrol, and stigmasterol were found in the steroid fraction. The structures of the purified compounds (37) and (38) were confirmed.

$$\beta\text{-D-Glc}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow 3')\text{-sitosterol}$$
(37)

$$\beta\text{-D-Glc}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow 3')\text{-sitosterol}$$
(38)

The concentrations of diglycosyl-, monoglycosyl-, and sulphoquinovosyldiglycerides decrease markedly in tobacco leaves on flue-curing.[119] The authors postulated that the high content of linolenate in these fractions may have contributed to this reduction.

The glycolipid fraction isolated from Ginkgo nuts (*Ginkgo biloba*) accounted for 3.12% of the total lipid content.[120] The di-D-galactosyldiglyceride fraction was 64.1% of the total glycolipid, mono-D-galactosyldiglyceride 32.2%, and cerebroside 4.7%. The major D-glucocerebroside was identified as *N*-α-hydroxypalmitoyl-D-glucosyl-octadecasphingadiene.

The cotyledons of germinating soybeans contained mono-D-galactosyldiglycerides, di-D-galactosyldiglycerides, and acylsterol-D-glucosides in the glycolipid fraction.[121] A pre-treatment of immature soybeans with 0.25% hot acetic acid was used to avoid the formation of artifacts during lipid extraction.[122] Complete recovery of lipid was obtained by chloroform–methanol mixtures, including the highly polar glycolipids.

The antibiotic amphomycin blocked the transfer of D-mannose residues from GDP-D-mannose into D-mannosylpyrophosphoryldolichol by a particulate enzyme preparation from mung beans.[123] It also inhibited the transfer of 2-acetamido-2-deoxy-D-glucose from UDP-2-acetamido-2-deoxy-D-glucose into 2-acetamido-2-deoxy-D-glucosylphosphorylpolyisoprenol and into glycoproteins. It did not block the transfer of D-glucose residues from UDP-D-glucose into β-D-glucans. The incorporation of D-mannose residues into glycolipids and glycoproteins was also inhibited in carrot roots but the incorporation of L-lysine into protein was not inhibited.

[116] Y. Fujino and T. Miyazawa, *Biochim. Biophys. Acta*, 1979, **572**, 442.
[117] T. Miyazawa and Y. Fujino, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 1979.
[118] Y. Fujino and M. Ohnishi, *Biochim. Biophys. Acta*, 1979, **574**, 94.
[119] A. Koiwai and T. Kisaki, *Agric. Biol. Chem.*, 1979, **43**, 597.
[120] H. Kameyama and C. Urakami, *J. Am. Oil Chem. Soc.*, 1979, **56**, 549.
[121] H. Yoshida and G. Kajimoto, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 1323.
[122] F. C. Phillips and O. S. Privett, *Lipids*, 1979, **14**, 949.
[123] M. C. Ericson, J. T. Gafford, and A. D. Elbein, *Arch. Biochem. Biophys.*, 1978, **191**, 698.

Particulate preparations from the chlorophyta, *Prototheca zopfii*, catalysed the incorporation of D-mannose and 2-acetamido-2-deoxy-D-glucose into glycolipids.[124] The products were similar to those produced by both animal and plant tissue and they also served as precursors for glycoprotein synthesis as well as for cell wall D-mannan. The reactions leading to the formation of glycoprotein took place in the rough endoplasmic reticulum while those synthesizing the D-mannans occurred in the Golgi vesicles.

Dolichylphosphate and dolichylpyrophosphate-activated sugar intermediates were both synthesized by membrane-bound glycosyltransferases in the alga, *Volvox canteri*.[125]

Little differences were observed in the glycolipid contents of cells of the fungus *Aureobasidium pullulans* S-1 when grown in shaking flasks at pH values of 6.0 and 2.5.[126] In jar fermentors, however, the glycolipid content was greater at pH 2.5 than at pH 6.0. The glycolipids were mainly D-glucolipids which were easily hydrolysed to D-glucose, D-gluco-oligosaccharides, and lipid.

A new cerebroside has been isolated from the fungus, *Fusicoccus amygdali*.[127] Its structure was confirmed by chemical degradation studies and application of n.m.r. spectroscopy and m.s. and was found to be *N*-2′-hydroxy-3-*trans*-octadecenoyl-1-*O*-β-D-glucopyranosyl-9-methyl-*cis*-4, χ-8-sphingadiene.

5 Microbial Glycolipids

The cord factor from *Mycobacterium tuberculosum* could be permethylated with methyl iodide and sodium hydride in a diethyl ether–dimethylformamide mixture in the presence of a molecular sieve.[128] It was confirmed that the mycolic acids were esterified at the 6,6′ positions of the αα-trehalose molecule. Using 4-palmitoyl-D-glucose as a model compound, it was verified that acyl migration did not occur in this system.

The cell walls of *M. lepraemurium* have been harvested from the livers of moribund rats that had been infected with the above organism. The walls contained a cord factor (αα-trehalose-6,6′-dimycolate) that was similar in structure to the well-characterized cord factor from *M. tuberculosum*.[129] This is the first unequivocal identification of a cord factor as a product of *in vivo* derived mycobacteria.

O-6 esters of methyl α-D-glucopyranoside and O-6, O-6′ diesters of αα-D-trehalose with *threo*- and *erythro*-mycolic acids containing both 18 and 32 carbon atoms in the chain and a C_{80}-*erythro*-mycolic acid were synthesized and, through a monolayer study at the air–water interface, these glycolipids were shown to form well organized phases in which the two hydrocarbon chains of

[124] P. A. Romero, H. E. Hopp, and R. Pont-Lezica, *Biochim. Biophys. Acta*, 1979, **586**, 545.
[125] E. Bause and L. Jaenicke, *FEBS Lett.*, 1979, **106**, 321.
[126] K. Ono, Y. Kawahara, and S. Ueda, *Agric. Biol. Chem. (Jpn)*, 1977, **41**, 2313.
[127] A. Ballio, C. G. Casinovi, M. Framondino, G. Marino, G. Nota, and B. Santurbano, *Biochim. Biophys. Acta*, 1979, **573**, 51.
[128] M. B. Goren and R. Toubiana, *Biochim. Biophys. Acta*, 1979, **574**, 64.
[129] M. B. Goren, O. Brokl, and P. Roller, *Biochim. Biophys. Acta*, 1979, **574**, 70.

mycoloyl residues must be in interaction.[130] The molecular packing of these glycolipids depends on the presence of the hydroxy-groups of mycoloyl residues and their stereochemistry. In particular, intermolecular hydrogen bonding is predicted for the bis-C_{32} ester. The activity of the diacyl trehaloses on mitochondria was studied with succinate as substrate.[131] The acyl groups had similar activities on phosphorylation but differences were noted of the effects on respiration. Electron microscopy studies suggested that cord factors weakened the peripheral part of the inner membrane but the christae looked unchanged. These observations led the authors to propose a model for the interaction of cord factors with mitochondria.

Pseudo cord factors have been prepared from α-D-glucopyranosyluronic acid-(1 → 1)-α-D-glucopyranosyluronic acid as bis-amides and di-esters of intermediate to high molecular weight.[132] The i.r. spectra of these compounds were similar to authentic cord factors. Several of the compounds had an impressive toxicity to mice and tumour-regression activity; the latter does not depend on the former.

The purification procedure of 6,6′-esters of αα-trehalose from *Corynebacterium diphtheria* has been modified and the products were analysed by m.s. of the permethylated derivative.[133] The constituent fatty acids have been extensively examined and the detailed structure of the major acids have been confirmed by a variety of techniques. Novel glycolipids in the cord factor fraction from *C. diphtheria* were a 6,6′-bis(3-oxo-acyl)-αα-D-trehalose and a 6′,6′-mixed ester of αα-trehalose with the 3-oxo-acyl group and a corynomycolic acid.[134] The metabolic implications of these structural studies suggest that condensation of the common fatty acids by a Claisen-like process *via* the oxo-ester pathway may not be the only pathway for the synthesis of the cord factors.

αα-Trehalose di-mycolates have been isolated from *Nocardia asteroides*, *N. farcinia*, *Gordona lentifragmenta*, and *G. bronchialis*,[135] whereas both mono- and di-mycolates were isolated from *N. caviae*.[136]

The incorporation of *Proteus mirabilis* lipopolysaccharide I or *Salmonella minnesota* Rc lipopolysaccharide into phospholipid vesicles protected these model membranes from disruption by detergents.[137] This suggested that the lipopolysaccharides in the outer membrane of enteric bacteria might contribute a similar protective function against the action of surfactants in the normal intestinal habitat.

A phosphatidyl-α-D-glucopyranosyldiacylglycerol has been isolated from the polar lipid fraction of *Pseudomonas vesicularis* as well as the glycolipids (39)–

[130] E. Durand, M. Welby, G. Laneelle, and J. F. Tocanne, *Eur. J. Biochem.*, 1979, **93**, 103.
[131] E. Durand, M. Gillois, J.-F. Tocanne, and G. Laneelle, *Eur. J. Biochem.*, 1979, **94**, 109.
[132] M. B. Goren and K.-S. Jiang, *Chem. Phys. Lipids*, 1979, **25**, 209.
[133] D. W. Thomas, A. K. Matilda, C. L. Silva, and T. Ioneda, *Chem. Phys. Lipids*, 1969, **23**, 267.
[134] G. Puzo, G. Tissie, H. Aurelle, C. Lacave, and J.-C. Prome, *Eur. J. Biochem.*, 1979, **98**, 99.
[135] C. L. Silva, J. L. Gesztesi, and T. Ioneda, *Chem. Phys. Lipids*, 1979, **24**, 17.
[136] M. T. Pommier and G. Michel, *Chem. Phys. Lipids*, 1979, **24**, 149.
[137] K. Nixdorff, J. Gmeiner, and H. H. Martin, *Biochim. Biophys. Acta*, 1978, **510**, 87.

1,2-di-*O*-acyl-3-*O*-α-D-Glc*p*-glycerol
(39)

1,2-di-*O*-acyl-3-*O*-α-D-Glc*p*UA-glycerol
(40)

1,2-di-*O*-acyl-3-*O*-[β-D-Glc*p*-(1 → 4)-α-D-Glc*p*UA]-glycerol
(41)

(41).[138] A novel lipid containing a heptose residue, 1,2-di-*O*-acyl-3-*O*-α-D-*glycero*-D-*gluco*-heptopyranosylglycerol has also been isolated and characterized.

Two glycolipids, D-glucosyldiglyceride and D-galactosyl-D-glucosyldiglyceride, were the major components of the polar lipid fraction from the membranes of the wild type and an aminopterin-resistant strain of *Streptococcus pneumonia.*[139] No relationship could be established between resistance to aminopterin and either polar lipid or fatty acid composition. However, in the presence of aminopterin, the wild type synthesized a higher proportion of unsaturated fatty acid.

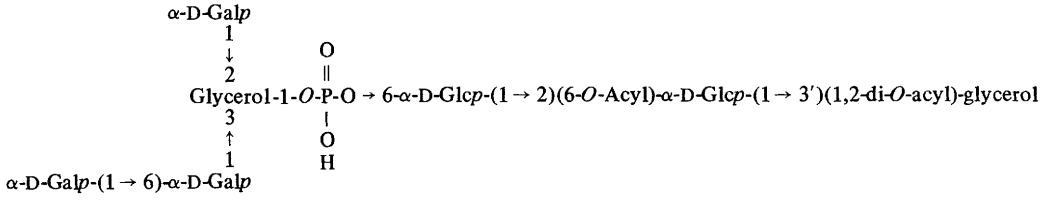
The glycolipid content of *S. faecium* cells accumulated relative to the cellular mass produced when the culture mass doubling time was increased from 30 to 80 min.[140] The accumulation rate of diacylglycerol was even faster.

At least six unusually polar glycolipids have been isolated from *S. lactis.*[141] The predominant one had the structure (42) but they were all based on a backbone of glycerophospho-acylkojibiosyldiacylglycerol.

D-Glucose could be transferred from UDP-D-glucose to endogenous receptors by a particulate enzyme fraction from the cellular slime mould, *Dictyostelium discoideum.*[142] When H_4-edta was incorporated into the medium, the sole product was a mono-D-glucosylceramide that contained a hydroxy fatty acid. Divalent cations were not essential but they could stimulate the reaction, whereas other sugars had little effect on the reaction.

Inositol (2.5%), 17-methylsphinganine (4.8%), and sphinganine (1.5%) have been reported to be constituents of the lipopeptidophosphoglycan from epimastigote forms of the protozoon *Trypanosoma cruzi.*[143]

[138] S. G. Wilkinson and L. Galbraith, *Biochim. Biophys. Acta*, 1979, **575**, 244.
[139] M.-C. Trombe, M.-A. Laneelle, and G. Laneelle, *Biochim. Biophys. Acta*, 1979, **574**, 290.
[140] D. Carson, R. A. Pieringer, and L. Daneo-Moore, *Biochim. Biophys. Acta*, 1979, **575**, 225.
[141] W. Fischer, D. Schuster, and R. A. Laine, *Biochim. Biophys. Acta*, 1979, **575**, 389.
[142] E. V. Crean and E. F. Rossomando, *Arch. Biochem. Biophys.*, 1979, **196**, 186.
[143] R. M. de Lederkremer, O. Casal, C. T. Tanaka, and W. Colli, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 1268.


α-D-Galp
1
↓
2
Glycerol-1-O-P-O → 6-α-D-Glcp-(1 → 2)(6-O-Acyl)-α-D-Glcp-(1 → 3′)(1,2-di-O-acyl)-glycerol
3
↑
1
α-D-Galp-(1 → 6)-α-D-Galp
O
O
H

(42)

8

Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, and Glycolipids

BY C. M. STURGEON

1 Synthesis of Polysaccharides, Oligosaccharides, Glycoproteins, Glycopeptides, and Glycolipids

Recent advances in the synthesis of disaccharides, glycosphingolipids, and oligosaccharides have been described.[1] Progress in synthesizing human blood group antigenic determinants has been reviewed.[2]

Polysaccharides. — Poly(urea-urethane)s containing 2-acetamido-2-deoxy-D-glucosyl residues in the main chain have been prepared by direct addition polymerization of 2-acetamido-2-deoxy-D-glucose and di-isocyanates.[3] The polymers were soluble in polar solvents and exhibited good reactivity toward acetylation and very high water absorption.

Several copolymers of styrene and 2,3 : 4,5-di-*O*-isopropylidene-1-vinylbenzyl-β-D-fructopyranose have been prepared by free-radical initiation.[4] The specific rotation of the copolymers was linearly related to their molar compositions, which also affected the glass transition temperatures.

1,6-Anhydro-2,4-di-*O*-benzyl-3-*O*-but-2-enyl-β-D-glucopyranose has been copolymerized with 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose.[5] Crotyl groups were removed quantitatively and side chains introduced by reaction of the hydroxylated polymers with 2,3,4-tri-*O*-(4-methyl-benzyl)-6-*O*-(*N*-phenyl-carbamoyl)-1-*O*-tosyl-D-glucopyranose. Decarbanilation and debenzylation yielded a family of stereoregular dextrans with randomly distributed 3-*O*-(α-D-glucopyranosyl) side chains, and these dextrans have been compared with naturally occurring microbial dextrans.

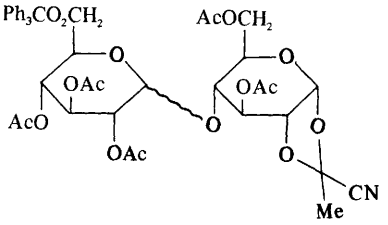
Poly(5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose) has been obtained by anionic ring-opening polymerization of 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose.[6] Phosphorus pentafluoride catalysis of the ring-opening polymerization of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose has been investigated using ^{31}P and ^{19}F n.m.r. spectroscopy, and a polymerization

[1] *Methods Enzymol.*, 1978, **50**.
[2] R. U. Lemieux, *Chem. Soc. Rev.*, 1978, **7**, 423.
[3] K. Kurita, N. Hirakawa, and Y. Iwakura, *Makromol. Chem.*, 1979, **180**, 2331.
[4] W. K. Busfield, F. P. Franke, and R. D. Guthrie, *Aust. J. Chem.*, 1978, **31**, 2559.
[5] H. Ito and C. Schuerch, *J. Am. Chem. Soc.*, 1979, **101**, 5797.
[6] T. Uryu, K. Kitano, and K. Matsuzaki, *Makromol. Chem.*, 1979, **180**, 1135.

mechanism proposed.[7] The cationic ring-opening polymerization of 3,5-anhydro-1,2-*O*-isopropylidene-α-D-xylofuranose has been reported.[8]

Novel polymers which show a high degree of water absorption have been synthesized by direct addition polymerization of α,α-trehalose with di-isocyanates.[9]

Polycondensation of (1) yields a stereoregular polymer (DP 20) identified as a glucan with alternating (1→4)-α-D and (1→6)-β-D linkages.[10] 2,3,6,2′,3′,4′-Hexa-*O*-acetyl-α-D-maltosyl bromide has been polymerized to give a polymer containing maltosyl structures.[11]



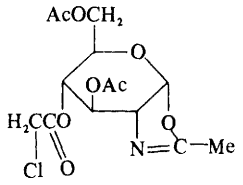
(1)

Oligosaccharides. – A new method of glycosylation involving anomeric hydroxy-group activation by iminium salts has been investigated.[12] Starting from a free hemiacetalic hydroxy group, glycosylation is performed by means of the DMF-carbonyl chloride Vilsmeier-Haack reagent and silver tosylate. The 1,2-*trans*-octa-acetates of cellobiose, lactose, and maltose have been converted into β-glycosides of 8-ethoxycarbonyloctanol in good yield by a single step reaction catalysed by tin(IV) chloride in dichloromethane at −10°.[13] β-Glycosides were initially obtained *via* 1,2-orthoacetate intermediates, but *in situ* anomerization occurred during reaction at room temperature and provided a preparative route to the α-glycosides. Hydrazide derivatives of the disaccharide glycosides were prepared for conversion into artificial carbohydrate antigens.

By reaction of the acetylated glycosyl bromides of lactose, cellobiose, and maltose with silver trifluoromethanesulphonate in *NN*-tetramethylurea, considerably better yields of 1,2-*trans*-glycosides of 8-ethoxycarbonyloctanol are obtained than by conventional Köenigs-Knorr and Helferich conditions.[14] Use of trimethylpyridine as proton acceptor provided good yields of the corresponding 1,2-orthoacetates, which were rearranged to the 1,2-*trans*-glycosides using tin(IV) chloride.

[7] T. Uryu, K. Ito, K. I. Kobayashi, and K. Matsuzaki, *Makromol. Chem.*, 1979, **180**, 1509.
[8] T. Uryu, Y. Koyama, and K. Matsuzaki, *J. Polym. Sci., Polym. Lett.*, 1979, **17**, 673.
[9] K. Kurita, N. Hirakawa, H. Morinaga, and Y. Iwakura, *Makromol. Chem.*, 1979, **180**, 2769.
[10] I. V. Obruchnikov and N. K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1977, **26**, 2574.
[11] N. K. Kochetkov, E. M. Klimov, and M. V. Ovchinnikov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1977, **26**, 1864.
[12] V. Dourtoglou, J. C. Ziegler, and B. Gross, *Tetrahedron Lett.*, 1979, 4371.
[13] J. Banoub and D. R. Bundle, *Can. J. Chem.*, 1979, **57**, 2085.
[14] J. Banoub and D. R. Bundle, *Can. J. Chem.*, 1979, **57**, 2091.

A facile synthesis of [3,6-di-*O*-acetyl-4-*O*-(chloroacetyl)-1,2-dideoxy-α-D-glucopyrano]-[2,1-*d*]-2-methyl-2-oxazoline (2) has been reported.[15] The compound is a valuable intermediate for the sequential synthesis of complex sugar molecules.



(2)

A two-step procedure for the preparation of 4-nitrophenyl-β-D-mannopyranoside from D-mannose involves formation of 2,3 : 4,6-di-*O*-cyclohexylidene-α-D-mannopyranose.[16] Treatment with triphenylphosphine, 4-nitrophenol, and diethyl diazocarbonate, followed by mild acid hydrolysis, yields the crystalline glycoside.

Significant improvement in the synthesis of disaccharides by cycloaddition may be achieved by reaction of diethyl mesoxalate with the dienyl ethers of protected sugars.[17] This more active dienophile adds to both *cis*- and *trans*-ethers, unlike compounds hitherto reported, and more efficient utilization of the protected starting sugar is possible.

The utility of secondary trityl ethers of sugars as aglycones in oligosaccharide synthesis has been demonstrated by glycosylation of various monosaccharide trityl ethers with 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-cyanoethylidene)-α-D-glucopyranose.[18]

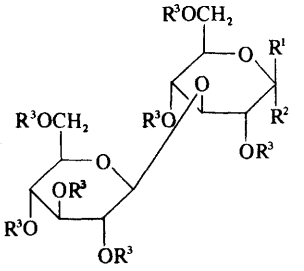
Syntheses of deoxy-derivatives of nigerose have been reported.[19]

A convenient synthetic method for the preparation of laminaribioside (3) and some of its derivatives, *e.g.* methyl α-laminaribioside (4) and methyl (5), benzyl (6), and phenyl (7) β-laminaribioside, involves condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (8) with methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (9).[20]

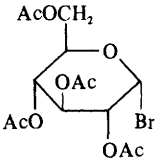
Methyl 3-*O*-β-D-quinovosyl-α-D-olivoside has been synthesized from laminaribiose and characterized as its peracetate.[21]

Using the Helferich modification of the Köenigs–Knorr reaction, methyl 4,6-*O*-benzylidene-α-D-mannopyranoside has been treated with tetra-*O*-acetyl-α-D-mannopyranosyl bromide, yielding methyl 3-*O*-α-D-mannopyranosyl-α-D-mannopyranoside.[22] Benzyl 2,4-di-*O*-benzyl-α-D-mannopyranoside has been

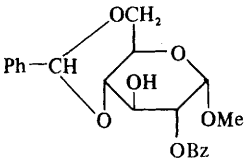
[15] V. K. Handa, J. J. Barlow, and K. L. Matta, *Carbohydr. Res.*, 1979, **76**, C10.
[16] P. J. Garegg, T. Iversen, and T. Norberg, *Carbohydr. Res.*, 1979, **73**, 313.
[17] S. David, J. Eustache, and A. Lubineau, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1795.
[18] Y. V. Wozney, L. V. Backinowsky, and N. K. Kochetkov, *Carbohydr. Res.*, 1979, **73**, 282.
[19] J. Thiem and J. Schwentner, *Chem. Ber.*, 1979, **112**, 3139.
[20] K. Takeo, *Carbohydr. Res.*, 1979, **77**, 245.
[21] J. Thiem and H. Karl, *Chem. Ber.*, 1979, **112**, 1046.
[22] E. E. Lee and J. O. Wood, *Carbohydr. Res.*, 1979, **75**, 322.



(3) R^1, R^2 = H, OH; R^3 = H
(4) R^1 = R^3 = H, R^2 = OMe
(5) R^1 = OMe, R^2 = R^3 = H
(6) R^1 = OBn, R^2 = R^3 = H
(7) R^1 = OPh, R^2 = R^3 = H



(8)



(9)

condensed with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannosyl chloride in toluene–nitromethane, using a mixture of mercury(II) bromide–mercury(II) cyanide as promoter, in another modification of the Köenigs–Knorr reaction which gives high yield of α-D-mannosides.[23] After deacetylation and catalytic hydrogenation 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannose was obtained.

Hepta-*O*-acetyl-α-kojibiosyl bromide has been converted into the β-chloride form by treatment with tetraethylammonium chloride, and then condensed immediately with benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside in an $AgClO_4$–Ag_2CO_3 catalysed reaction.[24] *O*-α-D-Glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl-(1 → 6)-D-glucose was obtained on hydrogenolysis and Zemplén deacetylation.

2-[4-(4-Toluenesulphonamido)phenyl] ethyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside has been condensed with 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-1-*O*-tosyl-D-glucopyranose to yield 2,4-(4-toluene-sulphonamido)-phenyl ethyl 2,3,4,2′,3′,4′-hexa-*O*-benzyl-6′-*O*-(*N*-phenylcarbamoyl)-α-isomaltoside.[25] The disaccharide was decarbanilated in ethanol with sodium ethoxide. The sequence of coupling with the 1-*O*-tosyl-D-glucose derivative followed by decarbanilation was repeated to form the tri- and tetra-saccharide derivatives.

The synthesis of 3-amino-3-deoxy-α-D-glucopyranosyl α-D-glucopyranoside (3-amino-3-deoxy-α,α-trehalose) from 4,6:4′,6′-di-*O*-benzylidene-α,α-trehalose has been reported.[26]

Syntheses have been devised for a number of disaccharides of interest as reference compounds in the study of polysaccharide and glycoprotein structure. Condensation of benzyl 2-acetamido-2-deoxy-3,6-di-*O*-benzyl-α-D-glucopyrano-

[23] J. Arnarp and J. Lönngren, *Acta Chem. Scand., Ser. B*, 1978, **32**, 696.
[24] V. Pozsgay, P. Nánási, and A. Neszmélyi, *Carbohydr. Res.*, 1979, **75**, 310.
[25] R. Eby, *Carbohydr. Res.*, 1979, **70**, 75.
[26] H. H. Baer and A. J. Bell, *Carbohydr. Res.*, 1979, **75**, 175.

side with 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*N*-methyl)acetimidoyl-β-D-glucopyranose followed by catalytic hydrogenation yielded crystalline 2-acetamido-2-deoxy-4-*O*-α-D-glucopyranosyl-α-D-glucopyranose (*N*-acetyl-maltosamine).[27] An alternative route was also reported. 2-Acetamido-2-deoxy-4-*O*-β-D-glucopyranosyl-α-D-glucopyranose has been synthesized by condensation of benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of mercury(II) bromide, followed by deacetylation and catalytic hydrogenolysis of the condensation product. A simple synthetic method for the preparation of 2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-D-glucose (Scheme 1) and -D-mannose from 3-*O*-β-D-galactopyranosyl-D-arabinose has been reported.[28] Using this method the products are 4 to 1 in favour of the 2-epimer (10), while previous methods (Kiliani reaction or nitromethane addition) favour the other epimer.

2-Acetamido-2-deoxy-3-*O*-, -4-*O*-, and -6-*O*-α-D-mannopyranosyl-D-glucoses have been prepared by condensation of tetra-*O*-acetyl-α-D-mannopyranosyl bromide with benzyl 2-acetamido-2-deoxy-4,6-*O*-(4-methoxybenzylidene)-α-D-glucopyranoside, benzyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside, and 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose, respectively.[29] 4-Methoxy-benzylidene groups were then removed with 80% acetic acid at 100 °C, *O*-acetyl groups by methanolic triethylamine or ammonia, and *O*-benzyl by hydrogenolysis. The anomeric configuration of the α-D-mannopyranosyl linkage in the disaccharides was confirmed on the basis of their susceptibility to hydrolysis by the α-D-mannosidase from *Aspergillus niger*.

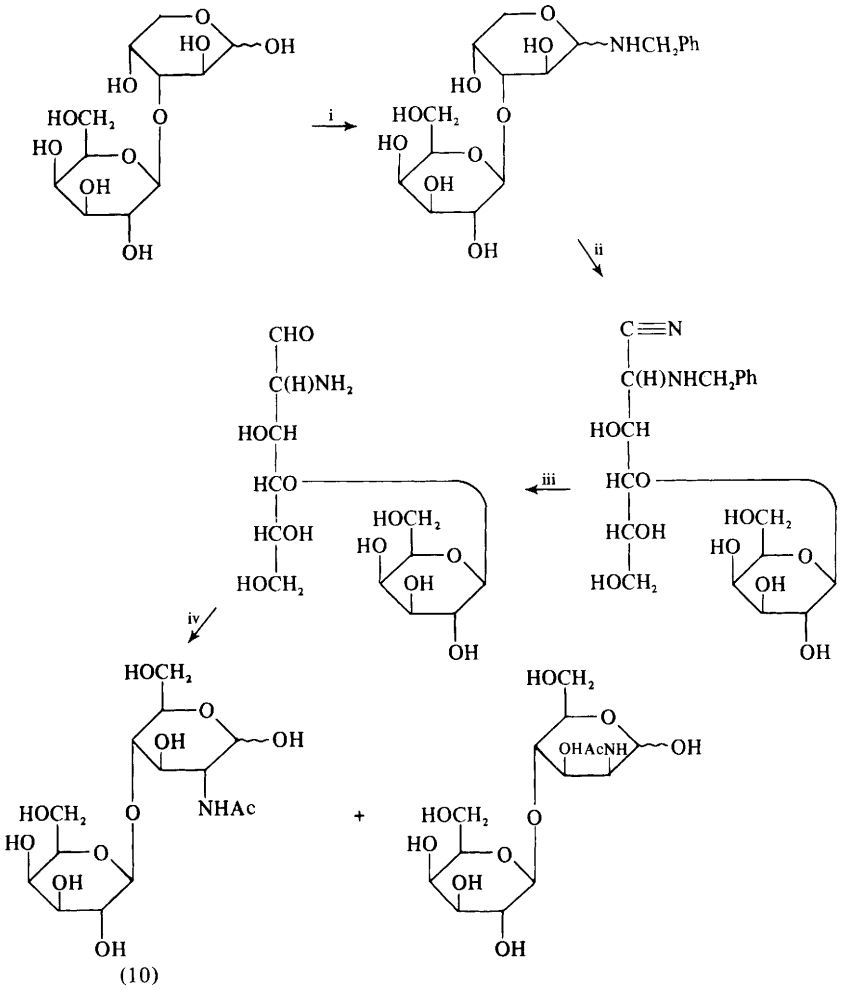
2-*O*-β-D-Galactopyranosyl-D-galactose has been synthesized for the first time.[30] The product obtained after reaction of methyl 4,6-*O*-benzylidene-α-D-galactopyranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, deprotection and separation from contaminants yielded D-galactose only on hydrolysis with crystalline β-D-galactosidase from *Escherichia coli*.

6-*O*-Acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl chloride reacts with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose or 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose with good stereoselectivity to yield α,α-trehalosamine or α,α-mannotrehalosamine (Scheme 2).[31] Several other α,α-(1 → 1)-linked disaccharides of the trehalosamine type and containing additional azido-groups were also synthesized.

Modification of mannobiose (4-*O*-α-D-mannopyranosyl-D-mannose) yielded the di-iodo compounds (11) and (12), which may be reduced with nickel(II) chloride–sodium borohydride to yield the glycosides (13) and (14) of 4-*O*-(β-D-rhamnopyranosyl)-α-D-rhamnopyranose or 4-*O*-(β-D-rhamnopyranosyl)-α-D-olivopyranose, respectively.[32]

Synthesis of (1 → 4)-linked disaccharide glycosides of the 2,3-anhydro-4-*O*-(hex-2-enopyranosyl)-α-D-allopyranoside type has been reported (Scheme 3).[33]

[27] M. A. M. Nassr, J. C. Jacquinet, and P. Sinaÿ, *Carbohydr. Res.*, 1979, **77**, 99.
[28] R. T. Lee and Y. C. Lee, *Carbohydr. Res.*, 1979, **77**, 270.
[29] K. L. Matta, R. H. Shah, and O. P. Bahl, *Carbohydr. Res.*, 1979, **77**, 255.
[30] T. Toba and S. Adachi, *Carbohydr. Res.*, 1979, **75**, C24.
[31] H. Paulsen and B. Sumfleth, *Chem. Ber.*, 1979, **112**, 3203.
[32] J. Thiem and A. Sievers, *Chem. Ber.*, 1979, **112**, 1035.
[33] J. Thiem, J. Schwentner, E. Schüttpelz, and J. Kopf, *Chem. Ber.*, 1979, **112**, 1023.



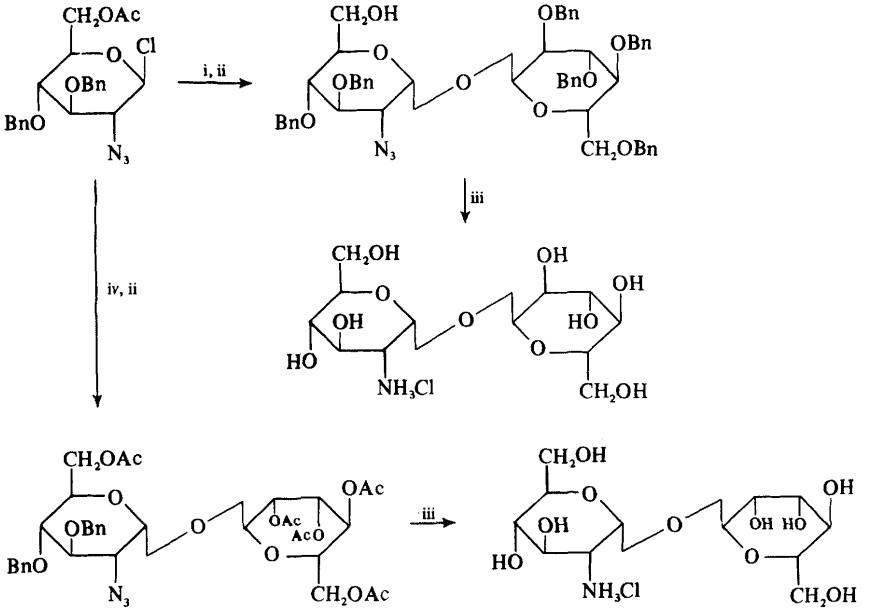
(10)

Reagents: i, $PhCH_2NH_2$; ii, HCN; iii, H_2–Pd; iv, Ac_2O

Scheme 1

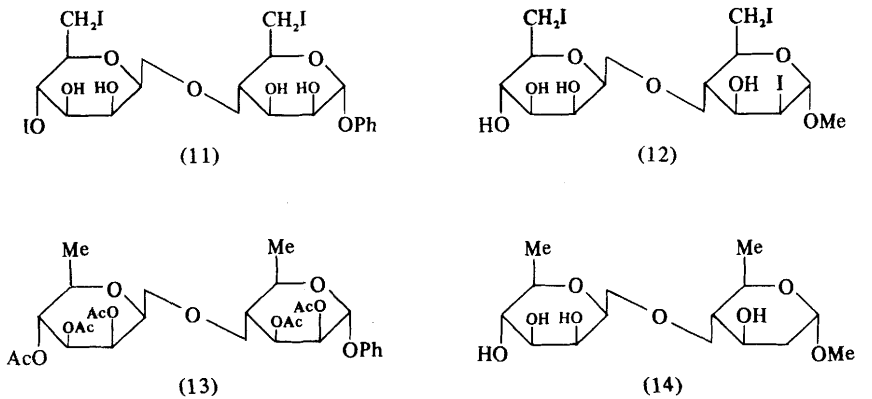
1,2,3,6,2′,3′-Hexa-*O*-acetyl-β-lactose has been synthesized from lactose and treated with 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide in a Köenigs–Knorr condensation.[34] After Zemplén deacetylation *O*-β-L-fucopyranosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose was obtained. Preparation of 2,4,6-tri-*O*-methyl-D-galactose by hydrolysis of the trisaccharide and from methyl α-D-galactopyranoside was also described.

[34] H. H. Baer and S. A. Abbas, *Carbohydr. Res.*, 1979, **77**, 117.



Reagents: i, 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose; ii, $AgClO_4$–*sym*-collidine; iii, Na-liq. NH_3; iv, 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose

Scheme 2

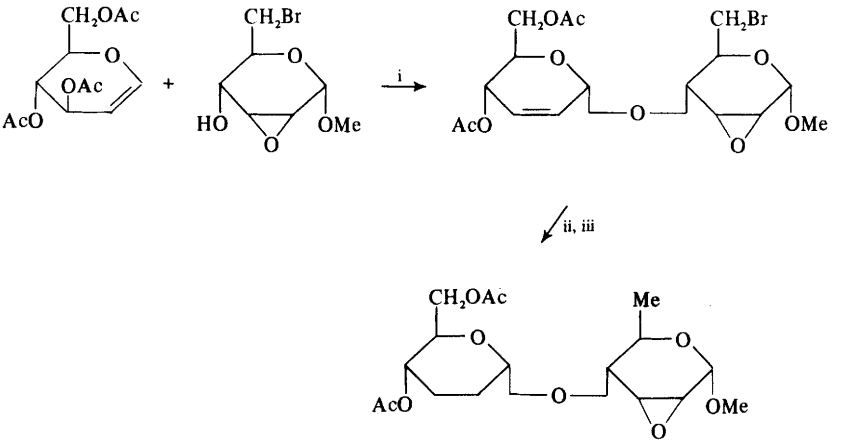


(11) (12) (13) (14)

The synthesis of methyl *O*-β-D-galactopyranosyl-(1 → 6)-*O*-α-D-galactopyranosyl-(1 → 6)-*O*-α-D-galactopyranosyl-(1 → 6)-*O*-β-D-glucopyranoside from stachyose has been reported.[35]

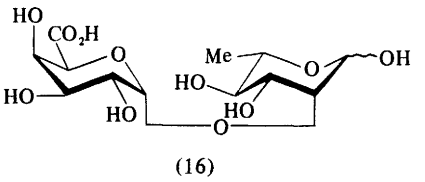
The 1,2′:1′,2-dianhydride of 3,4-di-*O*-acetyl-β-L-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-D-galactopyranuronate (15) has been synthesized from

[35] T. G. Chung, H. Ishihara, and S. Tejima, *Chem. Pharm. Bull.*, 1979, **27**, 1589.

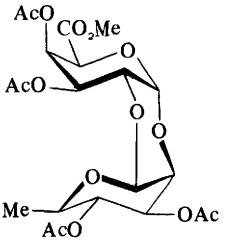


Reagents: i, $BF_3 \cdot Et_2O$; ii, Pd–C–$BaCO_3$–H_2; iii, Raney Ni–H_2

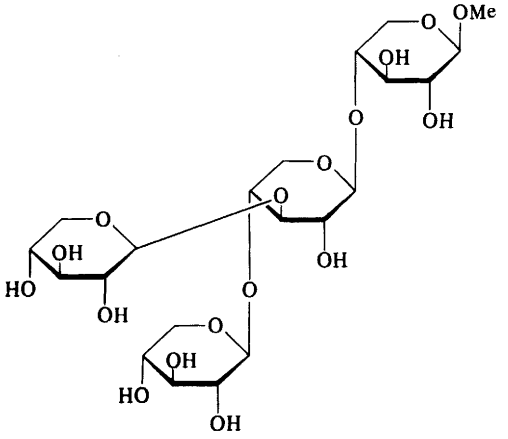
Scheme 3



(16)



(15)



(17)

2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnopyranose (16), demonstrating that the dianhydride is a cyclization product of (16).[36]

Methyl 2-*O*-β-D-xylopyranosyl-D-galactopyranuronate, a pseudoaldobiouronic ester, has been synthesized by a route in which the key step was the condensation of methyl (benzyl 3,4-*O*-isopropylidene-β-D-galactopyranosid)uronate with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide, followed by removal of the protecting groups.[37]

The reaction of methyl 3-*O*-benzyl-β-D-xylopyranoside with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide in acetonitrile in the presence of mercury(II) cyanide yields crystalline methyl 3-*O*-benzyl-2,4-di-*O*-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)-β-D-xylopyranoside.[38] Debenzylation and deacetylation gave 2,4-di-*O*-β-D-xylopyranosyl-β-D-xylopyranoside. Methyl 4-*O*-(2-*O*-β-D-xylopyranosyl-β-D-xylopyranosyl)-β-D-xylopyranoside was also synthesized from methyl 3-anhydro-(3,4-di-*O*-acetyl-β-D-xylopyranosyl)-β-D-ribopyranoside and 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide.

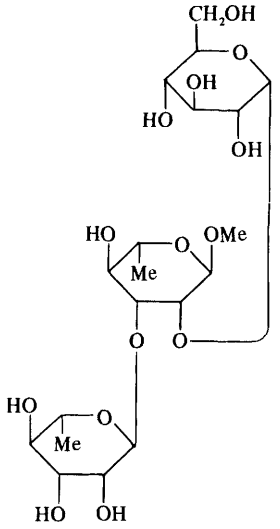
The stepwise synthesis of methyl 4-*O*-[3,4-di-*O*-(β-D-xylopyranosyl)-β-D-xylopyranosyl]-β-D-xylopyranoside (17), a methyl xylotetraoside related to branched xylans, has been achieved by stereoselective condensation of 2,4-di-*O*-acetyl-2-*O*-benzyl-α-D-xylopyranosyl bromide with methyl 2,3-anhydro-β-D-ribopyranoside and reaction of the deacetylated product with 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide.[39]

Considerable interest continues to be taken in the synthesis of the repeating units and antigenic fragments of bacterial polysaccharides for use in structural investigations, and in immunological studies of the relationship between structure and biological specificity, and as artificial antigens. An improved synthesis of β-D-mannopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 3)-D-galactose, the repeating unit of the specific polysaccharide of *Salmonella newington*, has been reported.[40] The trisaccharide methyl α-L-glycoside (18), representing the branching point of the immunogenic determinant of the *Streptococcus pneumoniae* type II antigenic polysaccharide, has been synthesized.[41]

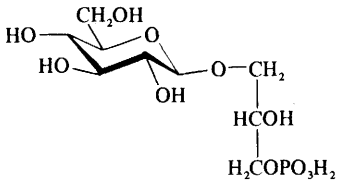
Stereocontrolled synthesis of 1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3-*O*-isopropylidene-D-glycerol has been achieved in good yield by use of the modified orthoester method.[42] The compound was then transformed into 1-deoxy-3-*O*-phosphono-D-glycerol-1-yl β-D-glucopyranoside (19) in a regiocontrolled way and unambiguously shown to be identical to the repeating unit of the teichoic acid isolated from the cell wall of *Bacillus subtilis* var *niger*.

Use of 1,2-isopropylidene-4,6-*O*-ethylidene-α-D-galactopyranose for the synthesis of 3-*O*-substituted galactopyranosyl residues has been described and

[36] T. Fujiwara and K. Arai, *Carbohydr. Res.*, 1979, **69**, 107.
[37] D. J. Poll and T. Doornbos, *Carbohydr. Res.*, 1979, **73**, 67.
[38] J. Hirsch and P. Kováč, *Carbohydr. Res.*, 1979, **77**, 241.
[39] P. Kováč, J. Hirsch, and V. Kováčik, *Carbohydr. Res.*, 1979, **75**, 109.
[40] N. K. Kochetkov, B. A. Dmitriev, and A. V. Nikolaev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1977, **26**, 2578.
[41] D. Schwarzenbach and R. W. Jeanloz, *Carbohydr. Res.*, 1979, **77**, C5.
[42] T. Ogawa, K. Katano, and M. Matsui, *Carbohydr. Res.*, 1979, **70**, 37.



(18)



(19)

α-L-Rha*p*-(1 → 3)-β-D-Glc*pN*Ac-(1 → R′) (20)

α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → R′) (21)

β-D-Glc*pN*Ac-(1 → 2)-α-L-Rha*p*-(1 → R′) (22)

} where R′ = $O(CH_2)_8CO_2Me$

the reagent has been applied to the synthesis of bacterial antigenic polysaccharides.[43]

Three disaccharides [(20)–(22)] representing structural elements similar to portions of the repeating unit of the *Shigella flexneri* O-antigen have been synthesized.[44] The glycosylation reactions utilized silver trifluoromethane-

[43] N. K. Kochetkov, B. A. Dmitriev, A. V. Nikolaev, and N. E. Bairamova, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1977, **26**, 1609.

[44] D. R. Bundle and S. Josephson, *Can. J. Chem.*, 1979, **57**, 662.

sulphonate and *NN*-tetramethylurea or *sym*-collidine, conditions which generated the three 1,2-*trans*-linked glycosides in high yield and with good stereospecificity. Utilizing silver trifluoromethanesulphonate-promoted Köenigs–Knorr reaction conditions, the *Shigella flexneri* trisaccharide hapten 8-methoxycarbonyloctyl *O*-α-L-rhamnopyranosyl-(1 → 3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-α-L-rhamnopyranoside has been synthesized in good yield.[45] 4-Nitrophenyl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-L-rhamnopyranoside has been synthesized from 4-nitrophenyl α-L-rhamnopyranoside.[46]

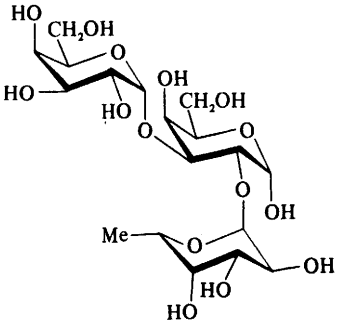
2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-1-*O*-tosyl-D-mannopyranose and 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-1-*O*-tosyl-D-glucopyranose have been treated with partially blocked 2-[4-(4-toluene sulphonamido)phenyl] ethyl α-D-manno- and D-gluco-pyranosides.[47] Disaccharides having α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranose, α-D-mannopyranosyl-(1 → 6)-α-D-glucopyranose, α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranose, and α-D-glucopyranosyl-(1 → 6)-α-D-mannopyranose structures, and a trisaccharide having the structure α-D-mannopyranosyl-(1 → 2)-[α-D-mannopyranosyl-(1 → 6)]-α-D-mannopyranose were synthesized.

The synthesis of blood-group antigenic determinants continues to arouse considerable interest.[2] The blood group B antigenic determinant, α-L-fucopyranosyl-(1 → 2)-[α-D-galactopyranosyl-(1 → 3)]-D-galactopyranose (23) has been prepared using the dibutylstannylene derivative (24) which was coupled with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride in *NN'N"*-hexamethylphosphorotriamide in the presence of lithium iodide.[48] These reaction conditions were found to be superior to those conventionally used for halide-catalysed glycosidation reactions. The product (25) was condensed with 2,3,4-tri-*O*-benzyl bromide and on deblocking the trisaccharide (23) was obtained.

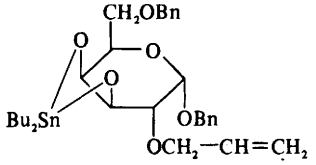
Use of stannylene compounds was not found to result in activation or selectivity in the reactions with benzylated glycosyl halides. The same trisaccharide has also been synthesized stereospecifically using the imidate procedure.[49] Allyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside was first α-L-fucosylated by 1-*O*-(*N*-methyl)-acetimidyl-2,3,4-tri-*O*-benzyl-β-L-fucopyranose and then, after *O*-benzoylation, α-D-galactosylated by 1-*O*-(*N*-methyl)-acetimidyl-2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranose. The resulting trisaccharide was also obtained from allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranoside after α-D-galactosylation, *O*-debenzoylation, and α-L-fucosylation. Deallylation followed by catalytic hydrogenolysis yielded (26).

Oligosaccharide antigenic determinants of blood-group substances A, B, and H have been synthesized by selective glycosidation reactions combined with a series of blocking and deblocking steps.[50] All three oligosaccharides selectively inhibited haemagglutination with the corresponding human antibodies or lectins.

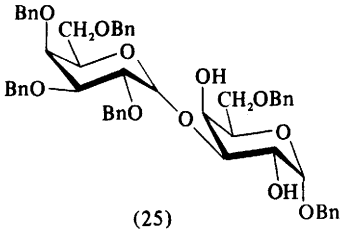
[45] D. R. Bundle and S. Josephson, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2736.
[46] P. J. Garegg and H. Hultberg, *Carbohydr. Res.*, 1979, **72**, 276.
[47] R. Eby and C. Schuerch, *Carbohydr. Res.*, 1979, **77**, 61.
[48] C. Auge and A. Veyrières, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1825.
[49] J. C. Jacquinet and P. Sinaÿ, *Tetrahedron*, 1979, **35**, 365.
[50] H. Paulsen and C. Kolář, *Chem. Ber.*, 1979, **112**, 3190.



(23)



(24)



(25)

α-L-Fuc*p*-(1→2)-D-Gal*p*
3
↑
1
α-D-Gal*p*

(26)

The synthesis of *O*-α-L-fucopyranosyl-(1→3)-[*O*-β-D-galactopyranosyl-(1→4)]-2-acetamido-2-deoxy-D-glucopyranoside, a trisaccharide located at the 'non-reducing end' of numerous oligosaccharides or glycoconjugates of human origin, has been reported.[51] The imidate procedure for α-glycoside production has been applied successfully to the preparation of 2-acetamido-2-deoxy-4-*O*-(α-L-fucopyranosyl)-3-*O*-(β-D-galactopyranosyl)-D-glucopyranose, 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-D-glucopyranose, and 2-acetamido-2-deoxy-4-*O*-(α-L-fucopyranosyl)-D-glucopyranose.[52]

Glycoproteins. — Pseudoglycoproteins have been synthesized by treating proteins with carbohydrates (see *e.g.*, Tables 1–5), chiefly for use in affinity chromatography. Oligosaccharides coupled to bovine serum albumin for use as immunogens include chito-oligosaccharides (also coupled to cytochrome *c*),[53] lactose covalently attached to 8-ethoxycarbonyloctanol,[54] 4-isothiocyanato-

[51] J. C. Jacquinet and P. Sinaÿ, *J. Chem. Soc., Perkin Trans. 1*, 1979, 314.
[52] J. C. Jacquinet and P. Sinaÿ, *J. Chem. Soc., Perkin Trans. 1*, 1979, 319.
[53] P. R. Anderas, D. Paul, J. T. Tomita, and B. Anderson, *Mol. Immunol.*, 1979, **16**, 341.
[54] D. R. Bundle, *Can. J. Biochem.*, 1979, **57**, 367.

phenyl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-L-rhamnopyranoside (artificial *Salmonella* O-factor 8),[46] 4-isothiocyanatophenyl-3-*O*-(3,6-dideoxy-D-*arabino*-hexopyranosyl) α-D-mannopyranoside (*i.e.* 4-isothiocyanatophenyl 3-*O*-α-tyvelopyranosyl-α-D-mannopyranoside, artificial *Salmonella* O-factor 9),[55,56] synthetic antigenic determinants for yeast D-mannans and a linear (1 → 6)-α-D-gluco-D-mannan,[47] and 2-(4-aminophenyl)ethyl α-isomalto-oligosaccharides.[25] The last were also coupled with edestin. Artificial *Salmonella* vaccines have been prepared by covalently linking the 2-(4-isothiocyanatophenyl)ethylamine derivative of an octasaccharide obtained by enzymic hydrolysis of the O-antigenic polysaccharide chain of *S. typhimurium* to diphtheria toxin, edestin, or purified outer membrane proteins ('porins') from *S. typhimurium*.[57] All vaccines thus prepared induced significant protection against experimental *S. typhimurium* infection in mice. Mannotetraose (27), lacto-*N*-fucopentaose III (28), and lacto-*N*-tetraose (29) have been coupled to edestin, and the specificity of the anti-carbohydrate antibodies that they elicit in rabbits investigated.[58]

α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*(1→2)-D-Man

(27)

β-D-Gal*p*-(1→4)-α-D-Fuc*p*-(1→3)-β-D-Glc*pN*Ac-(1→3)-β-D-Gal*p*-(1→4)-D-Glc

(28)

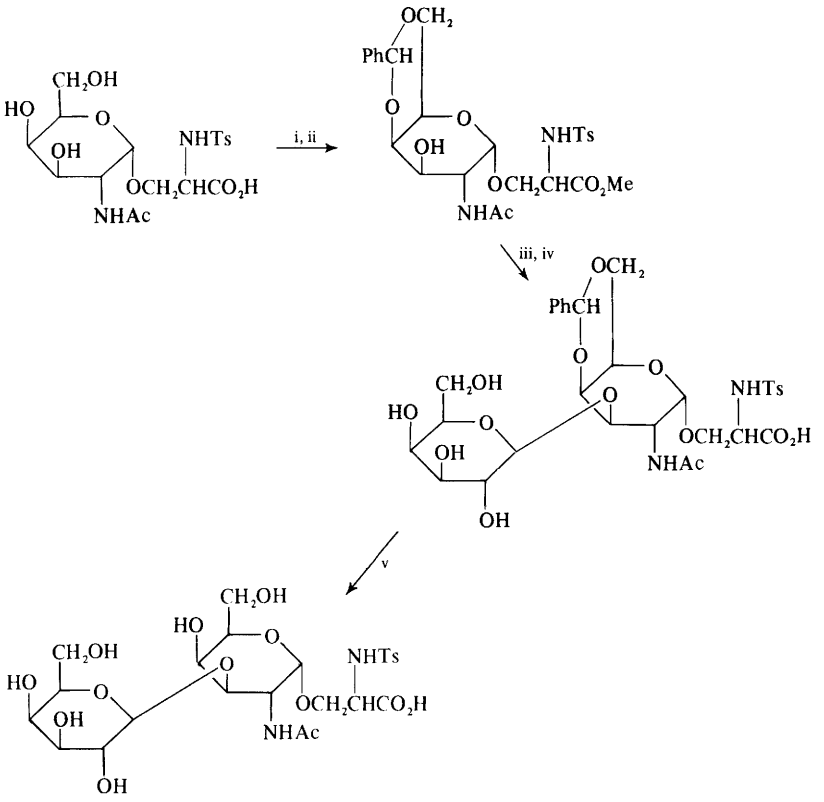
β-D-Gal*p*(1→3)-β-D-Glc*pN*Ac-(1→3)-β-D-Gal*p*-(1→4)-D-Glc

(29)

D-Mannose, D-glucose, and 2-acetamido-2-deoxy-D-glucose have been coupled to albumin to form pseudoglycoproteins for use in a study of receptor-mediated binding of glycoproteins by alveolar macrophages.[59] Fluoresceinyl derivatives of D-mannose, lactose, 2-acetamido-2-deoxy-α-D-galactose, and L-fucose have been covalently attached to albumin and used for direct visualization of membrane lectins *in situ*.[60]

The influence of glycosylation on tissue uptake of enzymes has been investigated. Lactose and *N*-acetylneuraminyl-lactose have been coupled to *Escherichia coli* L-asparaginase by reductive amination with sodium cyanoborohydride (see Scheme 2, Vol. 10, Part II, Chapter 8, p. 428).[61] Oligosaccharides obtained from

[55] H. E. Carlsson and A. A. Lindberg, *Acta Pathol. Microbiol. Scand.*, 1978, **C86**, 237.
[56] H. Jörbeck, H. E. Carlsson, S. B. Svenson, A. A. Lindberg, G. Alfredsson, P. J. Garegg, S. Svensson, and N. H. Wallin, *Int. Arch. All. Appl. Immunol.*, 1979, **58**, 11.
[57] S. B. Svenson, M. Nurminen, and A. A. Lindberg, *Infect. Immun.*, 1979, **25**, 863.
[58] D. A. Zopf, C. M. Tsai, and V. Ginsburg, *Arch. Biochem. Biophys.*, 1978, **185**, 61.
[59] D. Stahl, J. S. Rodman, M. J. Miller, and P. H. Schlesinger, *Proc. Natl. Acad. Sci., USA*, 1978, **75**, 1399.
[60] C. Kieda, A. C. Roche, F. Delmotte, and M. Monsigny, *FEBS Lett.*, 1979, **99**, 329.
[61] J. W. Marsh, J. Denis, and J. C. Wriston, *J. Biol. Chem.*, 1977, **252**, 7678.



(Ts = tosyl group)

Reagents: i, methylation; ii, $PhCHO–ZnCl_2$; iii, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide–$Hg(CN)_2$; iv, Dowex-1 ion exchange resin, elution with 0.1M HCl–30% aq. C_2H_5OH; v, approx. 16 h incubation, 16 °C

Scheme 4

acetolysis of bakers' yeast D-mannan have been coupled to the cross-linked dimer of ribonuclease A and to human serum albumin by the same method.[62]

Glycopeptides. – Treatment with sodium in liquid ammonia has been used as a novel method of benzyl- and benzylidene-group deprotection in glycopeptide synthesis.[63]

O^3-{*O*-β-D-Galactopyranosyl-(1→3)-*O*-[2-acetamido-2-deoxy-α (and β)-D-galactopyranosyl]}-*N*-tosyl-L-serine have been synthesized by the route shown in Scheme 4.[64] The interaction of the synthetic compounds with D-galactose-binding lectins was investigated.

[62] G. Wilson, M. Eidelberg, and V. Michalak, *J. Gen. Physiol.*, 1979, **74**, 495.

[63] M. Zaoral, J. Ježek, R. Straka, and K. Mašek, *Collect. Czech. Chem. Commun.*, 1978, **43**, 1797.

[64] R. Kaifu and T. Osawa, *Carbohydr. Res.*, 1979, **69**, 79.

Two dimers, the galactosyl bromide 2,4,6-tri-*O*-benzoyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-α-D-galactopyranosyl bromide, and the D-xylosyl-L-serine derivative, O^3-(2,3-di-*O*-benzoyl-β-D-xylopyranosyl)-*N*-carbobenzyloxy-L-serine benzyl ester, have been synthesized and then condensed and the product deblocked to yield (30).[65]

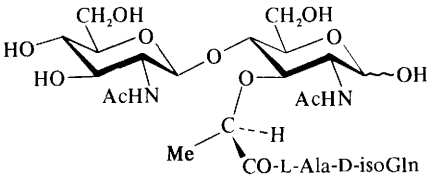
β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Xyl*p*-(1 → L-Ser

(30)

Syntheses have been reported for a number of glycopeptide derivatives containing the N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl) hydrogen L-asparaginate linkage and having the amino-acid sequences 32–34, 33–35, 33–37, and 33–38 of bovine ribonuclease.[66]

Di- and tri-peptides having an O^3-(2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-serine residue have been synthesized and the mechanism of the β-elimination reaction undergone by the derivatives investigated.[67]

O-(2-Acetamido-2-deoxy-β-D-glucosyl)-(1 → 4)-*N*-acetyl muramoyl-L-alanyl-D-isoglutamine (31), the dipeptide derivative of the disaccharide isolated from lysozyme digests of bacterial cell walls, has been prepared in a fourteen-step sequence from 2-acetamido-2-deoxy-D-glucose.[68] The dipeptide disaccharide derivative (32) isolated from lysostaphin *endo*-β-D-2-acetamido-2-deoxy-glucanase digests of cell walls has also been synthesized (Scheme 5).[69]

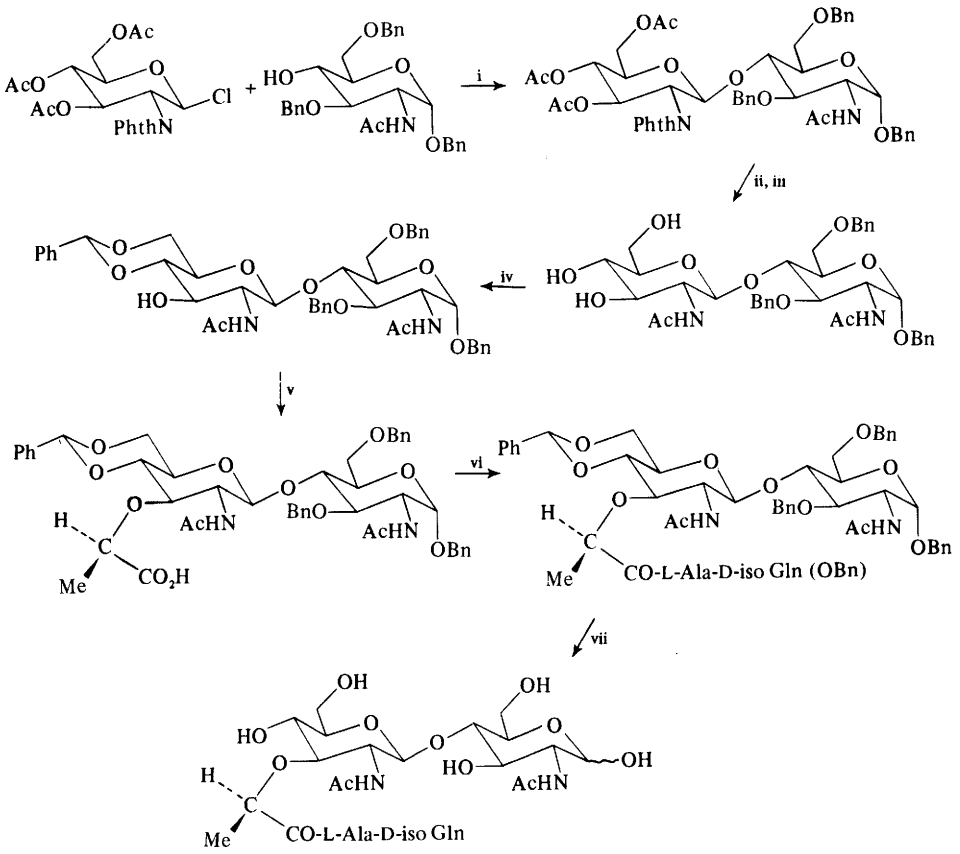


(31)

Glycolipids. — A convenient synthesis of D-glucosyl phosphatidyl glycerol *via* a phosphotriester intermediate has been reported (Scheme 6).[70] Use of the pivaloyl (*i.e.*, 2,2-dimethylpropanoyl) group for protection of one of the *sn*-glycerol primary hydroxy-groups was advantageous: it could be introduced selectively, no migration of the group was observed, and its removal could also be accomplished selectively to yield a free primary hydroxy-function.

Starting from 2-acetamido-2-deoxy-4,6-di-*O*-acetyl-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranose (33), a crystalline intermediate prepared by a conventional sequence of reactions, the total synthesis of

[65] P. J. Garegg, B. Lindberg, and T. Norberg, *Acta Chem. Scand., Ser.B*, 1979, **33**, 449.
[66] H. G. Garg and R. W. Jeanloz, *Carbohydr. Res.*, 1979, **70**, 47.
[67] H. G. Garg and R. W. Jeanloz, *Carbohydr. Res.*, 1979, **76**, 85.
[68] P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Carbohydr. Res.*, 1979, **77**, C1.
[69] P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Tetrahedron Lett.*, 1979, 4013.
[70] C. A. A. Van Boeckel and J. H. Van Boom, *Tetrahedron Lett.*, 1979, 3561.



(32)

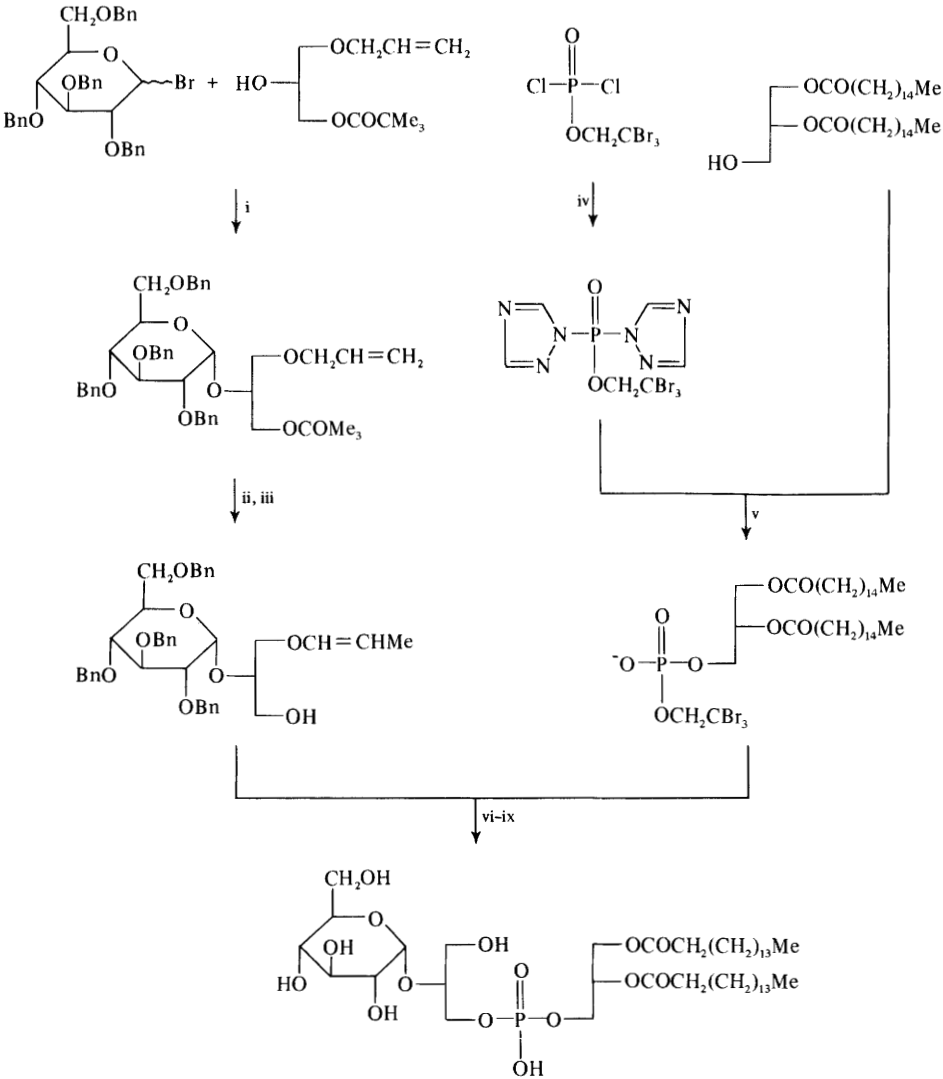
Phth = phthaloyl

Reagents: $MeNO_2$–silver triflate–collidine; ii, Bu^nNH_2–MeOH; iii, Ac_2O–MeOH; iv, PhCHO–$ZnCl_2$; v, (*s*)-2-chloropropionic acid–dioxane–NaH; L-Ala-D-isoGln benzyl ester hydrochloride–*N*-methylmorpholine–isobutyl chloroformate–DMF; vii, H_2–Pd–$MeCO_2H$

Scheme 5

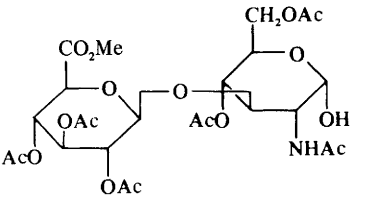
P^2-dolichyl-P^1-2-acetamido-2-deoxy-3-*O*-(D-glucopyranosyl uronic acid)-D-glucose pyrophosphate (34) has been achieved.[71] One of the key steps involved the transformation of (33) into the methyloxazoline (35) which was then converted into the stable crystalline disaccharide phosphate derivative in approximately 30% yield.

[71] E. Walker-Nasir and R. W. Jeanloz, *Carbohydr. Res.*, 1979, **68**, 343.

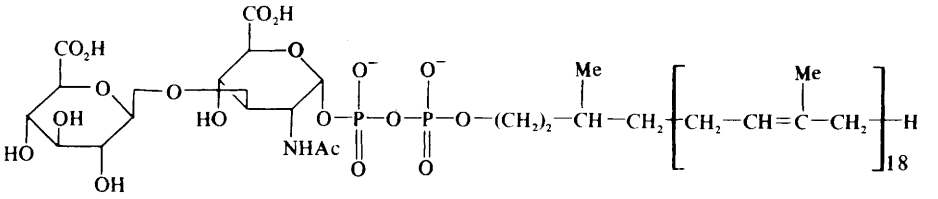


Reagents: i, $Pr^{i}_{2}NEt$–$Et_4\overset{+}{N}\overset{-}{Br}$; ii, $Bu_4\overset{+}{N}\overset{-}{O}H$; iii, $(Ph_3P)_3Rh\overset{-}{Cl}$; iv, triazole–$Et_3N$; v, H_2O; vi, 2,4,6-tri-isopropylbenzenesulphonyl-3-nitro-1,2,4-triazolide; vii, $HgCl_2$–HgO; viii, Zn–pyr–pentane-2,4-dione; ix, H_2–Pd–C

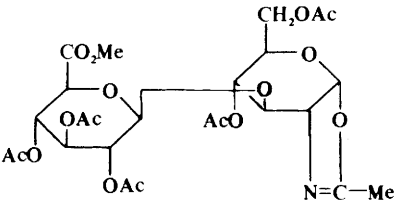
Scheme 6



(33)



(34)



(35)

2 Modification of Polysaccharides and Oligosaccharides, and Uses of Modified Polysaccharides and Oligosaccharides

Introduction. – Synthetic derivatives of polysaccharides have been discussed.[72] The use of polysaccharides as drug carriers in biology and medicine has been described.[73] Summaries of recent literature on molecular immobilization and bioaffinity phenomena, much of which involves use of polysaccharide matrices, are being published at regular intervals.[74–77] The proceedings of a Symposium concerning new developments in chromatography and electrophoresis have been published,[78] and progress in protein purification methods involving hydrophobic interactions has been reviewed.[79]

[72] J. F. Kennedy and C. A. White, in 'Comprehensive Organic Chemistry', Pergamon, Oxford, 1979, Vol. 5, p. 755.

[73] 'Drug Carriers in Biology and Medicine', ed. G. Gregoriadis, Academic Press, New York, 1979.

[74] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Technol.*, 1979, **1**, 53.

[75] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Technol.*, 1979, **1**, 129.

[76] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Technol.*, 1979, **1**, 210.

[77] C. M. Sturgeon and J. F. Kennedy, *Enzyme Microb. Technol.*, 1979, **1**, 290.

[78] 'Recent Developments in Chromatography and Electrophoresis', ed. A. Frigerio and L. Renoz, Elsevier, Amsterdam, 1979.

[79] R. J. Yon, *Int. J. Biochem.*, 1978, **9**, 373.

The importance of polysaccharides as support media in the field of affinity chromatography continues, as numerous reviews have indicated. Quantitative uses of affinity chromatography in purification, functional characterization, determination of rate constants, and measurement of macromolecule–ligand equilibrium binding have been discussed.[80] Improvement of affinity chromatographic separations may be achieved by use of mixed solvents at carefully controlled concentrations and at temperatures in the normal to sub-zero range.[81] Results of a theoretical study of the concentration dependence of elution volume for particular ligands have been presented.[82]

The measurement of the ligand content of affinity gels by an 'in-gel' method has been described.[83]

Review articles have appeared describing affinity chromatography using immobilized cyclic nucleotides for the purification of cyclic nucleotide receptor proteins,[84] using immobilized lectins for cell-membrane glycoprotein purification[85] and for cell fractionation,[86] using immobilized nucleic acids,[87] using immobilized pyridoxal 5′-phosphate for purification of pyridoxal 5′-phosphate-dependent enzymes,[88] and using immobilized spermidine.[89] Application of affinity chromatography to the purification of calcitonin has been discussed.[90] Protein purification with immobilized triazine dyes has been comprehensively reviewed.[91]

Theoretical aspects of affinity electrophoresis have been discussed and the applicability of the technique for determination of dissociation constants of protein–ligand complexes demonstrated.[92] The pH dependence of lectin interactions with sugars has been determined by affinity electrophoresis.[93] Association constants of dextrans and isomaltose oligosaccharides for myeloma anti-dextran have been determined using the technique.[94]

Agarose. – Agarose has been used without modification as an affinity chromatographic support for the separation of Abrin A and Abrin B, anti-tumour proteins from *Abrus precatorius*.[95] Abrin A binds to the support and is eluted with D-galactose but Abrin B does not bind.

[80] I. M. Chaiken, *Anal. Biochem.*, 1979, **97**, 1.
[81] P. Douzou, *Trends Biochem. Sci.*, 1979, **4**, N205.
[82] Y. Husimi, *Biopolymers*, 1979, **18**, 1023.
[83] T. Korpela and E. Mäkinen, *J. Chromatogr.*, 1978, **166**, 268.
[84] W. L. Dills, C. D. Goodwin, T. M. Lincoln, J. A. Beavo, P. J. Bechtel, J. D. Corbin, and E. G. Krebs, *Adv. Cyclic Nucleotide Res.*, 1979, **10**, 199.
[85] R. Lotan and G. L. Nicolson, *Biochim. Biophys. Acta*, 1979, **559**, 329.
[86] Y. Reisner and N. Sharon, *Trends Biochem. Sci.*, 1980, **5**, 29.
[87] H. Potuzak and P. D. G. Dean, *FEBS Lett.*, 1978, **88**, 161.
[88] S. Ikeda and S. Fukui, *Methods Enzymol.*, 1979, **62**, 517.
[89] O. Oleinik and D. R. Morris, *Adv. Polyamine Res.*, 1978, **1**, 69.
[90] A. H. Tashjian and E. F. Voelkel, in 'Methods of Hormone Radioimmunoassay', 2nd Edn., ed. B. M. Jaffe and H. R. Behrman, Academic Press, New York, 1978, p. 355.
[91] P. D. G. Dean and D. H. Watson, *J. Chromatogr.*, 1979, **165**, 301.
[92] V. Hořejši, *J. Chromatogr.*, 1979, **178**, 1.
[93] K. Hauzer, M. Tichá, V. Hořejší, and J. Kocourek, *Biochim. Biophys. Acta*, 1979, **583**, 103.
[94] S. Sugii, K. Takeo, and E. A. Kabat, *J. Immunol.*, 1979, **123**, 1162.
[95] J. Y. Lin, T. C. Lee, and T. C. Tung, *Int. J. Pept. Protein Res.*, 1978, **12**, 311.

Acid-treatment of agarose to expose D-galactose units has provided an affinity matrix for the purification of a lectin from slime mould (*Polysphondylium pallidum*).[96]

Deoxyribonucleic acid has been immobilized without chemical alteration by entrapment in agarose.[97] The gel was either heated or dissolved in sodium perchlorate to destroy its secondary structure and then gelled in the presence of DNA by cooling or dialysis, respectively. Some rotational constraint of the DNA molecule was observed as evidenced by the binding constant obtained for the drug ethidium; the nature of the constraint was discussed in terms of the known structure of agarose bihelices.

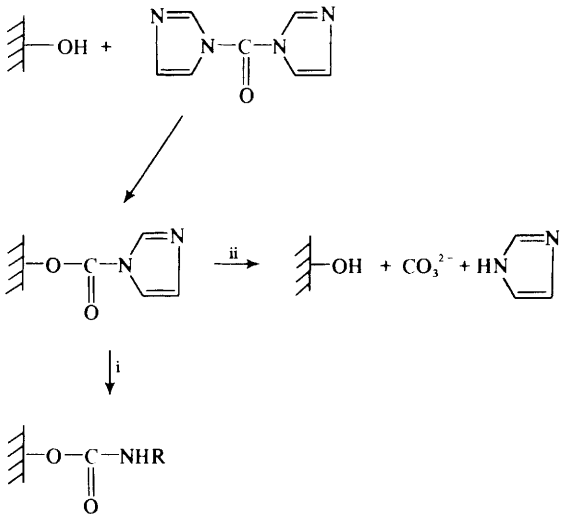
Alkyl- and aromatic-derivatives of agarose continue to be used in preparative techniques involving hydrophobic chromatography and for enzyme immobilization. Commercially available ω-aminoalkyl agarose has been used as a support for the immobilization of riboflavin kinase *via* an amide linkage.[98] Chlorophyllase has been adsorbed on to aminohexadecyl-, aminoundecyl- and heptyl-agarose.[99] Octylamine derivatives of agarose have been used for purification of murine onconaviral phosphoproteins,[100] for isolation of single chain bovine factor V,[101] and for resolution of mixtures of acyl-acyl carrier proteins by hydrophobic chromatography.[102] Decyl-agarose was also applied to the purification of murine onconaviral phosphoproteins.[100] Hydrophobic interaction chromatography utilizing phenyl-agarose has been applied to the purification of β-D-glucosidase isoenzymes,[103] the isolation of single chain bovine factor V,[101] and the fractionation of avian erythrocyte histones.[104] Six histones could be separated by use of appropriate salt concentrations or pH for elution. Phenyl-agarose provided a suitable hydrophobic (amphiphilic) matrix for crossed hydrophobic interaction immunoelectrophoresis, an analytical technique in which the principles of quantitative immunoelectrophoresis and hydrophobic interaction chromatography are directly combined.[105]

A 4-(*N*-acetyl-L-tyrosineazo)benzamidoethyl derivative of agarose has been synthesized and used for the selective immobilization of proteins.[106] The azo bond of the derivative can be reduced to liberate 4-aminobenzamidoethyl-agarose, which can be diazotized and recoupled to *N*-acetyl-L-tyrosine and subsequently to a fresh preparation of protein. Regeneration successively through five cycles is possible without detectable loss of functional groups.

[96] S. D. Rosen, J. Kaur, D. L. Clark, B. T. Pardos, and W. A. Frazier, *J. Biol. Chem.*, 1979, **254**, 9408.
[97] J. S. Lee and A. R. Morgan, *Can. J. Biochem.*, 1978, **56**, 585.
[98] A. H. Merrill and D. B. McCormick, *Biotechnol. Bioeng.*, 1979, **21**, 1629.
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A 4-aminobenzamidohexyl derivative of agarose has been diazotized and used for the immobilization of L-kynurenine.[107] Lysozyme and a synthetic lysozyme peptide have been immobilized on benzoquinone-activated agarose.[108]

Activation of cross-linked agaroses with 1,1′-carbonyl-di-imidazole yields a matrix for affinity chromatography devoid of additional charged groups.[109] The activated matrix (Scheme 7) is relatively stable to hydrolysis, but reacts smoothly with *N*-nucleophiles such as those present in affinity chromatography ligands and leashes. The matrix was treated with 1-aminobutane, 1,6-diaminohexane, or 6-aminohexanoic acid and then with soybean trypsin inhibitor or 4-aminobenzamidine *via* a carbodi-imide-mediated reaction, and the products were shown to be useful for the affinity chromatographic purification of trypsin.



R = Bu^n, $-(CH_2)_5CO_2H$, or $-(CH_2)_6NH_2$

Reagents: i, RNH_2, pH10; ii, 0.15 M NaOH

Scheme 7

The use of cyclic imidocarbonate derivatives of agarose for the immobilization of biologically active macromolecules and for preparation of media for affinity chromatography and immunoadsorption continues to increase. The nature of the coupling reaction has previously been described in these volumes (see Vol. 10, p. 433). The cyanogen bromide-activation of agarose has been modified and improved using a highly buffered reaction solution, and optimal reaction conditions for the activation proposed.[110]

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Recent references to the preparation of active immobilized forms of enzymes (for use as immobilized enzymes), of immunologically active macromolecules (for use as immunoadsorbents), and of various affinants (for use as affinity chromatographic matrices) using agarose cyclic imidocarbonate are summarized in the Tables which follow, the separate categories being the subject of Tables 1,[111-132] 2,[133-188] and 3,[189-330] respectively. The derivatives are listed according to the uses prescribed since, for example, the insolubilized form of an enzyme used for affinity chromatography may not be enzymatically active.

Table 1 *Use of agarose cyclic imidocarbonates for the preparation of active immobilized enzymes*

Enzyme coupled to agarose cyclic imidocarbonate	*EC No.*	*Ref.*
Alcohol dehydrogenase	1.1.1.1	111, 112
Alkaline phosphatase	3.1.3.1	113
D-Amino-acid oxidase	1.4.3.3	114
α-Amylase	3.2.1.1	115
L-Arginase	3.5.3.1	116
Carboxypeptidase Y		117, 118
Cholesterol oxidase	1.1.3.6	119
α-Chymotrypsin	3.4.21.1	120
Cyclic nucleotide phosphodiesterase	3.1.4.17	121
CMP-acyl neuraminate synthase (from frog liver)	–	122
β-D-Galactosidase ('Lactase')	3.2.1.23	123
Glucoamylase	3.2.1.3	117
D-Glucose oxidase	1.1.3.4	114, 117[a]
β-D-Glucuronidase	3.2.1.31	124
3α-Hydroxysteroid dehydrogenase	1.1.1.50	125
β-Hydroxysteroid dehydrogenase	1.1.1.51	125
20β-Hydroxysteroid dehydrogenase	1.1.1.53	125
Kallikrein	3.4.21.8	126[b]
Lysozyme	3.2.1.17	108
Malate dehydrogenase	1.1.1.37	127
Peroxidase	1.11.1.7	117
Pseudomonas primary amine dehydrogenase	–	128
Riboflavin kinase	2.7.1.26	98
α-Thrombin	3.4.21.5	129
Trypsin	3.4.21.4	130, 131, 132

[a] Scheme 8b (36); [b] See Scheme 8a (37).

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Table 2 *Use of agarose cyclic imidocarbonates for the preparation of immunoadsorbents*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
4-Aminophenyl isomaltoside	Isolation by immunoadsorption of anti-isomaltose antibodies from a rabbit immunized with pneumococcal type 20 vaccine	133
Anti-(A, B, H blood-group antigens) antibody	Detection by immunoadsorption of ABO blood-group specificity on carcinoembryonic antigen preparations	134
Anti-(AMP-deaminase) antibody	Purification by immunoadsorption of AMP-deaminases	135
Anti-(adenovirus capsid sub-unit) antibody	Preparation of specific antisera against adenoviruses by 'affinity bead immunization'	136
Anti-(alkaline phosphatase) antibody	Development of an immunoadsorbent method for the continuous purification of human placental alkaline phosphatase	137
Anti-[α-amylase inhibitor (*Phaseolus vulgaris*)] antibody	Purification by immunoadsorption of α-amylase inhibitor from *P. vulgaris*	138
Anti-(α-foetoprotein) antibody	Purification by immunoadsorption of human α-foetoprotein	139
Anti-(β_2-glycoprotein) antibody	Purification by immunoadsorption of an agglutinin in human sera	140
Anti-(β_2-microglobulin) antibody[a]	Isolation by immunoadsorption of HLA antigen from human tissue	141
Anti-(carboxypeptidase N) antibody	Purification by immunoadsorption of rat serum carboxypeptidase N	142
Anti-cellulase antibody	Purification by immunoadsorption of *Trichoderma viride* cellulase	143
Anti-(cholera toxin) antibody	Isolation by immunoadsorption of *Escherichia coli* LT enterotoxin	144
Anti-(factor VIII) antibody	Immunoadsorption of Factor VIII and fibrinogen from normal plasma; calibration of a one-stage Factor VIII assay	145
	Immunoadsorption and isolation of Factor VIII	146
Anti-fibronectin antibody	Purification by immunoadsorption of fibronectin	147
Anti-(α-D-glucosidase) antibody	Comparison of α-D-glucosidase activities in urine of normal subjects and those with Pompé's disease by immunoadsorption	148
Anti-(β-D-glucuronidase) antibody	Isolation by immunoadsorption of β-D-glucuronidase from human placenta	149
Anti-(D-glyceraldehyde 3-phosphate dehydrogenase) antibody or Fc fragments thereof	Immobilization by immunoadsorption of D-glyceraldehyde dehydrogenase	150
Anti-(hepatitis A virus) antibody	Purification by immunoadsorption of hepatitis A virus particles	151
Anti-(human chorionic gonadotrophin) antibody	Purification by immunoadsorption of human chorionic gonadotrophin–receptor complex	152

Table 2 *(cont.)*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Anti-(human immunoglobulin G)-antibody	Study of interaction between immobilized antibodies and antigen–peroxidase complex	153
	Isolation by immunoadsorption of human immunoglobulin G for use in enzyme immunoassay	154
Anti-(human serum) antibody	Isolation by immunoadsorption of C1q (a subcomponent of the first component of complement) from human serum	155
Anti-(infectious bovine rhinotracheitis antigen) antibody	Purification by immunoadsorption of infectious bovine rhinotracheitis antigen	156
Anti-interferon antibody	Study of nonspecific adsorption of interferon on immobilized serum immunoglobulin	157
	Purification by immunoadsorption of human interferon	158
Anti-(L-cell colony stimulating factor)antibody	Purification by immunoadsorption of L-cell colony stimulating factor	159
Anti-legumin antibody	Purification by immunoadsorption of legumin from *Pisum* seeds	160
Anti-lysozyme antibody	Assessment of immunological reactivity of synthetic lysozyme peptides	108
Anti-myosin antibody	Purification by immunoadsorption of rabbit ventricular myosin	161
Anti-(pregnancy zone protein)-antibody	Purification by immunoadsorption of pregnancy zone protein	162
Anti-(rabbit immunoglobulin G)-antibody	Immunoadsorption of anti-(human immunoglobulin G) antibody–β-D-galactosidase conjugates prepared for enzyme-immunoassay	154
Anti-(rat cell surface antigen)-antibody	Study by immunoadsorption of tritiated glycoproteins from the surface of rat lymphoid cells	163
Anti-(soluble egg antigen)antibody	Isolation by immunoadsorption of *Schistosoma mansoni* egg antigen	164
Anti-tetanolysin antibody	Purification by immunoadsorption of streptolysin O	165
Anti-thrombin III antibody	Separation of heparin into functionally distinct fractions on the basis of immunoreactivity	166
4-Azobenzenearsonate } Bovine serum albumin	Purification by immunoadsorption of anti-(bovine serum albumin) antibodies; assessment of a modified procedure for preparation of agarose cyclic imidocarbonate	110
3-(*O*-Carboxymethyl)-oximino-5α-dihydro-testosterone–bovine serum albumin conjugate 7-(*O*-Carboxymethyl)-oximino-5α-dihydrotestosterone–bovine serum albumin conjugate	Purification by immunoadsorption of antibodies against the steroid antigen	167

Table 2 *(cont.)*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Carcinoembryonic antigen	Analysis of human sera for carcinoembryonic antigen-binding proteins	168
	Isolation by immunoadsorption of a carcinoembryonic antigen-binding human immunoglobulin G from normal sera	169
2,4-Dinitrophenylated albumin	Isolation by immunoadsorption of anti-(2,4-dinitrophenyl) antibody preparations	170
Helix pomatia haemagglutinin	Selective immunoaffinity fractionation of murine cytotoxic T lymphocytes	171 172
7β-Hemisuccinamido-5α-dihydrotestosterone-bovine serum albumin conjugate 3β-Hemisuccinamido-5α-androstan-17β-ol–bovine serum albumin conjugate	Purification by immunoadsorption of antibodies with improved specificity for the steroid hapten	167
Histamine–rabbit serum albumin conjugate	Fractionation of human peripheral blood lymphocytes	173
Immunoglobulin E	Immunological study of surface glycoproteins and the immunoglobulin E receptor from rat basophilic leukaemia cells	174
	Purification by immunoadsorption of a cell-surface receptor for immunoglobulin E from rat basophilic leukaemia cells	175
Immunoglobulin G	Purification by immunoadsorption of C1r, an activated sub-component of the first component of the complement system	176
	Immunoadsorption of anti-(human immunoglobulin G antibody)–β-D-galactosidase conjugates prepared for enzyme immunoassay	154
Immunoglobulin G, Fc fragments	Isolation by immunoadsorption of immunoglobulin G Fc-fragment-binding glycoproteins from human blood platelets	177
Influenza A virus	Purification by immunoadsorption of antibodies to influenza A virus structural proteins	178
Lacto-*N*-difucaose[b] Lacto-*N*-tetraose[b]	Purification by immunoadsorption of anti-carbohydrate antibodies	179
Legumin	Isolation by immunoadsorption of anti-legumin	160
Lens culinaris phytohaemagglutinin	Investigation of mitogenic response of lymphocytes	180
	Immunoadsorption of surface proteins and glycoproteins of rat basophilic leukaemia cells	174
Lysozyme Lysozyme synthetic peptide IV	Isolation by immunoadsorption of anti-lysozyme antibodies	181

Table 2 *(cont.)*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Melanoma cell membranes	Isolation by immunoadsorption of immunoglobulins from sera of patients with melanoma, sarcoma, and carcinoma	182
Penicillin G[c]	Isolation by immunoadsorption of anti-benzyl penicilloyl antibodies	183
Protein A (*Staphylococcus aureus*)	Purification by immunoadsorption of goat immunoglobulin G1 and immunoglobulin G2 sub-classes	184
	Immunoadsorption of rabbit anti-haemocyanin for subsequent cross-linking and use in immunoaffinity chromatography (immunoadsorption)	185
	Isolation by immunoadsorption of antibodies specific for human chorionic gonadotrophin, carcinoembryonic antigen, α-foetoprotein, and casein	186
	Rapid separation by immunoadsorption of antibody–enzyme conjugates from free enzyme following conjugation reactions	187
	Separation by immunoadsorption of cell-dependent antibody and inhibitory antibody	188
Vicia villosa phytohaemagglutinin	Selective immunoaffinity fractionation of murine cytotoxic T lymphocytes	171

[a] See Scheme 8b (38); [b] See Scheme 8a (39); [c] See Scheme 8b (36).

Table 3 *Use of agarose cyclic imidocarbonates for the preparation of affinity chromatography materials without linkage extension*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
2-Acetamido-*N*-(6-aminohexanoyl)-2-deoxy-β-D-glucopyranosylamine	Affinity chromatography of β-D-2-acetamido-2-deoxy-hexosidases A and B	189
	Affinity chromatography of β-D-2-acetamido-2-deoxy-hexosidase P	190
Actin, glutaraldehyde-cross-linked	Affinity chromatography of light chain isoenzymes of myosin head region	191
Albumin	Investigation of binding of fatty acids, steroids and drugs to albumin	192
4-Aminobenzylphosphonic acid	Affinity chromatography of human red cell acid phosphatase	193
N-(6-Aminohexanoyl)-L-fucopyranosylamine	Affinity chromatography of human serum α-L-fucosidase	194, 195
	Purification of lectin II from *Ulex europaeus*	196
	Affinity chromatography of L-fucosidase	197

Table 3 *(cont.)*

Ligand of affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
N-(6-Aminohexanoyl)-β-D-galactosylamine	Affinity chromatography of neutral β-D-galactosidases from human liver	198
	Removal of traces of α-D-galactosidase activity during purification of β-D-mannosidase	199
N-[6-(6-Aminohexanoylamino)-hexanoylaminophenyl]-trimethylammonium bromide	Affinity chromatography of acetylcholinesterase from cobra venom	200
N-(6-Aminohexanoyl)-lacto-*N*-fucopentaosylamine	Affinity chromatography of α-L-fucosidase	201
N^6-(6-Aminohexyl)adenosine 2′,5′-diphosphate	Purification of glutathione reductase	202
	Affinity chromatography of light chain isoenzymes of myosin head region	191
	Affinity chromatography of H^+ translocating adenosine triphosphatase	203
N^6-(6-Aminohexyl)adenosine monophosphate	Affinity chromatography of *Bacillus subtilis* glutamine phosphoribosyl pyrophosphate aminotransferase	204
	Purification of adenosine kinase	205
	Analytical affinity chromatography of bovine liver glutamate dehydrogenase and investigation of its ligand-binding properties	206
	Affinity chromatography of acetate kinase	207
	Affinity binding studies with glycogen phosphorylase *b*	208
	Affinity chromatography of phosphorylase *a* from human platelets	209
	Affinity chromatography of bacterial malate dehydrogenases	210, 127
	Affinity chromatography of formaldehyde and formate dehydrogenases from pea seeds	211
N^6-(6-Aminohexyl)adenosine triphosphate	Affinity chromatography of *Bacillus subtilis* glutamine phosphoribosyl pyrophosphate amidotransferase	204
	Affinity chromatography of AMP-kinase	205
8-(6-Aminohexyl)-amino-adenosine monophosphate	Purification of alcohol dehydrogenase from *Drosophila*	212
	Analytical affinity chromatographic investigation of ligand binding properties of bovine liver glutamate dehydrogenases	206
	Purification of acetate kinase	207
	Purification of adenine phosphoribosyltransferase	213
8-(6-Aminohexyl)-amino-desulpho-coenzyme A	Affinity chromatography of phosphotransacetylase from *Clostridium kluyveri*	214
8-(6-Aminohexyl)-amino-guanosine monophosphate and diphosphate	Affinity chromatographic purification of polypeptide elongation factor II from rat and pig liver	215

Table 3 *(cont.)*

Ligand of affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
N^6-[*N*-(6-Aminohexyl)-carbamoylmethyl]-NADH	Affinity chromatography of alcohol dehydrogenases	216
N^6-[*N*-(6-Aminohexyl)-carbamoylmethyl]-*S*-(5-thio-2-nitrobenzoic acid)coenzyme A	Affinity chromatography of coenzyme A-dependent enzymes (citrate synthase and succinic thiokinase)	217
6-Aminohexyl 1-thio-β-D-galactopyranoside	Affinity chromatography of human liver acid β-D-galactosidase	218, 219
4-Aminophenyl 1-thio-β-D-galactopyranoside	Purification of β-D-galactosidase from rat mammary gland	220
N-(3-Aminopropyl)-1,3-diaminopropane	Affinity chromatography of putrescine oxidase and spermidine dehydrogenase	221
Anhydrotrypsin	Affinity chromatographic fractionation of naturally occurring peptides having L-arginine at the carboxy-terminus	222
Arachis hypogaea lectin	Affinity chromatography of antigenic determinants of membranes	223
L-Arginine	Purification of human fibronectin	224
L-Arginyl-L-tyrosyl-glycyl-glycine	Affinity chromatographic purification of papain	225
L-Aspartate (sodium salt)	Purification of plant aspartate trans-carbamoylase	226
Avidin	Affinity chromatography of biotin-containing enzymes	227
Bandeiraea simplicifolia lectin	Affinity chromatography of human liver hydrolases	228
	Affinity chromatography of soluble conjugates of *Salmonella* specific oligosaccharides and bovine serum albumin	229
Blood-group A substance	Affinity chromatography of *Vicia villosa* lectin	171
Blue Dextran	Purification of glycogen phosphorylase *b*	208
	Purification of UDP-*N*-acetylenolpyruvoyl-glucosamine reductase	230
	Affinity chromatography of dihydropterin reductase	231
	Purification of glyoxalase I from rabbit liver	232
	Purification of human pyruvate kinase	233
	Purification of lactate dehydrogenase and assessment of the homology of the enzyme sub-units	234
	Purification of D-glucan-binding proteins from *Streptococcus mutans*	235
	Purification of 6-phosphogluconate dehydrogenase from *Escherichia coli*	236
Ca^{2+}-dependent regulatory protein	Purification of adenylate cyclase	237

Table 3 *(cont.)*

Ligand of affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Calmodulin	Removal of contaminating proteins (which bind) during calmodulin purification	238
Carboxypeptidase inhibitor (potato)	Purification of pancreatic carboxypeptidase	239
	Purification of crayfish carboxypeptidase	240
N-(3-Carboxypropionyl)amino-decane	Purification of wheat-germ aspartate trans-carbamoylase	241
Casein	Fractionation of angiotensin I-generating activity of rat brain into two components	242
2-Chloro-10-(3-aminopropyl)-phenothiazine	Purification of calmodulin	238
Concanavalin A	Purification of bovine sperm forward motility protein	243
	Separation of peroxidase–immunoglobulin G conjugate from unconjugated immuno-globulin G for use in enzyme immunoassay	244
	Purification of β-D-2-acetamido-2-deoxy-hexosidases	189
	Purification of α-L-fucosidase	245
	Purification of 2-dansylamido-2-deoxy-D-glucose	246
	Affinity chromatography of soluble membrane receptors from mouse sub-maxillary glands	247
	Affinity chromatographic adsorption of activating factor of lymphocyte adenylate cyclase with consequent reversible deactivation of the enzyme	248
	Purification of mammalian serum haptoglobin	249
	Purification of *Schistosoma mansoni* soluble egg antigens	250
	Affinity chromatography of human alkaline phosphatases	251
	Purification of carcinoembryonic antigen	252
	Purification of D-glucocerebrosidase	253
	Purification of *Phaseolus vulgaris* phyto-haemagglutinin	254
	Affinity chromatography of human liver hydrolases	228
	Purification of human liver acid β-D-galactosidases	218
	Separation of cellulase and cellobiase from *Trichoderma viride*	255
	Purification of a myeloma immunoglobulin M and its tryptic Fab and $(Fc)_5$ fragments	256
	Fractionation of soluble brain glycoproteins	257
	Purification of rat kidney glycoproteins for production of heterologous antisera which induce congenital abnormalities	258

Table 3 *(cont.)*

Ligand of affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
	Affinity chromatographic separation of integral membrane glycoproteins E1 and E2 of Semliki Forest virus	259
	Separation of A- and B-type glycopeptides of Semliki Forest virus	260
	Purification of human placental α-D-galactosidase	261
	Affinity chromatography of antigenic fractions of human seminal plasma	262
	Affinity chromatography of glycopeptides obtained by 'pronase' digestion of immunoglobulin G from a patient with multiple myeloma	263
	Purification of 2-deoxy-D-glucoside 2-sulphamate sulphohydrolase	264
	Affinity chromatography of glycoproteins in purified myelin	265
	Affinity chromatographic purification of a D-galacto-D-mannan antigen from *Aspergillus fumigatus*	266
	Studies of the interaction of concanavalin A with tryptic fragments from plasma fibronectin	267
	Affinity chromatography of acid ribonuclease from HeLa cell lysosomes; demonstration that the enzyme is a glycoprotein	268
	Purification of human liver acid α-D-glucosidase	148
	Purification of two forms of α-D-2-acetamido-2-deoxy-galactosidase	269
	Study of specific binding of [^{35}S]-labelled proteoglycans from secretions of human skin fibroblasts	270
	Separation of two molecular variants of α-foetoprotein	271
	Purification of bull seminal plasma hyaluronidase	272
	Purification of ribonuclease B	59
	Purification of biliary glycoprotein I	273
	Separation of human amylase isoenzymes	274
	Purification of cathepsin D from rat spleen	275
	Purification of a cathepsin D-like acid proteinase from rat spleen	276
Cowper's gland mucus, desialylized	Purification of β-D-2-acetamido-2-deoxy-hexosidase from rabbit sperm cytoplasmic droplets	277

Table 3 *(cont.)*

Ligand of affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Cycloserine	Affinity chromatography of alanine aminotransferase at different temperatures	278
Cytidine diphosphate-hexanolamine	Purification of cytidine monophosphate-*N*-acetylneuraminate β-D-galactosyl glycoprotein sialyltransferase and cytidine monophosphate-*N*-acetylneuraminate-2-acetamido-2-deoxy-α-D-glucosyl glycoprotein sialyltransferase from porcine submaxillary gland	279
	Purification of cytidine monophosphate-*N*-acetylneuraminate-2-acetamido-2-deoxy-α-D-galactosyl glycoprotein sialyltransferase from porcine submaxillary gland	280
Deoxyribonucleic acid	Investigation of interaction of immobilized DNA with poly-(L-lysine) and copolymers of L-lysine and L-leucine, L-alanine, or glycine	281
Dextran sulphate	Investigation of inhibition of Factor VIII-associated platelet aggregation by dextran sulphate	282
1,12-Diaminododecane	Affinity chromatography of putrescine oxidase and spermidine dehydrogenase	221
1,2-Diaminoethane	Affinity chromatography of alanine aminotransferase at different temperatures	278
1,6-Diaminohexane 1,8-Diaminooctane	Affinity chromatography of putrescine oxidase and spermidine dehydrogenase	221
Enterotoxin	Investigation of antigenic sites of staphylococcal enterotoxins A and E	283
Factor VIII	Purification of low molecular weight Factor VIII	284
Factor X	Separation of heparin into functionally distinct fractions; investigation of mechanism of action of heparin	166
Fetuin	Purification of neuraminidase from *Streptomyces griseus*	285
Fetuin, desialylized	Purification of β-D-2-acetamido-2-deoxyhexosidase from rabbit sperm cytoplasmic droplets	277
Fibrin	Purification of fibrinogen plasminolysis fragment D	286
Gelatin	Demonstration of presence of fibronectin in human cerebrospinal fluid	287
	Purification of a collagen-binding peptide from tryptic digests of fibronectin from human plasma	267
	Purification of fibronectin from human plasma	224

Table 3 *(cont.)*

Ligand of affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Haptoglobin	Purification of the lymphocytosis-promoting factor haemagglutinin of *Bordetella pertussis*	288
Helix pomatia haemagglutinin	Purification of human and mouse lymphocyte surface glycoproteins	289
	Affinity chromatography of natural killer cells from other mouse lymphoid sub-populations	290
17-Hemisuccinyl-17β-oestradiol-ovalbumin conjugate	Purification of the oestrogen receptor from calf uterus cytosol	291
Heparin	Separation of human α_1-antitrypsin from antithrombin III antibodies	292
	Investigation of binding of porcine serum lipoproteins to heparin	293
	Purification of a high molecular weight glycoprotein from human blood platelets	294
	Isolation of Factor VIII from human plasma	146
	Purification of human and rat plasma fibronectin	147
	Investigation of different binding sites on heparin for platelet antiheparin proteins and antithrombin III antibodies	295
	Isolation of the oestrogen receptor from calf uterus cytosol	291
	Investigation of inhibition of Factor VIII-associated platelet aggregation by heparin	282
	Purification of RNA polymerases from avian liver	296
Hirudin	Fractionation of low molecular weight porcine heparin into 'active' and 'relatively inactive' forms	297
Histamine–albumin conjugate	Fractionation of human peripheral blood lymphocytes	173
Hog sulphated gastric mucin glycopeptides	General affinity chromatographic method for lectin purification	298
α-Lactalbumin	Purification of lactose synthase from pig thyroid	299
Lens culinaris lectin	Purification of a hyaluronidase from *Streptococcus pyogenes*	300
	Purification of an organ-specific tumour-associated antigen from rat mammary carcinoma	301
	Affinity chromatography of surface glycoproteins and immunoglobulin E receptors from rat basophilic leukaemia cells	174
	Affinity chromatography of membrane immunoglobulin M and murine lymphocyte immunoglobulin; study of their affinities for the lectin	302

Table 3 *(cont.)*

Ligand of affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
	Affinity chromatography of glycoprotein fractions from long-term cultured human lymphocytes	180
	Purification of *Trypanosoma cruzi* cell-surface protein	303
	Affinity chromatography of tritiated glycoproteins from the surface of rat lymphoid cells	163
	Purification of the major surface coat glycoprotein of *Trypanosoma brucei brucei*	304
Lotus tetragonolobus lectin	Affinity chromatography of glycoproteins in purified myelin	265
L-Lysine	Purification of plasminogen	305
Neurophysin	Extraction and concentration of oxytocin and other neurohypophyseal hormones from dilute samples of biological fluids	306
Noradrenaline	Affinity chromatography of L-arginine esterase of human blood plasma	307
Norleucine	Purification of hepatic phosphoenolpyruvate carboxykinase	308
Ovomucoid	Purification of a lectin from rice bran	309
Pepstatin	Purification of bovine brain cathepsin D	310
4-(Phenoxypropoxy)aniline hydrochloride	Affinity chromatography of avian oestradiol 17β-dehydrogenase, 3(17)β-hydroxysteroid dehydrogenase, and cortisone reductase	311
Phosphatidylserine	Purification of D-glucocerebrosidase	253
Plasmin	Purification of streptokinase	312
Poly(ethyleneimine)	Intermediate derivative prior to coupling with trypsin to yield an active immobilized enzyme	313
Poly(L-proline)	Purification of prolyl hydroxylase	314
Poly(uridylic acid)	Affinity chromatography of RNA-binding proteins from *Rana temporaria* oocytes	315
Prealbumin	Investigation of interaction between prealbumin and retinol-binding proteins	316
Protein A	Isolation of immunoglobulins by affinity chromatography	184–187
Prothrombin	Purification of staphylocoagulase	317
m-Ribonucleic acid	Affinity chromatography of eukaryotic initiation factors of ribosomal sub-units from embryonic chick muscle	318
Ricinus communis lectin	Affinity chromatography of human liver hydrolases	228
	Purification of a myeloma immunoglobulin M and its tryptic Fab and $(Fc)_5$ fragments	256
	Affinity chromatography of glycoproteins in purified myelin	265

Table 3 *(cont.)*

Ligand of affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
	Affinity chromatography of major cell-surface glycoproteins from normal and diabetic rat liver membranes	319
	Affinity chromatography of tyrosine tRNA's	320
Soybean agglutinin	Affinity chromatography of human liver hydrolases	228
Sulphobromophthalein–glutathione conjugate	Purification of rat plasma albumin	321
Tamm–Horsfall glycoprotein	Affinity chromatographic separation of *Phaseolus vulgaris* leucoagglutinin and haemagglutinin lectins	322
Tetragonolobus purpurea lectin	Affinity chromatography of human liver hydrolases	228
Thrombin	Fractionation of low molecular weight porcine heparin into 'active' and 'relatively inactive' forms	297
	Affinity chromatography of heparin	129
Thyroglobulin	Affinity chromatography of rabbit anti-thyroglobulin antibodies	323
	Purification of *Phaseolus vulgaris* isolectins	324
L-Thyroxine	Isolation of thyroxine-binding globulins	325
Transplantation antigens	Purification of *in vivo* activity of an H–Z alloantiserum	326
Tridacna maxima lectin	Affinity chromatographic purification of an arabinogalactan protein from *Gladiolus*	327
Ulex europaeus I lectin	Affinity chromatography of human liver hydrolases	228
Vicia faba lectin *Viscum album* lectin	Affinity chromatography of human serum proteins	328
Wheat germ agglutinin	Affinity chromatography of human liver hydrolases	228
	Affinity chromatographic investigation of interactions between wheat germ agglutinin and sialoglycoproteins	329
	Affinity chromatography of glycoproteins, development of improved coupling procedure for wheat germ agglutinin	330

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The extent of activation of the agarose matrix by cyanogen bromide treatment has been shown to affect the stability of immobilized malate dehydrogenase to both temperature and high salt concentration.[127] Preparations immobilized on agarose with a low degree of activation were found to be significantly less stable than those with a high degree of activation.

Use of immobilized protein A provides a rapid and efficient means of separating enzyme-labelled antibodies from free enzyme following conjugation reactions.[187] The method should be of use in the preparation of reagents for enzyme-linked immunoassay.

In a reinvestigation of an affinity chromatographic procedure for the purification of papain, it has been demonstrated that removal of the benzoyl blocking group from the L-tyrosine of the affinant considerably improves the specificity of the affinant glycyl-glycyl-L-tyrosyl-L-arginine for the purification of papain.[225] Using the deblocked tetrapeptide as affinant separate elution of active and non-activatable enzyme can be achieved.

Immobilized neurophysin has been shown to be suitable for the extraction and concentration of oxytocin from dilute samples of biological fluids.[306]

Agarose cyclic imidocarbonate has been treated with polyethyleneimine and then modified with β-1-[3,3-dimethyl-6-nitrospiro(indoline-2,2′-2*H*-benzopyran)]propionic anhydride.[313] Coupling of trypsin to the product yielded an active immobilized enzyme.

The presence of (1 → 4)-β-linked 2-acetamido-2-deoxy-D-glucose oligosaccharides during the coupling of wheat-germ agglutinin and agarose cyclic imidocarbonate yields an immobilized lectin with better binding capacity than preparations obtained by previous methods.[330]

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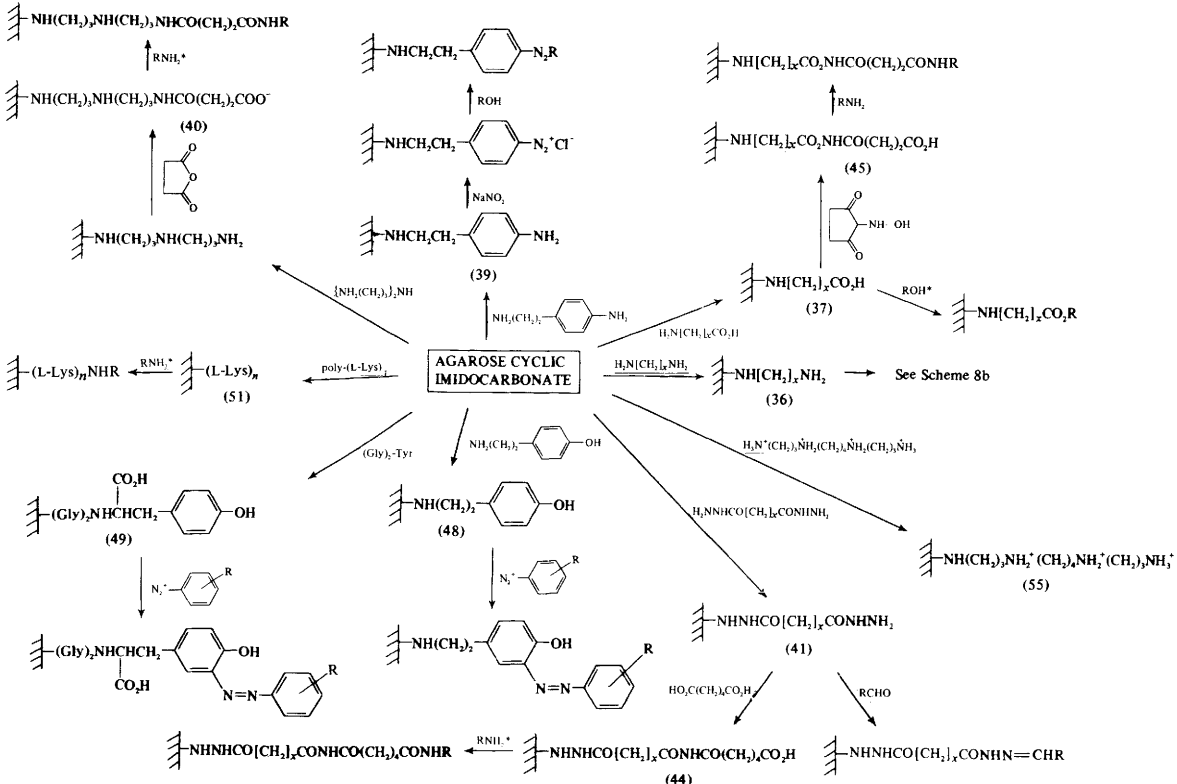
Direct attachment of an enzyme or an affinant to agarose cyclic imidocarbonate has frequently been unrewarding, since approach to the bound ligand may be sterically impeded. This difficulty can be overcome by inserting a covalent bridge between matrix and ligand. Bridging is also used when functional groups on the ligand do not react with those on the matrix, since it allows a new type of functionality to be introduced. Modifications recently used to provide bridges in the preparation of affinity chromatography matrices, *etc.*, from agarose cyclic imidocarbonates are summarized in Schemes 8a and 8b and Table 4.[331–384] Where bridging has been used to immobilize enzymes or to prepare immunoadsorbents, details are given in Table 1 or 2, respectively. The products obtained by coupling various substances to agarose cyclic imidocarbonate for diverse uses are given in Table 5.[385–403]

Coupling cholic acid or cholesterol to agarose cyclic imidocarbonate *via* a spacer containing a thiol ester bond (cystamine or cysteamine) yields a matrix for the affinity chromatography of human plasma low- and high-density lipoproteins.[348] Elution of bound lipoproteins from conventional supports is very difficult without using denaturing conditions, but with the present method the lipoproteins are eluted by cleavage of the thiol ester bond with hydroxylamine.

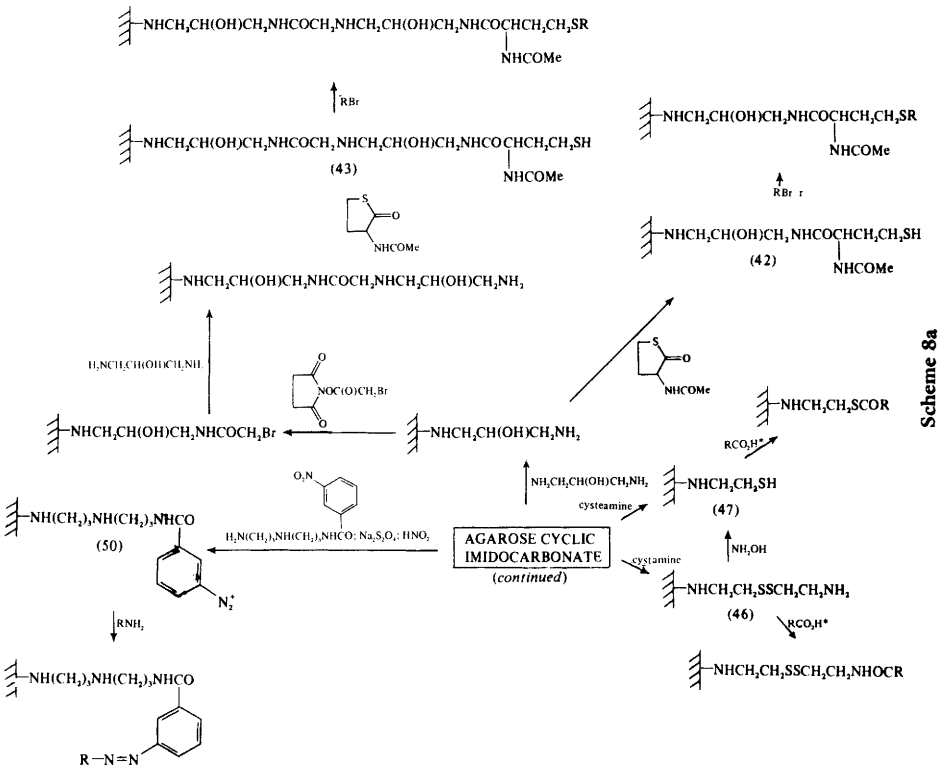
(2-Nitrosulphenyl-L-methionyl)-L-tyrosyl-L-phenylalanine has been treated with aminohexyl-agarose *via* a carbodi-imide mediator, and the 2-nitrosulphenyl group then removed with sodium dithionite.[372] The matrix is suitable both for the affinity chromatographic purification of neurophysins, and for measurement of their ligand-binding parameters by quantitative affinity chromatography.

In affinity chromatography of glycosaminoglycan polysaccharidases on chondroitin, dermatan sulphate, or heparin bound covalently to aminohexyl-agarose, additional non-covalent coating of the covalently-bound glycosaminoglycan with the same or another glycosaminoglycan has been found to be important.[387,397] A simple potentiometric titration method for the determination of the heparin content of immobilized heparin preparations has been developed using heparin–agarose conjugates.[399]

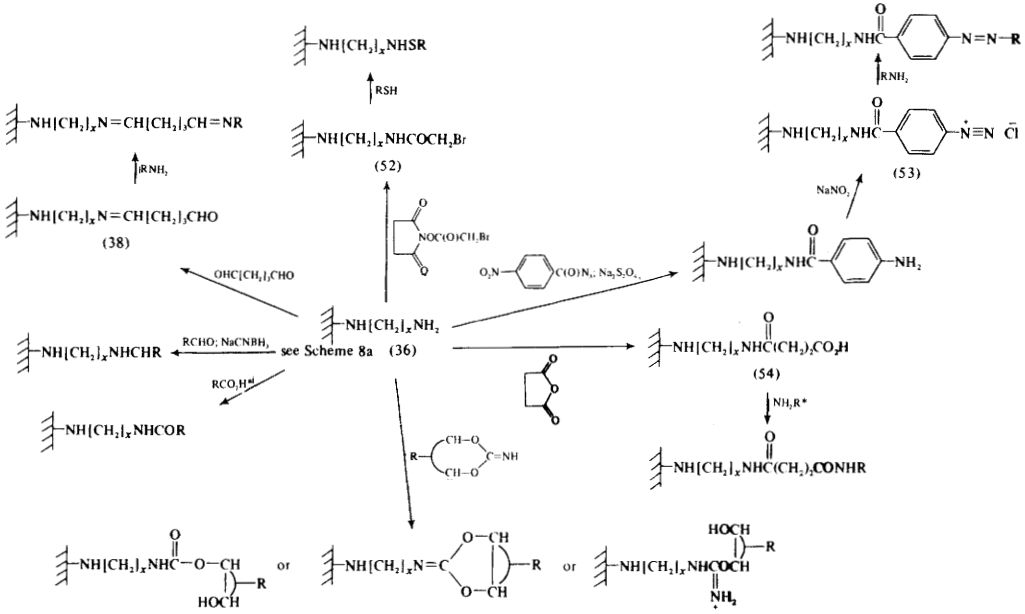
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AGAROSE CYCLIC IMIDOCARBONATE
(39)
(40)
(45)
(37)
(51)
(36)
(49)
(48)
(41)
(44)
(55)
See Scheme 8b

Scheme 8a



Scheme 81 (cont.)


NH[CH2]xNHSR
RSH
NH[CH2]xNHCOCH2Br
(52)
NH[CH2]xN=CH[CH2]3CH=NR
RNH2
NH[CH2]xN=CH[CH2]3CHO
(38)
OHC[CH2]3CHO
NH[CH2]xNH2
see Scheme 8a (36)
RCHO; NaCNBH3
NH[CH2]xNHCHR
RCO2H
NH[CH2]xNHCOR
NH[CH2]xNHC(CH2)2CO2H
(54)
NH2R*
NH[CH2]xNHC(CH2)2CONHR
NH[CH2]xNHC=O
NH[CH2]xNH2
NaNO2
NH[CH2]xNHC=O
N≡N Cl
(53)
NH[CH2]xNHC=O
N=N—R
RNH2
or
or

Scheme 8b

Table 4 *Use of agarose cyclic imidocarbonates for the preparation of affinity chromatography material* via *linkage extension*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Agarose intermediate*[a]	*Use of product*	*Ref.*
2-Acetamido-2-deoxy-D-galactose	(37)	Affinity chromatographic purification of a phytohaemagglutin from *Robinia pseudoacacia*	331
2-Acetamido-4-amino-2,4-dideoxy-D-glucose	(40)	Affinity chromatographic purification of a β-D-2-acetamido-2-deoxy-glucosidase from malted barley	332
4-(Acetoxymercuri)aniline	(36)	Affinity chromatography of dipeptidyl aminopeptidase I	333
N-Acetyl-L-tryptophan	(36)	Affinity chromatographic purification of indolyl-3-alkane α-hydroxylase	334
1-Acyl-2-(9-carboxy)nonyl-glycero-3-phosphatidylcholine	(36)	Affinity chromatographic purification of phosphatidylcholine exchange protein from liver	335
S-Adenosyl-L-homocysteine	(36)	Affinity chromatographic purification of *Ruta graveolens* *O*-methyltransferases	336
Adenosine triphosphate	(41)	Affinity chromatographic purification of rat liver AMP-deaminase	337
	(41)	Affinity chromatographic purification of pig liver phosphoglycerate kinase	338
Adriamycin, periodate-oxidized	(41)	Affinity chromatographic demonstration of presence of adriamycin-binding proteins in various tissues	339
Albumin, glutaraldehyde-cross-linked	(38)	Affinity chromatographic purification of hepatitis B surface antigen from human sera	340
Alprenolol	(42), (43)	Affinity chromatographic purification of the β_1-adrenergic receptor from turkey erythrocytes	341
6-Amino-2-naphthamidine	(37)	Affinity chromatographic purification of urokinase	342
4-Aminophenyl 1-thio-β-L-fucopyranoside	(40)	Affinity chromatographic purification of β-D-2-acetamido-2-deoxy-glucosidase	343
N-[^{14}C]-4-Androsten-3-on-17β-oxycarbonyl 6-aminohexanoic acid	(44)	Affinity chromatographic separation of apoenzyme and prosthetic group of 4-en-3-oxosteroid: (acceptor)-1-en-oxidoreductase from *Nocardia opaca*	344
	(36), (44)	Affinity chromatographic purification of the 4-en-3-oxosteroid 5α-reductase and its apoenzyme from *Mycobacterium smegmatis*	345

[a] See Schemes 8a and 8b, pp. 612–4.

Table 4 *(cont.)*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Agarose intermediate*[a]	*Use of product*	*Ref.*
Aprotinin ('Trasylol')	(37)	Affinity chromatographic removal of kallikrein from other 4-tosyl-L-arginine methyl ester ('TAME') esterases in human urine	346
D-Arginine	(45)	Purification and affinity chromatography of pancreatic carboxypeptidases	239
5-(3-Carboxypropanamido)xanthotoxin	(36)	Affinity chromatography of *Ruta graveolens O*-methyltransferases	336, 347
Cholesterol hydrogen succinate Cholic acid	(36), (46), (47)	Affinity chromatography of human low- and high-density lipoproteins	348
Dehydroepiandrosteron-17-(*O*-carboxymethyl)oxime	(36)	Affinity chromatographic purification of dehydroepiandrosterone sulphotransferase from human adrenals	349
Dermatan sulphate	(36)	Affinity chromatographic purification of chondroitinase B	350
4-Diazobenzylphosphonic acid	(48)	Affinity chromatographic purification of alkaline phosphatase from human liver	351
4-(4-Diazophenylazo)phenyl arsonic acid	(48)	Affinity chromatographic purification of alkaline phosphatases from hen	352
4-Diazophenyl hydroxamic acid	(49)	Affinity chromatographic isolation of neuraminidase	353
Diazophenyl pentyl fumaric acid	(48)	Affinity chromatography of pig heart fumarase	354
Ferulic acid	(36)	Affinity chromatography of *Ruta graveolens O*-methyltransferases	336
Folic acid	(36)	Affinity chromatographic purification of folate-binding protein in cow's whey	355
L-Fucose	(37)	Affinity chromatographic purification of human placental α-L-fucosidase	356
Glycogen	(41)	Affinity chromatographic isolation of glycogen phosphorylase *b* from cow uterus	357
Guanosine diphosphate		Affinity chromatographic purification of the 3.2×10^4 mol. wt. polypeptide from mitochondria of brown adipose tissue	358
Guanosine monophosphate		Affinity chromatographic purification of hepatic phosphoenolpyruvate carboxykinase	308
	(36a)	Affinity chromatographic purification of human adenine phosphoribosyltransferase	359

Haemin	(50)	Affinity chromatographic isolation of haemopexin from human serum 360
Heparan sulphate	(36)	Affinity chromatographic purification of chondroitinase B 350
Histamine	(51)	Affinity chromatographic purification of histamine-binding proteins from human serum 361
Hyaluronic acid	(36)	Affinity chromatographic purification of components of the hyaluronate–proteoglycan complex of cartilage 362
Hydroxocobalamin	(36)	After conversion into immobilized cyanocobalamin by CN^- wash, affinity chromatographic purification of human transcobalamin II–cyanocobalamin complex 363
Indoleacrylic acid Indole-3-carboxaldehyde	(36)	Affinity chromatographic purification of indolyl 3-alkane-α-hydroxylase 334
Lactose	(36)	Affinity chromatographic purification of α,α-trehalase from *Artemia salina* embryos 364
D-Mannan	(36)	Affinity chromatographic purification of β-D-mannanase from lucerne seeds 365
6-Mercaptopurine 9-D-riboside	(52)	Affinity chromatographic purification of *S*-adenosyl-L-homocysteine hydrolase from rat liver 366
Methotrexate	(36)	Affinity chromatographic purification of folate-binding protein in cow's whey 355
Nebularine 2′,3′-*O*-1-(2-carboxyethyl) ethylidene	(36)	Affinity chromatographic purification of adenosine deaminase 367
Nicotinamide adenine dinucleotide		Affinity chromatographic isolation of human erythrocyte membrane NADH: (acceptor) oxidoreductase 368
		Affinity chromatographic purification of sorbitol dehydrogenase 369
Nicotinamide adenine dinucleotide phosphate		Affinity chromatographic purification of UDP-*N*-acetylenol pyruvoyl 2-acetamido-2-deoxy-D-glucose reductase from *Escherichia coli* 230
		Affinity chromatographic purification of NADPH cytochrome *c* (P-450) reductase from house flies 370
		Affinity chromatographic purification of L-kynurenine 3-hydroxylase 371
(3-Nitrosulphenyl-L-methionyl)-L-tyrosyl-L-phenylalanine	(36)	Affinity chromatographic purification of neurophysins 372
2-Oxoglutaric acid	(36)	Affinity chromatography of L-alanine aminotransferase at different temperatures 278

Table 4 *(cont.)*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Agarose intermediate*[a]		*Ref.*
Pepstatin	(36)	Affinity chromatographic isolation of chymosin from rennin	373
	(36)	Affinity chromatographic isolation of bovine brain cathepsin D	374
Phaseolus vulgaris protease inhibitor	(36)	Affinity chromatographic purification of a trypsin-like protease from *Streptomyces griseus*	375
5S Ribosomal ribonucleic acid (rat liver), periodate-oxidized	(41)	Affinity chromatography of eukaryotic ribosomal proteins	376, 377
Soybean trypsin inhibitor	(36)	Affinity chromatographic purification of kallikrein from human serum	378
Thiamine pyrophosphate	(36)	Affinity chromatographic isolation of thiamine-binding protein from chicken egg-white	379
	(53)	Affinity chromatography of transketolase	380
	(36)	Affinity chromatography of thiamine pyrophosphokinase from rat brain	381
Trimethyl-(4-aminophenyl)-ammonium chloride	(54)	Affinity chromatographic purification of the mixed-type putative acetylcholine receptor from wild and mutant strains of house flies	382
1-(*NNN*-Trimethylammonium)-10-decylamine bromide hydrobromide	(55)	Affinity chromatography of cholinergic proteins	383
Tubercidin 2′,3′-*O*-1-(2-carboxyethyl) ethylidene	(36)	Affinity chromatographic purification of adenosine deaminase	367
Uridine diphosphate		Affinity chromatographic separation of UDP-2-acetamido-2-deoxy-D-glucose 2-epimerase and UDP-2-acetamido-2-deoxy-D-mannose dehydrogenase	384

Table 5 *Use of agarose cyclic imidocarbonates for the preparation of miscellaneous derivatives*

Species coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
2-Aminoethyl glycopyranosides (D-galactoside, D-glucoside)	Preparation of affinity adsorbents for carbohydrate-binding proteins	385
6-Aminohexanoyl-D-tryptophan methyl ester	Study of heterogeneity in binding of chymotrypsin and related proteins to affinity chromatography media	386
6-Aminohexyl glycopyranosides (D-galactoside, D-glucoside, D-fucoside, D-mannoside, D-xyloside, 2-acetamido-2-deoxy-D-glucoside)	Preparation of affinity adsorbents for carbohydrate-binding proteins	385
Chondroitin sulphate[a]	Study of interaction of glycosaminoglycan polysaccharidases with glycosaminoglycans	387
Concanavalin A	Study of inhibition of binding of immobilized concanavalin A to insulin receptors on isolated intact rat fat cells by inhibitors of concanavalin A	388
	'Anchoring units' for enzymes reversibly immobilized on the support for use in an analytical flow system	389
	Comparison of 4-methylumbelliferyl β-D-galactosidase and G_{M_1} β-D-galactosidase levels in normals and patients with I-cell disease	390
	Adsorption of soluble receptors from mouse submaxillary glands for use in a ligand-binding assay for epidermal growth factor-urogastrone	247
	Adsorption of *N*-[^{14}C]methyl ribonuclease B for use as substrate in a semiquantitative assay of *endo*-β-D-2-acetamido-2-deoxy-glucanase	391
Concanavalin A, succinylated	Adsorption of β-D-fructofuranosidase for use in enzyme immunoassay	392, 393
Cytochrome *c*	Demonstration of the utility of immobilized cytochrome *c* for study of the photochemical reaction of chloroplasts	394
Deoxycholic acid-poly(L-lysine)	Amphipathic matrix with binding affinity for integral membrane protein	395
Dermatan sulphate	Structural study of aggregating glycan chains and oligosaccharides with affinity for dermatan sulphate	396
	Study of interaction between immobilized dermatan sulphate and chondroitinase C	397
4-Diazophenylphosphoryl-choline	Quantitative affinity chromatographic evaluation of the expression of multivalency in the interaction of antibody with immobilized antigen	398

Table 5 *(contd.)*

Species coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Heparin	Study of interaction of glycosaminoglycan polysaccharidases with glycosaminoglycans	387
	Study of interaction between immobilized heparin and chondroitinase B	397
	Model arterial system for study of the kinetics and extent of binding of porcine serum lipoproteins to immobilized heparin	293
	Development of a potentiometric titration method for the determination of the bound heparin content of immobilized preparations	399
Insulin	Study of inhibition of binding of immobilized insulin receptors on isolated intact rat fat cells by inhibitors of concanavalin A	388
Lens culinaris lectin	'Anchoring units' for enzymes reversibly immobilized on the support for use in an analytical flow system	389
L-Lysine	Investigation of changes in prothrombin activity induced by plasmin adsorbed on immobilized L-lysine	400
	Investigation of the relationship between the amount of L-lysine immobilized and the capacity of the matrix for binding heparin	401
Noradrenaline	Investigation of direct hormonal regulation of the blood-clotting system by catecholamines	307
4-Phenylbutylamine	Study of heterogeneity in binding of chymotrypsin and related proteins to affinity chromatography media	386
Protyrosinase	Demonstration of DOPA oxidase activity of the immobilized proenzyme	402
Urinary glycoprotein	Purification of human urinary glycoprotein by sub-unit exchange chromatography	403

[a] See (36) in Scheme 8a, p. 612.

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[392] D. Pain and A. Surolia, *FEBS Lett.*, 1979, **107**, 73.
[393] S. Pillai and B. K. Bachhawat, *FEBS Lett.*, 1979, **101**, 301.
[394] M. S. Khristin, E. A. Akulova, and V. I. Surovtsev, *Biochemistry (USSR)*, 1977, **42**, 101.
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[397] N. Ototani and Z. Yosizawa, *Carbohydr. Res.*, 1979, **70**, 295.
[398] D. Eilat and I. M. Chaiken, *Biochemistry*, 1979, **18**, 790.
[399] M. Y. Varshavskaya, A. L. Klibanov, V. S. Goldmacher, and V. P. Torchilin, *Anal. Biochem.*, 1979, **95**, 449.
[400] I. P. Baskova and E. G. Mirkamalova, *Biochemistry (USSR)*, 1977, **42**, 224.
[401] M. W. C. Hatton, L. R. Berry, H. Kaur, A. Koj, and E. Regoeczi, *Can. J. Biochem.*, 1979, **57**, 1183.
[402] J. L. Iborra, A. Manjón, M. Tari, and J. A. Lozano, *Gen. Pharmacol.*, 1979, **10**, 143.
[403] G. Carrea, G. Lugaro, R. Niada, P. Vecchini, and E. Antonini, *FEBS Lett.*, 1979, **104**, 393.

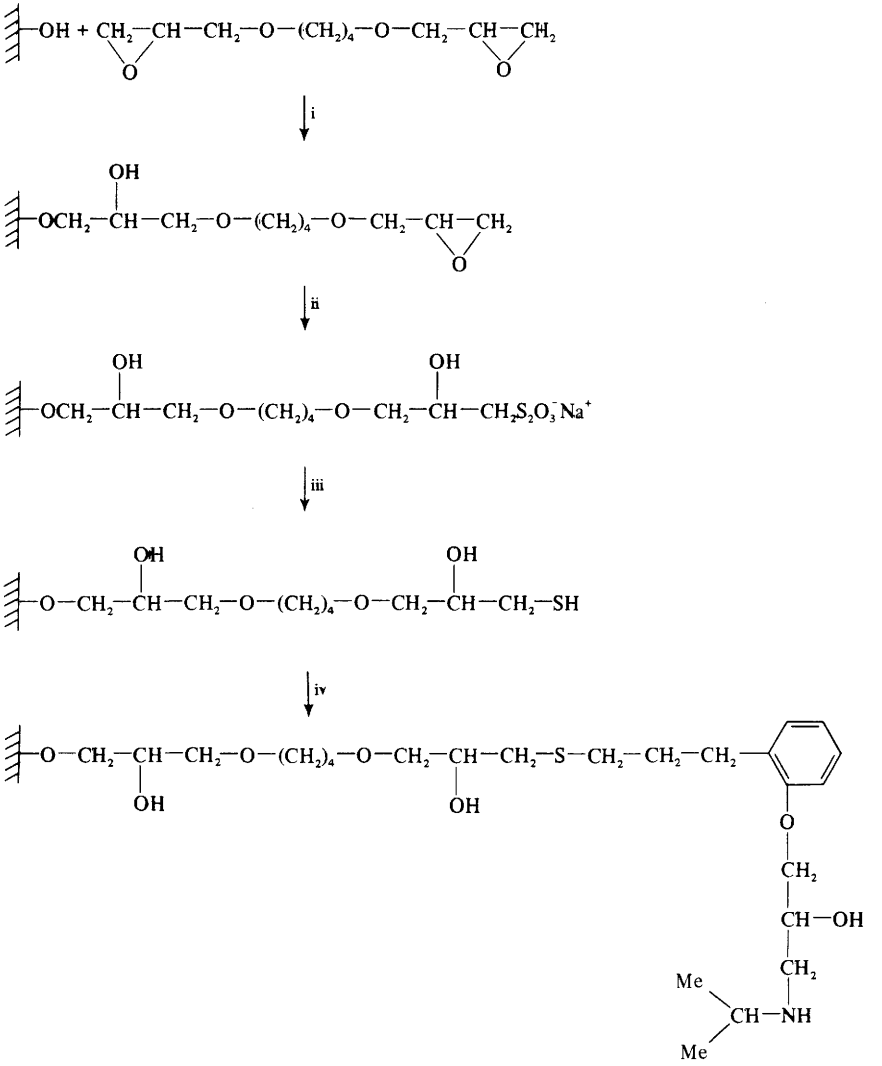
Agarose into which 4-aminobutyraldehyde had been incorporated as a ligand has been used to develop a method for measurement of the ligand content of affinity gels.[83] The method involves elution of a suitable reagent (here 3-benzyloxyamine) through a small column of the gel sample. The 3-benzyloxyamine reacts almost irreversibly with aldehydes, and its consumption per millilitre of the gel is determined. The method is of general applicability provided a reagent absorbed irreversibly by the gel can be selected.

Carboxymethyl-agarose has been treated with 5-(2-carboxyethyl)-6-azauridine 5 -phosphate using a carbodi-imide mediator, to provide an efficient affinity column for purification of orotidine-5′-phosphate decarboxylase from yeast.[404]

Divinylsulphone-cross-linked agarose has been used for the affinity chromatographic purification of an 'agarase' from a culture filtrate of a *Pseudomonas*-like bacterium.[405] Agarose activated with divinylsulphone has been treated with D-mannose and the matrix used for the purification of a lectin from *Onobrychis viciifolia*.[406]

Epoxy-activated agaroses continue to be widely used for preparation of affinity matrices. Agarose activated with butane-1,4-diol diglycidyl ether has been treated with L-alanyl-L-alanyl-L-alanyl-4-nitroanilide, a substrate for porcine pancreatic elastase.[407] Successful purification of the enzyme using this matrix could be achieved by performing the affinity chromatography at sub-zero temperatures where enzyme turnover was very low or halted. Ether-activated agarose has been treated with sodium thiosulphate, reduced with dithiothreitol, and coupled to the propylene side-chain of alprenolol in a free-radical chain addition reaction (Scheme 9).[408] The matrix has been successfully applied to the affinity chromatographic purification of β-adrenergic receptors from frog brain[408] and rat hepatic plasma membranes.[409] 4-Aminobenzamidine immobilized on epoxy-activated agarose has been used for the study of the binding of trypsin to this inhibitor.[410] Affinants coupled to epoxy-activated agarose have included chitin oligosaccharides for the affinity chromatographic isolation of a lectin from *Cucurbita pepo*,[411] chlorpromazine for the affinity chromatography of glutamate dehydrogenase,[412] dextran for the purification of D-glucan-binding proteins,[235] fluphenazine for the purification of calmodulin,[413] folic acid for affinity chromatography of ribonuclease A and glutamate dehydrogenase,[414] *S*-hexylglutathione for affinity chromatographic purification of glyoxalase

[404] R. S. Brody and F. H. Westheimer, *J. Biol. Chem.*, 1979, **254**, 4238.
[405] M. Malmqvist, *Carbohydr. Res.*, 1978, **62**, 337.
[406] K. D. Hapner and J. E. Robbins, *Biochim. Biophys. Acta*, 1979, **580**, 186.
[407] C. Balny, C. Le Doucen, P. Douzou, and J. G. Bieth, *J. Chromatogr.*, 1979, **168**, 133.
[408] M. G. Caron, Y. Srinivasan, J. Pitha, K. Kociolek, and R. J. Lefkowitz, *J. Biol. Chem.*, 1979, **254**, 2923.
[409] C. L. Wood, M. G. Caron, and R. J. Lefkowitz, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 1.
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[411] A. K. Allen, *Biochem. J.*, 1979, **183**, 133.
[412] F. M. Veronese, O. Schiavon, E. Boccu, R. Largajolli, and C. A. Benassi, *Il Farmaco*, 1979, **34**, 266.
[413] H. Charbonneau and M. J. Cormier, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 1039.
[414] F. Sawada, *Anal. Biochem.*, 1979, **98**, 184.



Reagents: i, NaOH; ii, $Na_2S_2O_3$; iii, H_4edta, dithiothreitol; iv, $K_2S_2O_8$, 90 °C, alprenolol

Scheme 9

I[232,415] and glutathione-*S*-transferases,[416] 8-(2′-hydroxyethyl)-thio-cyclicAMP for affinity chromatographic purification of protein kinase sub-units from rabbit skeletal muscle,[417] and *myo*-inositol for isolation of *myo*-inositol oxygenase

[415] A. C. Aronsson, G. Tibbelin, and B. Mannervik, *Anal. Biochem.*, 1979, **92**, 390.
[416] C. Guthenberg and B. Mannervik, *Biochem. Biophys. Res. Commun.*, 1979, **96**, 1304.
[417] W. Weber, C. W. Vogel, and H. Hilz, *FEBS Lett.*, 1979, **99**, 62.

from rat kidneys.[418] Evaluation of the binding of antipsychotic drugs to L-glutamate dehydrogenase has been achieved by competitive elution of the enzyme, adsorbed on perphenazine immobilized on epoxy-activated agarose, with buffers containing soluble phenothiazines and butyrophenones.[419] Epoxy-activated agarose has been treated with hexandioic acid dihydrazide and then with periodate-oxidized ribonucleic acid, and the binding of *Escherichia coli* and rat-liver ribosomal proteins to the matrix have been investigated.[420] *NN'N''*-Triacetylchitotriose, immobilized on epoxy-activated agarose and then treated with acetic anhydride to reacetylate any de-*N*-acetylated groups, provides a suitable matrix for the rapid purification of the lectin from *Solanum tuberosum*.[421] Various immobilized preparations of tryptophanol phosphate have been examined for their ability to bind tryptophan synthase.[422] The optimal gel was prepared by treating epoxy-activated agarose with an excess of hexandioic acid dihydrazide, followed by reaction with pyridoxal phosphate, treatment with 4-dimethylaminobenzaldehyde, and acetylation of the secondary hydrazide groups followed by deprotection of the primary hydrazide groups. The matrix was then coupled to the ligand, indole-propanol phosphate.

Epoxy-activated agarose has been coupled with iminodiacetic acid and converted into a zinc chelate form by equilibration with zinc chloride.[423] The column has been successfully applied to the purification of human plasma α_2-macroglobulin and α_1-proteinase inhibitor. The column can be stripped with H_4-edta and regenerated with zinc chloride.

Optimal conditions for the activation of agarose with epichlorohydrin have been established and absorbents prepared using 2-acetamido-2-deoxy-D-glucose, *NN'N''*-triacetylchitotriose, and *Ricinus communis* lectin.[424] The *NN'N''*-triacetylchitotriose matrix specifically adsorbed *Solanum tuberosum* agglutinin, which could be quantitatively recovered by elution with ammonia. The column could be repeatedly used without reduction of its binding capacity. Immobilized *R. communis* lectin was also used for the purification of porcine thyroglobulin by affinity chromatography. Cortisol immobilized on epichlorohydrin-activated agarose has been used to isolate human retroplacental blood plasma proteins.[425] L-Fucose reacts with epichlorohydrin-activated agarose in the presence of sodium borohydride to yield a matrix which binds the tetrameric form of L-fucosidase specifically, allowing its separation from monomeric enzyme.[426]

Agarose, cross-linked with epichlorohydrin to render the beads sturdier and more heat-resistant, has been activated with cyanogen bromide and coupled with glycine methyl ester.[427] The ester was then converted into the glycyl

[418] F. Koller and O. Hoffmann-Ostenhof, *Z. Physiol. Chem.*, 1979, **360**, 507.
[419] F. M. Veronese, R. Bevilacqua, and I. M. Chaiken, *Mol. Pharmacol.*, 1979, **15**, 313.
[420] I. Toots, A. Metspalu, A. Lind, M. Saarma, and R. Villems, *FEBS Lett.*, 1979, **104**, 193.
[421] N. N. Desai and A. K. Allen, *Anal. Biochem.*, 1979, **93**, 88.
[422] H. P. Gschwind, U. Gschwind, C. H. Paul, and K. Kirschner, *Eur. J. Biochem.*, 1979, **96**, 403.
[423] T. Kurecki, L. F. Kress, and M. Laskowski, *Anal. Biochem.*, 1979, **99**, 415.
[424] I. Matsumoto, Y. Mizuno, and N. Seno, *J. Biochem. (Tokyo)*, 1979, **85**, 1091.
[425] A. A. Akhrem, I. I. Vashkevich, G. V. Avvakumov, O. V. Sviridov, and O. A. Strel'chenok, *Biochemistry (USSR)*, 1979, **44**, 116.
[426] T. Alam and A. S. Balasubramanian, *Biochim. Biophys. Acta*, 1979, **566**, 327.
[427] S. H. Yu, P. P. L. Lau, and T. G. Spring, *Biochim. Biophys. Acta*, 1978, **517**, 31.

hydrazide and then into the azide before being treated with histones from chick erythrocytes and used for affinity chromatographic purification of calf thymus deoxyribonucleic acid and PM-2 viral deoxyribonucleic acid.

Commercially available 'Affi-gel' supports, agarose beaded gel included in a polyacrylamide support having different spacer arm lengths, have been used for the preparation of a number of affinity matrices. The spacers are anchored by extremely stable ether linkages. Ligands immobilized on 'Affi-gel 10' have included α-amylase for the affinity chromatographic purification of the proteinaceous α-amylase inhibitor from *Phaseolus vulgaris*,[138] *Ricinus communis* lectin for the affinity chromatography of tyrosine transfer ribonucleic acids,[320] and anti-(enterobacterial common antigen) antibody for purification of the antigen by immunoadsorption.[428]

2-(Ethyloxycarbonyl)-3-phenylpropanoyl-L-alanylglycine has been immobilized on 'Affi-gel 101' and used for the affinity chromatographic purification of thermolysin.[429] 'Affi-gel 201', which has an eight-carbon spacer arm, has been treated with 7-aminocephalosporanic acid and 6-aminopenicillanic acid and applied to the affinity chromatographic purification of β-lactamase from *Neisseria gonorrhoeae*.[430] A quantitative affinity chromatographic study of the multiple binding sites on carboxypeptidase B has been carried out using D-phenylalanine methyl ester pre-treated with 'Affi-gel 201' and then saponified.[431]

Purification of wheat-germ porphobilinogen deaminase has been achieved on an organomercurial agarose derivative, 'Affi-gel 501'.[432] Haemoglobin bound to 'Affi-gel 501' binds haptoglobin specifically.[249] Dissociation of the haptoglobin–haemoglobin complex from the matrix can be achieved with buffers containing dithiothreitol or other thiols: the pure complexes are very effective immunogens and yield monospecific antibody to haptoglobin.

A polyacrylamide–boronate affinity gel, 'Affi-gel 601', has been used for the separation of cyclic adenosine monophosphate, cyclic guanosine monophosphate, and cyclic cytidine monophosphate from their corresponding 5′-nucleotides and nucleosides.[433] A simple direct assay of 3′,5′-cyclic nucleotide phosphodiesterase activity has been developed, based on the use of the gel.

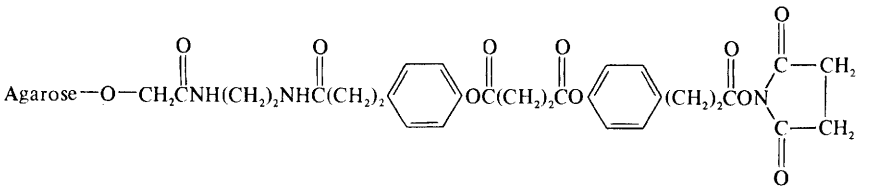
Thromboxane B_2 and prostaglandin derivatives immobilized on 'Affi-gel 701' have been prepared for use in new immunoassay methods for their determination.[434]

A mixed disulphide derivative of agarose, agarose-glutathione-2(5-nitropyridyl)-disulphide, has been used to immobilize bovine galactosyltransferase.[435] As the immobilized enzyme retains biosynthetic activity, the thiol group of the enzyme that is involved in the coupling must be physically distinct

[428] J. Measel, *Microbios Lett.*, 1978, **7**, 19.
[429] N. Nishino and J. C. Powers, *Biochemistry*, 1979, **18**, 4340.
[430] L. A. Griquez and R. F. D'Amato, *Antimicrob. Agents Chemother.*, 1979, **15**, 229.
[431] J. Danner, J. E. Somerville, J. Turner, and B. M. Dunn, *Biochemistry*, 1979, **18**, 3039.
[432] C. S. Russel and S. E. Pollack, *J. Chromatogr.*, 1978, **166**, 632.
[433] C. W. Davis and J. W. Daly, *J. Cyclic Nucleotide Res.*, 1979, **5**, 65.
[434] L. Levine, I. Alam, and J. J. Langone, *Prostaglandins Med.*, 1979, **2**, 177.
[435] J. Grunwald and L. J. Berliner, *Biochim. Biophys. Acta*, 1978, **523**, 53.

from the active site, because the bulky glutathione group does not obscure the α-lactalbumin-binding region.

A mixed polyacrylic agarose carrier has been used for immobilization of 4-aminophenyl L-lactic acid for affinity chromatography of lactate dehydrogenase.[436] 4-Phenylbutylamine, immobilized on succinylated poly(acrylic hydrazide) using a carbodi-imide reaction mediator, has been used in a quantitative affinity chromatographic study of the binding of ligands to chymotrypsin.[437] In contrast to earlier studies of competitive elution phenomena, where an added soluble ligand was shown to interfere with enzyme binding to an affinity matrix, benzyloxycarbonyl derivatives of aromatic acids were shown to facilitate binding of chymotrypsin to this matrix. These results suggest that it may be possible for affinity chromatographic methods to be 'fine-tuned' by appropriate selection of co-solutes.



(56)

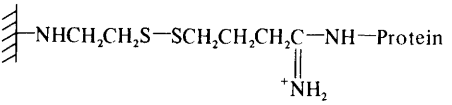
The preparation of a new succinimidyl ester agarose derivative (56) by reaction of *bis*{4-[2-(*N*-succinimidoxycarbonyl)ethyl]phenyl}succinate with an amino-ethylcarbamyl-methyl derivative of agarose has been described.[438] The derivative can be used for covalently linking proteins and other ligands containing amino-groups to agarose *via* phenyl ester linkages that can later be broken under mild conditions which should not alter other groups or residues which may be present in proteins, *e.g.* cystinyl residues and glycosyl residues. Trypsin and myoglobin have been immobilized on the derivatives and their properties investigated.

Glutathione has been conjugated with sulphobromophthalein and then treated with agarose cyclic imidocarbonate.[439] The matrix was used for the partial purification of ligandin.

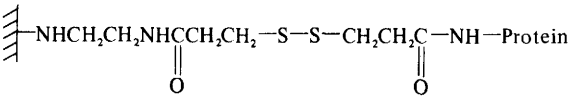
A commercially available thiol-containing agarose has been applied to the purification of procollagen type II from Swarm chondrosarcoma of rat.[440]

Alkaline phosphatase has been reversibly immobilized on cross-linked agarose by two methods, both based on disulphide linkages.[441] Prior to coupling with 5,5′-dithiobis-(2-nitro-benzoic acid)-activated thiol-agarose (57), the enzyme was modified by thiolation with 4-mercaptobutyrimidate. The unmodified enzyme could be coupled directly to dithiobis(succinimidyl propionate)-agarose (58).

[436] E. Brown and R. Joyeau, *Biochimie*, 1979, **61**, 437.
[437] B. M. Dunn and W. A. Gilbert, *Arch. Biochem. Biophys.*, 1979, **198**, 533.
[438] P. Singh, S. D. Lewis, and J. A. Shafer, *Arch. Biochem. Biophys.*, 1979, **193**, 284.
[439] A. W. Wolkoff, M. M. Bhargava, C. Chung, Z. Gatmaitan, *Proc. Soc. Exp. Biol. Med.*, 1979, **160**, 150.
[440] K. Angermann and H. J. Barrach, *Anal. Biochem.*, 1979, **94**, 253.
[441] S. McCracken and E. Meighen, *Can. J. Biochem.*, 1979, **57**, 834.



(57)



(58)

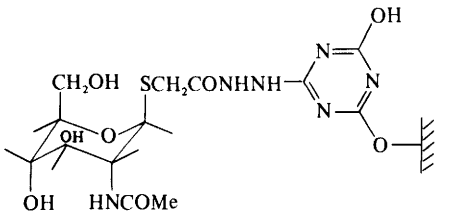
Triazinyl dyes (usually Cibacron Blue F-3GA) react with agarose *via* the chlorine atoms on the triazine ring, and have been used for the purification of numerous proteins, as has been reviewed.[91] Commercially available Cibacron Blue F-3GA is heterogeneous and a minor contaminant in the dye has been shown to inactivate phosphoglycerate kinase and isoleucyl-tRNA synthetase.[442] Purification of Cibacron Blue F-3GA to homogeneity results in a dramatic decrease in inactivation of these enzymes. Some caution is therefore required when purifying enzymes on immobilized Cibacron Blue F-3GA. An electrophoretic desorption cell that can contain large quantities of affinity matrix has been constructed and a method developed for the purification of preparative amounts of human serum albumin on Cibacron Blue F-3GA–agarose using the cell.[443]

Cibacron Blue F-3GA immobilized on agarose has been successfully applied to the purifications of equine liver alcohol dehydrogenase on a large scale,[444] porcine brain aldose reductase,[445] dihydropterin reductase,[231] the prosthetic group [6,7-dimethyl-8(1′-D-ribityl)lumazine] of the blue fluorescent protein from *Photobacterium phosphoreum*,[446] D-glucose 6-phosphate dehydrogenase from rat liver,[447] human leukocyte and lymphoblastoid interferons,[448] bacterial malate dehydrogenases,[210] myosin light chains,[449] NADP-malic enzyme from maize leaf,[450] and phosphoglycerate kinase from *Saccharomyces cerevisiae*.[338] The dye is also available immobilized on 'Affi-gel', and these preparations have been used to purify glutamine synthetase from *Azotobacter* species and other

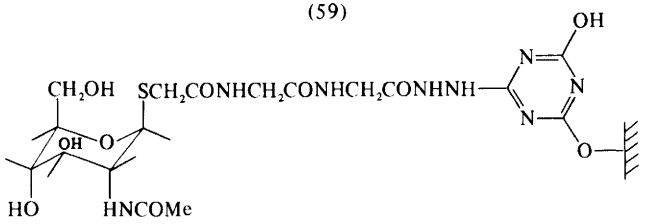
[442] B. H. Weber, K. Willeford, J. G. Moe, and D. Piszkiewicz, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 252.
[443] M. R. A. Morgan, N. A. Slater, and P. D. G. Dean, *Anal. Biochem.*, 1979, **92**, 144.
[444] S. K. Roy and A. H. Nishikawa, *Biotechnol. Bioeng.*, 1979, **21**, 775.
[445] R. A. Boghosian and E. T. McGuiness, *Biochim. Biophys. Acta*, 1979, **567**, 278.
[446] P. Koka and J. Lee, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3068.
[447] M. L. Dao, J. J. Watson, R. Delaney, and B. C. Johnson, *J. Biol. Chem.*, 1979, **254**, 9441.
[448] J. S. Erickson and K. Paucker, *Anal. Biochem.*, 1979, **98**, 214.
[449] A. P. Toste and R. Cooke, *Anal. Biochem.*, 1979, **95**, 317.
[450] S. Asami, K. Inoue, K. Matsumoto, A. Murachi, and T. Akazawa, *Arch. Biochem. Biophys.*, 1979, **194**, 503.

prokaryotes,[451] NADP-specific isocitrate dehydrogenase from *Escherichia coli*,[452] and adenylate cyclase.[237]

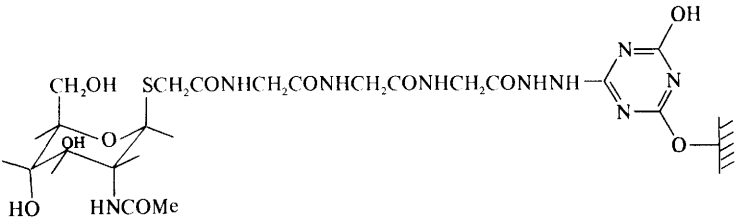
Three biospecific adsorbents with weak ion-exchange properties have been prepared by treating the second available chlorine group on trichloro-*sym*-triazine, already coupled by reaction of the first chlorine with agarose, with the hydrazide group of 2-*S*-(2′-acetamido-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranosyl)-2-thioglycolyl hydrazide (59), 2-*S*-(2′-acetamido-2′-deoxy-β-D-glucopyranosyl-2-thioglycolyl-glycylglycyl hydrazide (60), or 2-*S*-(2′-acetamido-2′-deoxy-β-D-glucopyranosyl)-2-thioglycolylglycylglycylglycyl hydrazide (61).[453] The adsorbents were used in an affinity chromatographic investigation of the active site of β-D-2-acetamido-2-deoxy-hexosidase. Human urinary protease inhibitor has been isolated on chymotrypsin immobilized in the presence of β-phenylpropionate by reaction with agarose previously activated with trichloro-*sym*-triazine and coupled to aniline.[454] Heparin has been immobilized by reaction with trichloro-*sym*-triazine-activated agarose and used for the purification of bull seminal plasma hyaluronidase.[272]



(59)



(60)



(61)

[451] J. E. Lepo, G. Stacey, O. Wyss, and F. R. Tabita, *Biochim. Biophys. Acta*, 1979, **568**, 428.
[452] B. Vasquez and H. C. Reeves, *Biochim. Biophys. Acta*, 1979, **578**, 31.
[453] A. A. Artyukov and N. V. Molodtsov, *J. Solid-phase Biochem.*, 1978, **3**, 33.
[454] A. Kessner, L. T. Hodgins, and W. Troll, *Anal. Biochem.*, 1979, **92**, 383.

Agarose has been treated with 2,4,6-trifluoro-5-chloropyrimidine ('FCP'), a reactive group in reactive dyes.[455] Under appropriate conditions a thermally stable product with unimpaired beaded structure was obtained and was reactive toward amines and mercaptans. The stability of immobilized ligands (1,6-diaminohexane, aniline, and ethanethiol) bound to FCP-agarose between pH 4 and 8 was about 200 times higher than similar products obtained by coupling with agarose cyclic imidocarbonate. There was very little ligand leakage from the matrix. A high degree of substitution can be achieved and the FCP–agarose can be stored in the wet state at 4 °C without substantial decrease in coupling capacity. 2,4,5,6-Tetrachloro- and 2,4,5,6-tetrafluoro-pyramidines can also be used for the activation of agarose.

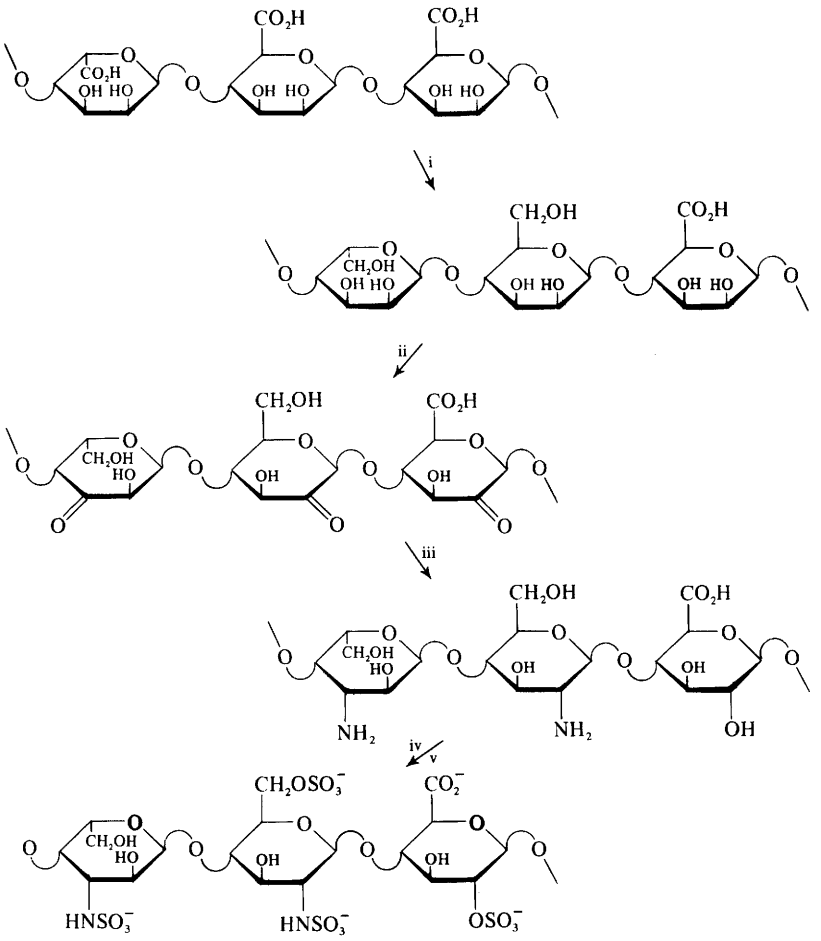
Alginic Acid and Alginates. – Alginic acid has been converted into a polysaccharide containing the residues present in heparin (Scheme 10).[456] The heparin analogue thus obtained (62) showed low whole-blood anticoagulant activity, which was increased by purifying the material on a column of antithrombin III-agarose.

Columns of calcium alginate gel pellets have excellent physical properties when used as supports for cell immobilization.[457] *Saccharomyces uvarum* cells have been mixed with high concentrations of sodium alginate, precipitated by addition of calcium chloride, and extruded as discrete droplets which were found to be very resistant to compression and abrasion during passage of high concentrations of sucrose at high flow rate.

Amylopectins. – The effects of acrylamide graft copolymerization on the solution properties of amylopectin have been discussed.[458] Amylopectin has been dyed with 'DyAmyl-L' and used in this form as a substrate for the assay of α-amylase.[459] Amylopectin has been treated with isocyanate derivatives of 4-amino-(1,1-dimethyl ethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one ('metribuzin') or acid chloride derivatives of 2,4-dichlorophenoxyacetic acid ('2,4-D') and 2,2-dichloropropionic acid ('dalapon'), to produce controlled-release polymeric pesticide systems.[460] The solvent system utilized for these reactions, a lithium chloride or bromide salt in *NN*-dimethylacetamide, allows dissolution of the reactant salt and facilitates analysis of the polymer product by such techniques as i.r., u.v., and n.m.r. spectroscopies and gel permeation chromatography. Derivatives of other naturally occurring polysaccharides, including amylopectin, cellulose, chitin, and dextran, were also prepared.

Amylose. – The effects of acrylamide graft copolymerization on the solution properties of amyloses have been discussed.[458] As part of a study of the blue

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[456] O. Larm, K. Larsson, E. Scholander, L. O. Andersson, E. Holmer, and G. Söderström, *Carbohydr. Res.*, 1979, **73**, 332.
[457] P. S. J. Cheetham, K. W. Blunt, and C. Bucke, *Biotechnol. Bioeng.*, 1979, **21**, 2155.
[458] D. R. Owen and T. C. Shen in 'Structure–Solubility Relationships in Polymers', ed. F. W. Harris and R. B. Seymour, Academic Press, New York, 1977.
[459] J. A. Carney, S. A. Osibelumo, J. A. Clements, and R. F. Jay, *Ann. Clin. Biochem.*, 1977, **14**, 350.
[460] C. L. McCormick and D. K. Lichatowich, *J. Polym. Sci., Polym. Lett.*, 1979, **17**, 479.



(62)

Reagents: i, carbodi-imide, $NaBH_4$; Br_2–H_2O, pH 7.0; iii, $\overset{+}{N}H_4A\bar{c}$, $NaCNBH_3$; iv, glacial AcOH; dmf, SO_3–pyr

Scheme 10

colour of tri-iodide ions in starch and starch fractions, the characterization and assignments of absorption and circular dichroism spectra of tri-iodide ions in amylose have been recorded.[461] An electron diffraction study of single crystals of amylose complexes with butanol has been reported.[462]

[461] T. Handa and H. Yajima, *Biopolymers*, 1979, **18**, 873.
[462] F. P. Booy, H. Chanzy, and A. Sarko, *Biopolymers*, 1979, **18**, 2261.
[463] F. R. Seymour, R. D. Knapp, T. E. Nelson, and B. Pfannemüller, *Carbohydr. Res.*, 1979, **70**, 125.

^{13}C N.m.r. spectra have been recorded for previously reported comb-like derivatives of amylose produced by orthoester and Helferich condensations of D-glucose and amylose.[463] It was anticipated that, for these polymer condensations, the Helferich and orthoester condensations would favour α- or β-D-glycosylation, respectively, as for monomeric systems. However, the spectra indicate extensive, if not exclusive, β-D-glycosylation for both the Helferich and the orthoester conditions.

The crystal and molecular structure of a tri-*O*-ethylamylose polymorph, TEA-3, has been solved by stereochemical conformation and packing analysis, combined with *X*-ray fibre diffraction analysis.[464] The unit cell is orthorhombic, space group $P2_12_12_1$, with $a = 15.36$ (±0.03) Å, $b = 12.18$ (±0.05) Å, and c (fibre repeat) = 15.48 (±0.01) Å. The actual chain conformation is a 4_3 helix with the 6-ethoxy-group in the *tg* position, as was previously found for the polymorph TEA-1.

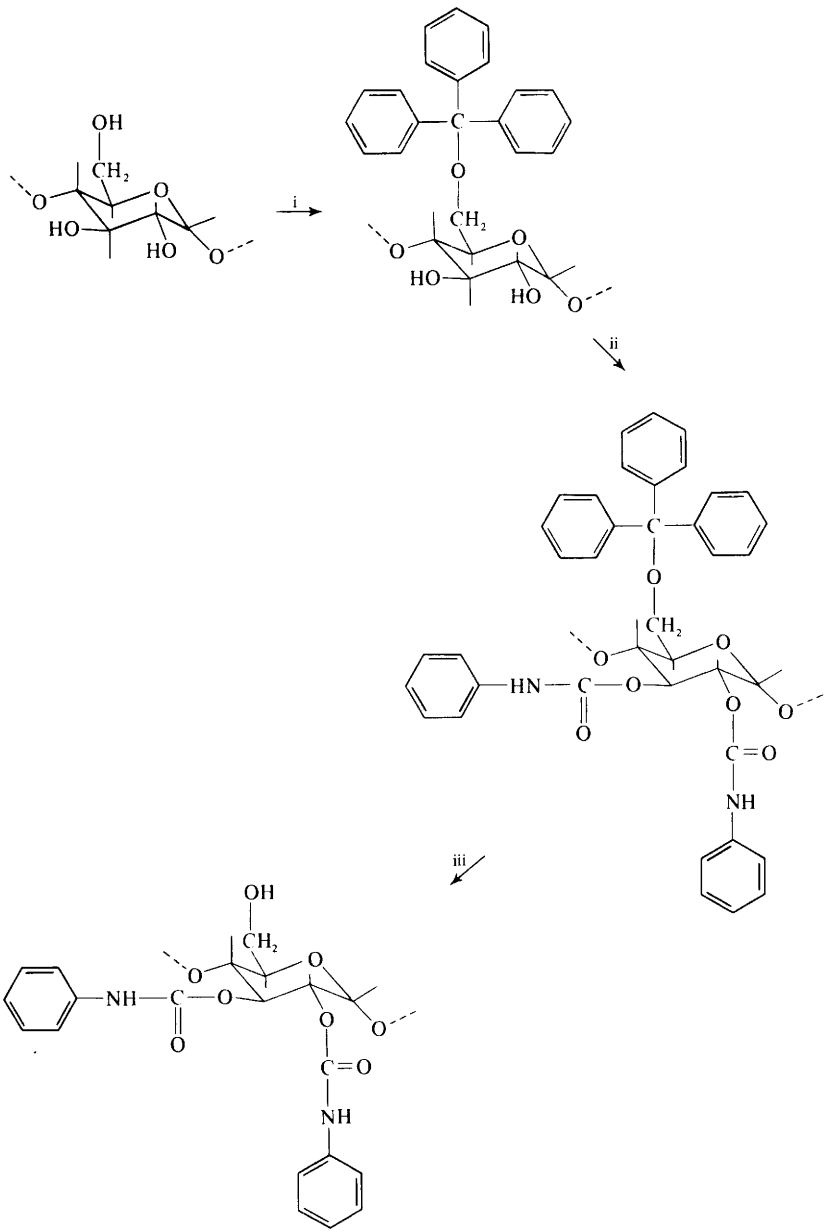
An investigation of the interaction of amylose with the hydrophobic fluorescent probe 2-(4′-toluidinylnaphthalene)6-sulphonate has been reported.[465]

6-Trityl-2,3-dicarbanilyl and 2,3-dicarbanilyl derivatives of amylose (Scheme 11) may be used as a backbone to introduce oligosaccharide branches *via* (1→6)-D-glycosidic bonds.[466] Possible degradation prior to and during the glycosidic coupling has been followed by light scattering measurements at each step of derivatization. Some degradation was shown to occur in the tritylation reaction and possibly in the conversion into the 6-trityl-2,3-dicarbanilate, but virtually none was observed in subsequent steps. Carbanilated and tritylated derivatives have been characterized by measurement of optical rotary dispersion and c.d. spectra.[467] No anomalous o.r.d. and only a weak c.d. spectrum was found for 6-tritylamylose in 1,4-dioxane, indicating a widely unordered structure, which is in accord with the low viscosity of the compound. 2,3-Dicarbanilylamylose is distinct from other carbanilated derivatives having substituents in the C-6 position in having an unusually high Cotton effect, suggesting a helical chain which may adopt a more regular orientation.

Potato amylose (DP *ca.* 800) has been oxidized with sodium metaperiodate and used as a substrate for glucoamylase free of α-amylase activity.[468] The Michaelis constant and maximal velocity for the enzyme were found to decrease with increasing oxidation of the amylose substrate. These observations were explained on the basis of competitive inhibition by the oxidized non-reducing end of the (1→4)-α-D-glucan chain.

6-*O*-Methylamylose and 6-deoxyamylose have been studied as substrates for the crystalline liquefying amylase of *Bacillus subtilis*.[469] The oligosaccharides obtained from the enzyme digests were separated by paper chromatography and some of the fractions identified after acetylation by electron-impact m.s. Neither

[464] T. L. Bluhm and P. Zugenmaier, *Carbohydr. Res.*, 1979, **68**, 15.
[465] H. Nakatani, K. I. Shibata, H. Kondo, and K. Hiromi, *Biopolymers*, 1977, **16**, 2363.
[466] B. Pfannemüller and A. Berg, *Makromol. Chem.*, 1979, **180**, 1183.
[467] B. Pfannemüller and A. Berg, *Makromol. Chem.*, 1979, **180**, 1201.
[468] L. Bezerra Carvalho, *Carbohydr. Res.*, 1979, **75**, 257.
[469] C. E. Weill, M. Kaminsky, and J. Guerrera, *Carbohydr. Res.*, 1979, **73**, 337.



Reagents: i, trityl chloride, pyr; ii, phenyl isocyanate, pyr; iii, conc. HCl, MeOH

Scheme 11

6-*O*-methyl-D-glucose nor 6-deoxy-D-glucose was detected in any of the hydrolyzates, in agreement with results previously obtained with other chemically modified amyloses for which a chemically modified monomer has never yet been obtained as a product of the amylase activity.

Amylose has been treated with isocyanate or acid chloride derivatives of pesticides to produce controlled-release polymeric pesticide systems (see also Amylopectin, p. 629).[460]

Amylose cross-linked with epichlorohydrin has been used for the affinity chromatographic purification of the extracellular isoamylase of *Pseudomonas amyloderamosa*.[470]

Cellulose. – A comprehensive treatise on the modification of cellulose has appeared.[471] The book is sub-divided into five parts: a summary of previous work on modified cellulosics, a discussion of cotton and wood cellulose, a review of the accessibility and reactivity of cellulose, a discussion of modification of cellulose by grafting of vinyl monomers, and a description of additional techniques for cellulose modification.

By exploiting the good dissolution properties of compressed gases in the supercritical state in a flow apparatus, thermal degradation of cellulose has been achieved without the secondary reactions and strong carbonization usual in conventional pyrolysis methods.[472] Cellulose could be degraded to the extent of 98%.

Cellulose has been dissolved in *N*-methylmorpholine *N*-oxide at high temperature and the recrystallization of the polysaccharide upon cooling investigated.[473] The crystallization system is analogous to the solidification of binary mixtures of polymer and diluent, which present unusual thermodynamic and morphological properties. The system provides a new method of texturing cellulose.

The kinetics of the deuteration of cellulose have been studied in an i.r. spectroscopic study employing D_2O–H_2O vapours.[474] D_2O is rapidly adsorbed by cellulose, whereas hydrogen bonds in the cellulose are observed to break on swelling. Deuteration of only a fraction of the amorphous regions occurs rapidly, while the remainder are deuterated slowly, as was demonstrated by m.s. studies.

The swelling and ion-exchange properties of diethylaminoethyl- and benzoylated diethylaminoethyl-cellulose in water–methanol–1M-hydrochloric acid mixtures have been compared.[475] Benzoylation changes these properties of diethylaminoethyl-cellulose: in aqueous solution the water uptake of the benzoylated polysaccharide is much less than that of diethylaminoethyl-cellulose, while its ability to adsorb metal ions (Cd^{2+}, Hg^{2+}, Bi^{3+}) is much better. At high

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[472] P. Koll and J. Metzger, *Angew. Chem. Int. Ed. Engl.*, 1978, **17**, 754.

[473] H. Chanzy, M. Dubé, and R. H. Marchessault, *J. Polym. Sci., Polym. Lett.*, 1979, **17**, 219.

[474] M. Wahba, *Chem. Scr.*, 1977, **11**, 158.

[475] A. Lasztity and M. Nemeth, *Acta Chim. Acad. Sci. Hung.*, 1978, **98**, 285.

methanol concentrations, however, the two weakly basic anion exchangers have similar sorption properties.

Crystal data on cellobiose octa-acetate and cellotriose undeca-acetate have been compared in order to analyse what information available from crystal structures of oligosaccharides can be used to deduce the three-dimensional structure of the related polysaccharide.[476]

The ^{13}C n.m.r. spectra of peracetylated α-cellotriose, α-cellotetraose, and α-cellopentaose have been reported.[477]

Grafting of vinyl acetate onto cellulose has been achieved by using azobisisobutyronitrile (AIBN) and benzoyl peroxide (BPO) as initiators.[478] The chain-transfer mechanism occurring in these systems was verified by carrying out several grafting experiments in the presence of propane-1-thiol, a common chain-transfer agent. Kinetic studies in an aqueous medium were carried out to determine the relative abilities of AIBN and BPO in effecting graft copolymerization. AIBN-initiated grafting proceeds as a higher overall rate, and gives a higher percentage of grafting since side reactions are minimized. Studies of grafting of acrylamide and acrylonitrile onto cellulose,[479] and of methyl methacrylate onto cotton using a redox initiator,[480] have also been reported.

The composition of the water-soluble portion of cellulose cigarette smoke condensate has been studied and forty-two compounds identified.[481] Semi-quantitative estimation showed that 1,6-anhydro-D-glucopyranose ('levoglucosan') was the most abundant component.

Investigations of the interaction of cellulose with antimicrobial compounds have been reported and the properties of the products obtained described.[482]

Glacial acetic acid, acetyl chloride, and acetic anhydride have been compared as acetylating agents for solutions of cellulose in dimethylsulphoxide–paraformaldehyde.[483] Mixtures of pyridine and acetic anhydride were demonstrated to acetylate the cellulose rapidly to yield beige to amber acetone-soluble cellulose acetates which were partially oxidized. These thermoplastic resins exhibited softening points between 80 and 110 °C and thermal stabilities similar to those of native cellulose.

The preparation of water-soluble cyanoethyl and carboxyethyl derivatives of native cellulose has been described.[484] 6-Chloro-6-deoxy- and 6-iodo-6-deoxy-cellulose have been prepared by treatment of cellulose 2,3-di-*O*-acetate with *N*-chlorosuccinimide or *N*-iodosuccinimide, respectively, followed by de-*O*-acetylation.[485]

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[478] B. N. Misra, J. K. Jassal, and C. S. Pande, *J. Polym. Sci., Polym. Chem. Ed.*, 1978, **16**, 295.
[479] S. Oprea, S. Dumitriu, and V. Bulagovischi, *Cellul. Chem. Technol.*, 1979, **13**, 3.
[480] A. K. Mukerjee and S. Sanyal, *Cellul. Chem. Technol.*, 1979, **13**, 13.
[481] H. Sakuma and S. Sugawara, *Agric. Biol. Chem.*, 1979, **43**, 1585.
[482] Z. A. Rogovin, A. D. Virnik, G. S. Kondrashowa, V. N. Kolokolow, N. A. Andronowa, and N. S. Plotkina, *Cellul. Chem. Technol.*, 1979, **13**, 441.
[483] R. B. Seymour and E. L. Johnson, *J. Polym. Sci., Polym. Chem. Ed.*, 1978, **16**, 1.
[484] B. Lukanoff, B. Philipp, and H. Schleicher, *Cellul. Chem. Technol.*, 1979, **13**, 417.
[485] A. I. Usov, R. G. Krylova, and F. R. Suleimanova, *Izv. Acad. Nauk SSSR Ser. Khim.*, 1977, **26**, 2158.

6-Trityl-2,3-dicarbanilyl and 2,3-dicarbanilyl derivatives of cellulose have been used as backbones onto which oligosaccharide branches can be introduced *via* (1 → 6)-glycosidic bonds. Light scattering measurements on each step of this derivation have shown that degradation occurs mainly during the tritylation step.[467] Carbanilated and tritylated derivatives of cellulose have been studied by measurement of o.r.d. and c.d. spectra.[468] The conformation of the modified cellulose was found to exhibit a clear steric dependence on the size of the C-6 substituent.

The distribution of methyl groups after methylation of celluloses containing phosphate groups is quite different from that obtained after methylation of cellulose.[486] Results indicated a probable high level of phosphorylation at positions 2 and 6 of the D-glucopyranosyl ring and provided supporting evidence for the recognized fibrillar models for cotton. Cellobiose and cellotriose have been methylated using methyl iodide in dimethyl sulphoxide containing potassium t-butoxide.[487] Studies on the cellulose methylation process under laboratory conditions have been reported.[488] Cellulose has been treated with isocyanate or acid chloride derivatives of pesticides to produce controlled-release polymeric pesticide systems (see also Amylopectin, p. 629)[460]

Cellulose phosphonate has been treated with *NN*-dimethylacrylamide or 4-vinylpyridine in the presence of sodium ethoxide to yield cellulose 2-(*NN*-dimethyl)carbamoyl-ethylphosphonate or cellulose 2-pyridinylethylphosphonate, respectively.[489] Cellulose phosphonate fabrics modified with *NN*-dimethyl-acrylamide had powerful flame-retardant properties, suggesting that the combination of phosphoryl and amide groups was an effective flame retardant.

The preparation of water-soluble cellulose sulphates having a degree of substitution of 0.3–2.0 has been reported using a homogeneous method involving cellulose nitrite as a reactive intermediate.[490] Products obtained differed substantially from those prepared *via* the customary heterogeneous method. Principal chemical differences were the uniform distribution of the substituents, the exceptionally high molecular weights of the sulphate esters, and the location of the substituents on the D-glucosyl residues. These differences result in a number of chemical and physical properties not exhibited by other cellulose derivatives.

Derivatives of cellulose continue to be used as supports for new affinity chromatography media and for immobilization of enzymes. Trypsin has been immobilized on 4-aminobenzyl-cellulose.[132] A penicillinase from *Bacillus cereus* has been immobilized by glutaraldehyde-mediated reaction with aminoethyl-cellulose.[491] Tannin has been activated with cyanogen bromide and coupled with

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[487] N. V. Bovin, V. A. Nesmeyanov, and A. Y. Khorlin, *Izv. Akad. Nauk SSSR Ser. Khim.*, 1977, **26**, 2622.
[488] B. Philipp, K. H. Bischoff, and F. Loth, *Cellul. Chem. Technol.*, 1979, **13**, 23.
[489] N. Inagaki and K. Katsuura, *J. Polym. Sci., Polym. Chem. Ed.*, 1978, **16**, 2771.
[490] R. G. Schweiger, *Carbohydr. Res.*, 1979, **70**, 185.
[491] Y. Klemes and N. Citri, *Biotechnol. Bioeng.*, 1979, **21**, 897.

aminohexyl-cellulose to provide a matrix for the immobilization by adsorption of aminoacylase,[492] and a number of other enzymes.[493]

Aminohydroxybutyl-cellulose has been used to immobilize tRNA's *via* their oxidized 3′-termini for use in the affinity chromatography and study of tRNA-methylases.[494,495] Benzoylated diethylaminoethyl-cellulose has been used for the fractionation of DNA.[496] D-Glucose isomerase and β-D-galactosidase have been immobilized by entrapment in a cellulose fibre of highly hydrophilic character.[497] α-Cellulose was solubilized in a mixture of *N*-ethylpyridinium chloride and dimethylformamide and enzyme added to the suspension, from which the enzyme-containing fibres could then be produced.

Carboxymethyl-cellulose has been used in the development of an affinity elution technique to study the conformational changes of yeast phosophoglycerate kinase on binding of substrates.[498] Carboxymethyl-cellulose activated with a carbodi-imide and cellulose activated with cyanogen bromide have been used to immobilize L-lysine for the affinity chromatography of plasminogen.[499] L-Asparaginase has been immobilized on cellulose fibres activated with cyanogen bromide, and used in an extracorporeal reactor dialyser for enzyme therapy.[500] Cellulose coated with chitin has provided an affinity chromatographic support for lysozyme and its chemically modified derivatives.[501]

The reactivity of various proteins and enzymes with dialdehyde-cellulose has been investigated.[502]

Cellulose activated with epichlorohydrin has been treated with glycine and then coupled with α-amylase *via* a carbodi-imide mediator.[503] Glucoamylase was also immobilized by a carbodi-imide-mediated reaction with epichlorohydrin-activated cellulose, which had previously been treated with 6-aminohexanoic acid, or by reaction with diazotized benzidine previously coupled to epichlorohydrin-activated cellulose. Immobilized 4-chloromercuribenzoate was prepared by reaction with 1,6-diaminohexane coupled to epichlorohydrin-activated cellulose, and used for the affinity chromatographic purification of α-amylase and glucoamylase.

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[493] T. Watanabe, M. Fujimara, T. Mori, T. Tosa, and I. Chibata, *J. Appl. Biochem.*, 1979, **1**, 28.
[494] A. A. Nedospasov, R. M. Khomotov, A. S. Gambaryan, I. A. Morozov, and T. V. Venkstern, *Mol. Biol.*, 1979, **12**, 851.
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[499] S. O. Kudinov, E. V. Eretska, T. M. Pozdnyakova, N. E. Panchenko, S. P. Matsuy, and I. M. Babenko, *Ukr. Biokhim. Zh.*, 1979, **51**, 330.
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The utility for enzyme immobilization of several hydrophobic cellulose esters (phenoxyacetyl, phenylpropionyl, phenylacetyl, benzoyl, hexanoyl, and palmitoyl) has been investigated as a function of solvent composition, extent of esterification, and enzyme.[504] Phenoxyacetylcellulose was also used for the immobilization of rat-liver microsomes, for hydrophobic chromatography of proteins and for removal of Triton X-100 from protein solutions. Phenoxyacetyl groups esterified to cellulose were much less subject to enzymatic hydrolysis than were soluble phenoxyacetyl esters. Phenoxyacetyl-cellulose has been used as a support for immobilization of lactoperoxidase for use in the radioactive iodination of proteins and small molecules.[505] However, adsorption and desorption phenomena encountered with the immobilized preparation limit its applicability. Methods have been described for the preparation of cellulose esters in gel forms suitable for the removal of the bitter principle limonin from orange juice without requiring prior centrifugation of the juice and without introducing sorbent fines into the product.[506] Cellulose acetate gel beads were used to debitter eight successive batches of juice, the sorptive capacity of the beads being regenerated by washing with water between treatments.

Columns of mercuriated cellulose have been applied to the separation of thiol-containing RNA from cells treated with 6-thioguanosine or 4-thiouridine.[507] Mercuriated cellulose was prepared by treating cellulose with allyl glycidyl ester followed by mercuric acetate. Prior to modification RNA was purified on oligo(dT)–cellulose columns.

Palmitoyl-cellulose has been used for the purification of *Streptomyces chromofuscus* phospholipase D by hydrophobic chromatography.[508]

Chitin. – The primary product of the thermal degradation of chitin, namely 2-acetamido-1,6-anhydro-2-deoxy-β-D-glucopyranose, has been detected for the first time and isolated on a preparative scale.[472] Thermal degradation was achieved with few secondary reactions by using compressed gases in a supercritical state in a flow apparatus.

Chitin has been reacted with isocyanate or acid chloride derivatives of pesticides to produce controlled-release polymeric pesticide systems (see also Amylopectin, p. 629).[460]

The action of lysozyme on partially deacetylated chitin and chitin oligosaccharides has been studied.[509] Chitin was de-*N*-acetylated at about 70% of its 2-acetamido-2-deoxy-D-glycopyranosyl residues, and lysozyme digests of this substrate were fractionated by gel chromatography, paper chromatography, and paper electrophoresis.

An *endo*-chitinase from wheat germ has been purified by affinity chromatography on chitin, and its activity on nascent and preformed chitin investigated.[510] Mink lysozyme has been purified by affinity chromatography on

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[505] C. N. M. Bakker and F. M. Kaspersen, *Int. J. Appl. Radiat. Isotopes*, 1979, **30**, 320.
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deaminated chitin,[511] and chitin itself has been used for the affinity chromatographic purification of a chitin-binding lectin from *Neurospora crassa* cell walls.[512]

Urease has been immobilized successfully by glutaraldehyde-mediated reaction with chitin.[513]

Chitosan. – A method has been developed for the rapid identification and quantitative determination of chitosan oligosaccharides using an amino-acid analyser with a linear gradient.[514]

Chitosan, dissolved in aqueous oxalic acid solutions by heating in a boiling water bath, forms novel gels of chitosan salts on cooling to room temperature.[515] The gels melt on further heating and re-set on cooling. This is the first report of thermally reversible gelation of basic glycosaminans. It was suggested that the gel may have a framework similar to those of alginate and low methoxyl pectin gels, which are formed with divalent cations (Ca^{2+} *etc.*) and uronic residues of polyuronide chains by salt formation.

The influence of manufacturing variables on the characteristics and effectiveness of chitosan products have been examined, with particular reference to the chemical composition, molecular weight distribution, and viscosity of the products,[516] the effectiveness of the chitosan products for coagulation of activated sludge suspensions,[517] and the effectiveness of the products for the coagulation of cheese whey solids.[518] Preparations of higher molecular weight were found to be most effective for coagulation of activated sludge suspensions, whereas the effectiveness for coagulation of cheese whey solids was found to be inversely related to molecular weight.

Lysozyme has been isolated by affinity chromatography on chitosan.[519] Chitosan activated with a water-soluble carbodi-imide has been used as a support for the immobilization of D-glucose isomerase, urease, glucoamylase, trypsin, and D-glucose oxidase.[520] A novel matrix for enzyme immobilization has been developed: chitosan was dissolved in 2% v/v acetic acid and mixed with pepsin or alkaline phosphatase.[521] Glutaraldehyde or acrolein was then added as a cross-linking agent, and the suspension solidified to yield a yellow gel containing the active immobilized enzyme.

[511] G. M. Malinina, I. A. Cherkasov, N. A. Kravchenko, and V. A. Berestov, *Biochemistry (USSR)*, 1977, **42**, 680.
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[521] S. Hirano and O. Miura, *Biotechnol. Bioeng.*, 1979, **21**, 711.

Cycloamyloses. – The Raman spectrum of cyclohexa-amylose has been compared with those of maltotriose and maltose, and structural implications of the spectra discussed.[522]

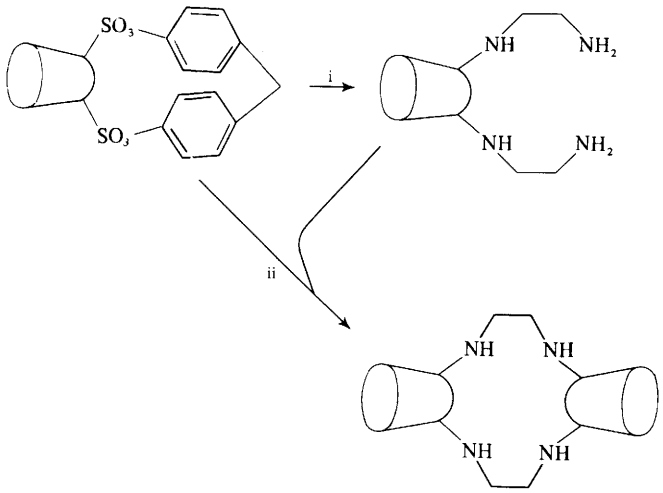
Diffusion of cyclohexa- and cyclohepta-amyloses has been studied in aqueous solutions of poly(methacrylic acid), sodium poly(styrene sulphonate) having three different degrees of sulphonation, and copoly(styrene–methacrylic acid) containing three different amounts of styrene.[523] A decrease of the diffusion coefficients of the cycloamyloses in these polymer solutions was found to be dependent on the polymer content, the degree of sulphonation, the styrene content, and the degree of neutralization. The results were interpreted by assuming a 1:1 complex formation between the cycloamylose and an appropriate residue in the polymer.

By substituting ω-aminoethylamino-groups in place of one of the primary hydroxy groups of cyclohepta-amylose, a simple binding model of rhodopsin has been obtained.[524] The modified cyclohepta-amylose has a hydrophobic and a carbonyl recognition site and binds retinal very strongly in aqueous solution. The spectrum of the hepta(ω-aminoethylamino)-cyclohepta-amylose–retinal complex was determined and discussed with respect to theories concerning the retinal–rhodopsin interaction in biological systems. The cyclohepta-amylose model system appears to be of value for further study of the rhodopsin mechanism.

'Duplex cyclodextrin', a dimer of capped cyclohepta-amylose which has two hydrophobic binding sites together with another recognition site, has been prepared (Scheme 12).[525] The duplex cyclodextrin shows unique and interesting binding characteristics towards guest molecules having two hydrophobic recognition sites. Considerable shifts in fluorescence maximum and association constant have been demonstrated for 6-(4-toluidinylnaphthalene)-2-sulphonate and Methyl Orange, respectively, on complex formation with duplex cyclodextrin.

Formation of crystalline cyclohepta-amylose inclusion complexes has been followed by recording continuously the turbidity of the aqueous solution of cyclohepta-amylose and the guest molecule (solvent, drug, or aromatic compound) from 60 °C downwards.[526] The concentrations of the guest molecules were such that they could not form crystals above the crystallization temperature of pure cyclohepta-amylose. The appearance of turbidity caused by crystal formation before the characteristic temperature of cyclohepta-amylose crystallization is therefore considered to be evidence for inclusion complex formation. The influence of parameters such as concentration, pH, cooling speed, presence of inorganic salts, and inoculating crystals was studied.

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Reagents: i, 1,2-diaminoethane, 50 °C, 3 h; ii, dmf–pyr, 80 °C, 72 h

Scheme 12

Cycloamylose polymers have been characterized and their chromatographic behaviour investigated.[527]

2,3,6-Tri-*O*-methyl and 2,6-di-*O*-methyl derivatives of cyclohexa- and cyclohepta-amylose have been prepared by Hakomori methylation and shown to be versatile complexing agents, forming complexes in aqueous solution which are more stable than the corresponding complexes of unsubstituted cycloamyloses.[528] The methylated cycloamyloses also form crystalline complexes, the stability of which depends on the size and shape of the guest molecules. The decomposition temperature of the crystalline complexes of unsubstituted cycloamyloses is relatively high and increases with increasing chain-length of the hydrocarbon.

Cyclohepta-amylose very effectively catalyses a variety of specific allylation–oxidation reactions of hydroquinone derivatives, and appears to mimic the action of allylquinone synthase.[529] Possible explanations for the molecule's catalytic ability were described.

Inclusion complex formation with cycloamylose has been shown to be a technically simple process by which the ratio of saturated to unsaturated fatty acids may be altered under mild conditions in fatty acid mixtures obtained from natural sources.[530] Complex formation was demonstrated by the results of *X*-ray diffraction spectra, the low solubility of the formed complexes, and the protection of the complexed fatty acids against atmospheric oxidation.

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[529] I. Tabushi, Y. Kuroda, K. Fujita, and H. Kawakubo, *Tetrahedron Lett.*, 1978, 2083.
[530] J. Szejtli, E. Bánky-Elöd, A. Stadler, P. Tétényi, I. Héthelyi, and L. Kernóczy, *Acta Chim. Acad. Sci. Hung.*, 1979, **99**, 447.

Powdered inclusion complexes have been obtained by freeze-drying together cyclohepta-amylose and anti-inflammatory drugs (indomethacin, flufenamic acid, or phenylbutazone).[531] Significantly less irritation was caused to the stomach of rats by phenylbutazone on oral administration when the drug was given as a freeze-dried inclusion compound, but no such effect was observed for the other two drugs.

The stability constants (K_c) have been determined by h.p.l.c. for 1:1 inclusion complexes of cyclohexa- and cyclohepta-amyloses with barbiturates, phenothiazines, sulphonylamides, and sulphonylureas.[532] Results were in good agreement with those obtained by solubility and spectroscopic methods. Use of h.p.l.c. is particularly suitable for chemically unstable compounds like phenothiazine.

A ^{13}C n.m.r. spectroscopic study of 1:1 complexes between cycloamyloses and azo dyes having a naphthalene nucleus has been reported.[533] Almost all carbons of the guest molecules showed low field shifts on inclusion, consistent with ^{1}H n.m.r. spectroscopic results. The shift may be due not to a simple steric polarization but to hydrophobic interaction with the host molecule, whose cavity uniformly affects the guest molecule as if it is dissolved in a hydrophobic solvent. The host molecule was shown to be deformed by the bulky naphthalene nucleus at the glycosidic linkage rather than at the small diameter side of the cavity.

The diffusion behaviour of cyclohexa- and cyclohepta-amyloses in aqueous poly(methacrylic acid), copoly(methacrylic acid–styrene), and copoly(styrene–sulphonic acid) solutions has been investigated.[534] The diffusion coefficients decreased with the concentration of the polymers, and the descending tendencies were successfully explained by means of obstruction and interaction effects.

Epichlorohydrin-cross-linked cyclohexa- and cyclohepta-amylose gels have been used for the chromatographic resolution of racemic mandelic acid and its derivatives.[535] Modified cyclohepta-amylose bound the L-(+)-isomers preferentially, and resolved D,L-methyl mandelate to give the D-(−)-isomer of 100% optical purity in the first fraction. Cross-linked cyclohexa-amylose bound D-(−)-isomers more strongly than L-(+)-isomers, resolving D,L-methyl mandelate to a smaller extent than cross-linked cyclohepta-amylose. Binding was studied quantitatively by the equilibrium method.

Dextrans. – Measurements of the penetration of porous glasses by dextrans have yielded penetration curves relating the degree of penetration to molecular

[531] M. Nambu, K. Kikuchi, T. Kikuchi, Y. Takahashi, H. Ueda, and T. Nagai, *Chem. Pharm. Bull.*, 1978, **26**, 3609.
[532] K. Uekama, F. Hirayama, S. Nasu, N. Matsuo, and T. Irie, *Chem. Pharm. Bull.*, 1978, **26**, 3477.
[533] M. Suzuki, Y. Seraki, and M. Sugiura, *Chem. Pharm. Bull.*, 1979, **27**, 1797.
[534] T. Moro, S. Kobayashi, K. Kainuma, T. Uemura, T. Harada, J. Komiyama, and T. Iijima, *Carbohydr. Res.*, 1979, **75**, 345.
[535] A. Harada, M. Furue, and S. I. Nozakura, *J. Polym. Sci., Polym. Chem. Ed.*, 1978, **16**, 189.

size.[536] Penetration was found to be dependent only on the relative size of the molecule and the pore.

The effects of acrylamide graft copolymerization on the solution properties of dextran have been described.[458]

Polyuridine has been coupled to agarose *via* an aminoethylcarbamoyl-dextran spacer to yield a new and highly effective biospecific adsorbent for affinity chromatography of polyadenylated mRNA.[537]

Chymotrypsin has been covalently bound to water-soluble oxidized dextran through Schiff bases, and the stability of the modified enzyme investigated.[538] The increase in stability observed was attributed to an increase in the enthalpy of activation of the denaturation reaction.

The hydrolysis of maltose by glucoamylase has been studied in the absence and presence of dextran and dextran sulphate.[539] Dextran became bound to glucoamylase and was a non-competitive inhibitor of the enzyme. The reaction rate was hardly affected at pH 4.0–4.5 by dextran sulphate addition but depended considerably on dextran sulphate at pH 3.5, suggesting some kind of interaction between the two. The transport and metabolism of dextran [^{35}S] sulphates of various molecular weights and sulphur contents in rat intestinal mucosa, and the effects of calcium thereon, have been investigated.[540] Results suggested that dextran sulphates are transferred by pinocytosis and other mechanisms and are then desulphated mainly in microsomes, and subsequently become depolymerized.

Insoluble streptococcal dextran has been used for the affinity chromatographic purification of the extracellular D-glucosyltransferase from *Streptococcus mutans* 6715,[541] and *Lathyrus odoratus* (sweet pea) lectins have been purified by affinity chromatography on crosslinked dextran (Sephadex®).[542]

Isocyanate or acid chloride derivatives of pesticides have been treated with dextran to produce controlled-release polymeric pesticide systems (see also Amylopectin, p. 629).[460]

A homologous series of water-soluble alkyl-dextrans varying in the length of their hydrocarbon side-chain (63) have been synthesized by reaction of the appropriate alkylamine with dextran cyclic imidocarbonate.[543] The derivatives were used in an affinity electrophoretic study of the interaction between phosphorylase and hydrophobic groups. As the length of the hydrocarbon

$$-NH-(CH_2)_n-Me$$

(63; n = 1–5)

[536] J. C. Day, B. Alince, and A. A. Robertson, *Can. J. Chem.*, 1978, **56**, 2951.
[537] B. A. Klyashchitskii, G. E. Koroleva, V. K. Mitina, R. P. Alyokhin, I. B. Zborovskaya, and A. V. Likhtenstein, *Bioorg. Khim.*, 1979, **5**, 92.
[538] N. N. Molchanova and L. V. Kozlov, *Biochemistry (USSR)*, 1978, **43**, 797.
[539] S. Adachi, K. Nakanishi, R. Matsuno, and T. Kamikubo, *Agric. Biol. Chem. (Jpn.)*, 1977, **41**, 1673.
[540] K. Kagawa, M. Nishiyama, and S. Tomizawa, *Biochem. Pharmacol.*, 1978, **27**, 1931.
[541] W. R. Figures and J. R. Edwards, *Carbohydr. Res.*, 1979, **73**, 245.
[542] M. Sakakibara, H. Noguchi, and S. Makino, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 1647.
[543] K. Nakamura, A. Kuwahara, and K. Takeo, *J. Chromatogr.*, 1979, **171**, 89.

side-chains of the alkyl groups increased, the affinity of the phosphorylases for the alkyl groups increased.

Preparations of pancreatic ribonuclease, ribonuclease from *Actinomyces rimosus*, and the nuclease from *Serratia marcescens* have been covalently bound to 4β-hydroxyethylsulphonylanisole-, 4β-hydroxyethylsulphonylaniline, and 3-Cl-2-hydroxypropyl derivatives of dextran and dialdehyde-dextran and to the 4-aminobenzyloxymethyl ester of dextran.[544] The yield and thermal stability of the modified nucleases depends both on the amount and the character of the functional groups activating the polysaccharide.

Amino-oxybutyl-dextran has been prepared by alkylation of a sodium salt of dextran followed by treatment with hydrochloric acid.[545] Amino-oxybutyl-Sephadex® was similarly prepared.

Blue Dextran® immobilized by reaction with agarose cyclic imidocarbonate has been used to purify lactate dehydrogenase and to investigate the homology of the enzyme's sub-units.[234] The interaction of Blue Dextran® with calf spleen NAD^+ glycohydrolase has been investigated in an attempt to demonstrate structural analogies between the NAD^+-binding domain of this enzyme and enzymes possessing the dinucleotide fold.[546]

Bromohydroxypropyl-derivatives of dextran have been treated with several different 8-(6-aminohexyl)amino-adenine nucleotide coenzymes and their derivatives.[547] The bound derivatives were purified on Sephadex® G-50 and then studied using n.m.r. spectroscopy. Results of such studies may explain why polymer-linked coenzymes exhibit lower biological activity than the unmodified coenzymes but similar binding affinity for most enzymes.

Copper–Sephadex® complexes have been prepared and an improved method developed for the separation of peptides and α-amino-acids on the matrix.[548]

Dextran cyclic imidocarbonate has been used to prepare soluble conjugates of α-amylase from *Bacillus amyloliquefaciens* and of bovine liver catalase for use in experimental enzyme therapy.[549] The results suggest that by coupling the enzymes with a poorly antigenic macromolecule (dextran) anaphylactic reaction in animals pre-immunized with dextran may be suppressed or eliminated. Trypsin has been immobilized by reaction with dextran cyclic imidocarbonate.[132]

A study of the effect of diethylaminoethyl-dextran and dextran sulphate on [^{35}S]-sulphate incorporation into glycosaminoglycans of normal and SV40 virus-transformed 3T3 cells has been reported.[550]

The interaction of diethylaminoethyl-dextran and bovine plasma albumin at pH values above albumin's isoelectric point has been investigated.[551] Turbidity

[544] B. M. Kurinenko, N. V. Kalacheva, G. A. Penzikova, and I. F. Skokova, *Biochemistry (USSR)*, 1978, **43**, 1567.
[545] A. A. Nedospasov and R. M. Khomutov, *Izv. Akad. Nauk SSSR Ser. Khim.*, 1978, **27**, 692.
[546] F. Schuber and M. Pascal, *Biochimie*, 1977, **59**, 735.
[547] C. Y. Lee, *J. Solid-phase Biochem.*, 1978, **3**, 49.
[548] E. Rothenbühler, R. Waibel, and J. Solms, *Anal. Biochem.*, 1979, **97**, 367.
[549] J. J. Marshall and J. D. Humphreys, *J. Appl. Biochem.*, 1979, **1**, 88.
[550] K. Ehrlich and M. Murray, *Life Sciences*, 1979, **25**, 1557.
[551] K. Gekko, H. Harada, and H. Noguchi, *Agric. Biol. Chem. (Jpn.)*, 1978, **42**, 1385.

and electrophoretic measurements showed the mixture formed a coacervate, formation of which was sensitively affected by pH, ionic strength, temperature, and the weight ratio of both compounds. The interaction appears to be chiefly electrostatic and is enhanced by hydrophobic interaction.

Iron–dextran, a synthetic therapeutic complex of iron oxide coated with dextran (Imferon®), has been shown to have properties similar to those of ferritin, a natural complex of iron oxide encased in protein.[552] Both maintain high concentrations of iron in solution at physiological pH, and release iron relatively slowly *in vivo*. Extended *X*-ray absorption fine structure analysis showed that the near-neighbour environment around the average iron atom in ferritin and iron–dextran is identical for the first three shells, which may explain the similarity of iron release *in vivo*. The results suggest that iron–dextran will be a useful model compound in studies of the relation of the iron core and protein in ferritin to function.

Succinic esters of dextran have been prepared and transformed into the corresponding imidazolides, which react smoothly with alcohols or amines to yield the corresponding polymeric esters or amides.[553] These activated amides may be of value for the preparation of polymer–drug adducts with a main backbone degradable to safe metabolites in the body fluids.

Sulphopropyl-Sephadex® has been used for the purification of β-D-2-acetamido-2-deoxy-hexosidases A and B from human kidney,[554] and of a (1→4)-β-D-glucan glucanohydrolase from the cellulolytic fungus *Trichoderma viride* QM 9414.[555]

Glycogen. – The affinity of yeast glycogen synthase to glycogen cross-linked with epichlorohydrin has been studied under various experimental conditions.[556] It was found that the higher the degree of cross-linking, the lower the affinity of glycogen synthase to glycogen. The amount of glycogen synthase absorbed from the solution depends on the amount of cross-linked glycogen added and is inversely proportional to the concentration of the soluble glycogen.

Glycogen immobilized by reaction with agarose cyclic imidocarbonate has been used for the affinity chromatographic purification of smooth muscle glycogen phosphorylase *b*.[557]

Poly(uridine) has been immobilized on agarose *via* a glycogen hydrazido-succinyl spacer arm and used for the affinity chromatographic purification of polyadenylated mRNA.[537]

Glycosaminoglycans. – A thermodynamic study of the partition of chondroitin sulphate–hexadecyl pyridinium complexes in butanol–aqueous salt biphasic solutions has been reported.[558]

[552] E. Theil, D. E. Sayers, and M. A. Brown, *J. Biol. Chem.*, 1979, **254**, 8132.
[553] P. Ferruti, *Makromol. Chem.*, 1979, **180**, 375.
[554] D. V. Marinkovic and J. N. Marinkovic, *Biochem. J.*, 1977, **163**, 133.
[555] U. Håkansson, L. Fägerstam, G. Pettersson, and L. Andersson, *Biochim. Biophys. Acta*, 1978, **524**, 385.
[556] J. Zemek, S. Bauer, and L. Kuniak, *Biopolymers*, 1979, **18**, 2135.
[557] L. N. Viktorova, B. A. Klyashchitskii, and E. V. Ramensky, *Bioorg. Khim.*, 1979, **5**, 100.
[558] R. E. Hurst, *Biopolymers*, 1978, **17**, 2601.

Chondroitin sulphate and methylated chondroitin methyl ester, modified by oxidation with bromide-water or by reduction with sodium borohydride, have been used as substrates for chondroitinases from *Arthrobacter* and *Flavobacterium* species.[559]

By heating the pyridinium salts of chondroitin 4- and 6-sulphates in dimethyl sulphoxide containing 10% water or methanol at 80 °C for 1–5 hours, several chondroitin preparations with sulphur contents of 0.02–1.05% have been prepared in 83–96% yields.[560] Desulphation by this method occurs smoothly in a homogeneous medium under mild conditions, unlike previous methods. The products of the previous and present methods were compared.

N-[^{35}S]Heparin and [^{35}S,^{3}H]heparin have been used as substrates in a study of the action of an *endo*-glycanase from rat-liver lysosomes.[561]

The thermodynamic and co-operativity parameters of the Acridine Orange–heparin system have been reported.[562]

Sulphated disaccharides from the sodium nitrite–acetic acid deamination of porcine and whale heparins have been isolated and characterized and the products compared.[563]

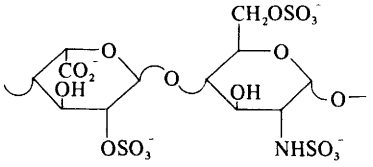
The stability of ester sulphates in porcine intestinal heparin to solvolytic desulphation in dimethyl sulphoxide containing 2% pyridine has been compared to their stability when treatment is carried out in dilute acid.[564] The modified preparations were deaminated and fractionated. The yields of disulphated disaccharide (64) and of monosulphated disaccharides (65) and (66) indicated that L-iduronic acid residues of heparin were more labile than the 6-*O*-sulphates in 2-acetamido-2-deoxy-D-glucosyl residues when treated with dilute acid, while the opposite was the case for the solvolysis.

Chymotrypsin has been successfully immobilized by carbodi-imide-mediated reaction with heparin.[565] Catalytic properties of the modified enzyme, which is of potential importance therapeutically, were unchanged while the enzyme's thermal stability was improved.

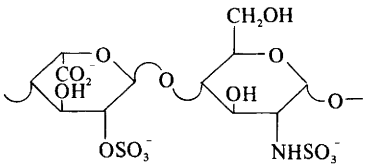
Low molecular weight heparin has been fractionated into 'active' and 'relatively inactive' forms by affinity chromatography on immobilized thrombin and hirudin.[297]

Mannans. — D-Galacto-D-mannan dyed with Remazol Brilliant Blue has been used in a simple assay procedure for D-mannanase.[566] The procedure is quantitative, sensitive, and highly specific.

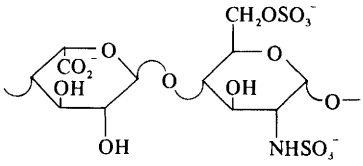
[559] K. Hiyama and S. Okada, *J. Biochem. (Tokyo)*, 1976, **80**, 1201.
[560] K. Nagasawa, Y. Inoue, and T. Tokuyasu, *J. Biochem. (Tokyo)*, 1979, **86**, 1323.
[561] B. Arbogast, J. J. Hopwood, and A. Dorfman, *Biochem. Biophys. Res. Commun.*, 1977, **75**, 610.
[562] J. M. Menter, R. E. Hurst, N. Nakamura, and S. S. West, *Biopolymers*, 1979, **18**, 493.
[563] M. Kosakai, F. Yamauchi, and Z. Yosizawa, *J. Biochem. (Tokyo)*, 1978, **83**, 1567.
[564] M. Kosakai and Z. Yosizawa, *J. Biochem. (Tokyo)*, 1979, **86**, 147.
[565] V. P. Torchilin, *Bioorg. Khim.*, 1978, **4**, 566.
[566] B. V. McCleary, *Carbohydr. Res.*, 1978, **67**, 213.



(64)



(65)



(66)

Pancreatic ribonuclease, ribonuclease from *Actinomyces rimosus*, and the nuclease from *Serratia marcescens* have been covalently bound to the 3-aminobenzyloxymethyl ester of D-mannan.[567]

An acetylated methyl-D-mannan polysaccharide from *Streptomyces griseus* has been purified using palmitoyl chloride immobilized on aminoethyl-Bio-Gel®.[568]

Miscellaneous Polysaccharides, Oligosaccharides, and Glycopeptides. – Laminaribiose has been modified to form the β-methyl and β-benzyl glycosides,[21] and 4-*O*-α-D-mannopyranosyl-D-mannose has been chemically modified also.[32]

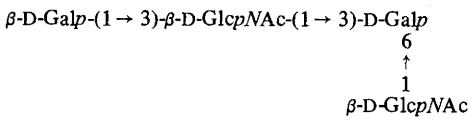
The blood group I and i activities of five synthetic oligosaccharides [(67)–(71)] have been investigated by radioimmunoassay.[569]

Sucrose has been activated by reaction with cyanuric chloride to yield a [^{14}C]sucrose–dichlorotriazine adduct which was stable for at least six hours and which could be covalently linked to low density lipoprotein.[570] A general method utilizing radiolabelled sucrose covalently linked to protein is described for assessment of the degradation of metabolized proteins.

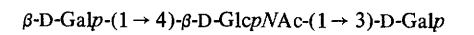
Malto-oligosaccharides radioactively labelled at their reducing ends have been used to elucidate the mechanisms by which *Aspergillus oryzae* α-amylase degrades malto-oligosaccharides.[571]

The reducing ends of oligosaccharides have been coupled to 2-aminopyridine, a fluorescent compound, by reductive amination with sodium cyanoborohydride.[572,573] The fluorescent oligosaccharides are then easily visualized after

[567] B. M. Kurinenko, N. V. Kalacheva, G. A. Penzikova, and I. F. Skokova, *Biochemistry (USSR)*, 1978, **43**, 1994.
[568] B. E. Kari and G. R. Gray, *J. Biol. Chem.*, 1979, **254**, 3354.
[569] E. Wood and T. Feizi, *FEBS Lett.*, 1979, **104**, 135.
[570] R. C. Pittman, S. R. Green, A. D. Attie, and D. Steinberg, *J. Biol. Chem.*, 1979, **254**, 6876.
[571] J. D. Allen and J. A. Thoma, *Biochemistry*, 1978, **17**, 2338.
[572] S. Hase, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Tokyo)*, 1979, **85**, 989.
[573] S. Hase, T. Ikenaka, and Y. Matsushima, *J. Biochem. (Tokyo)*, 1979, **85**, 995.



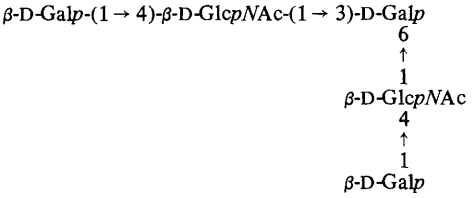
(67)



(68)



(69) (70)



(71)

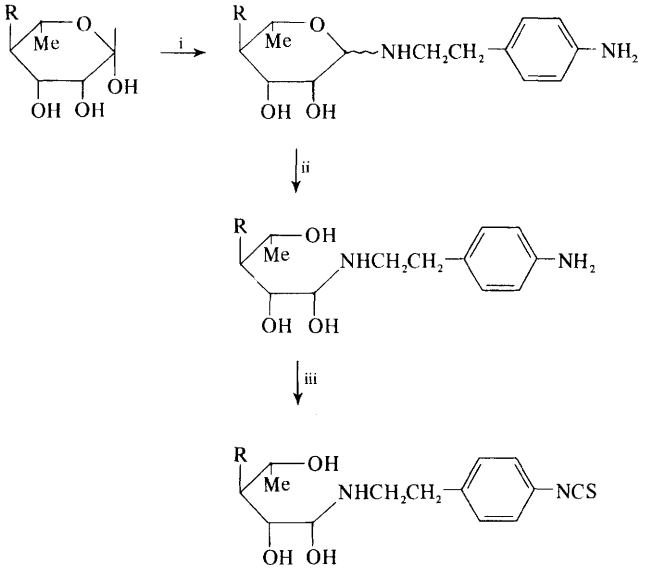
electrophoresis. The compounds were used both for the detection of oligosaccharides derived from proteins,[572] and for linkage point analysis when the electrophoresed oligosaccharides were hydrolysed, methylated, and analysed by g.c.–m.s.[573]

The terminal reducing end groups of acid-labile *Salmonella* species oligosaccharides have been treated with 2-(4-aminophenyl)ethylamine converted to the corresponding succinylphenylisothiocyanate derivatives and covalently linked to the free ϵ-lysyl groups of carrier proteins (Scheme 13).[574] The conjugates were found to be highly immunogenic. 'Reductive amination', *i.e.*, reaction in the presence of sodium cyanoborohydride in aqueous solution under mild conditions, has been used to link lactose to aminohexyl-agarose.[364] α,α-Trehalase from *Artemia salina* embryos was purified by affinity chromatography on a column of immobilized lactose.

The 2-aminoethylaminocarbamylmethyl α- and β-ketosides of neuraminic acid have been prepared and immobilized by reaction with agarose cyclic imidocarbonate.[575] The immobilized derivatives were used for affinity chromatographic purification of influenza virus.

Allyl α-L-rhamnoside, immobilized by copolymerization with acrylamide and *NN'*-methylene bisacrylamide, has provided a matrix for the large-scale affinity chromatographic separation of *Ricinus communis* agglutinin (which binds) and toxin (which does not bind).[576] A simple method has been developed for the

[574] S. B. Svenson and A. A. Lindberg, *J. Immunol. Methods*, 1979, **25**, 323.
[575] L. Holmquist and G. Nilsson, *Acta Pathol. Microbiol. Scand.*, 1979, **87B**, 129.
[576] V. Hořejší, *J. Chromatogr.*, 1979, **169**, 457.



R = remainder of polysaccharide

Reagents: i, 2-(4-aminophenyl)ethylamine; ii, $NaCNBH_3$; iii, $CsCl_2$

Scheme 13

preparation of acrylamide derivatives containing thioglycosides.[577] The synthetic scheme involves the preparation of an *O*-acetylated 1-thiosugar *via* a pseudothiourea derivative, the controlled addition of the thiosugar to *NN'-bis*-methyleneacrylamide to form a monoaddition product in which one of the acrylamide groups remains unchanged, and finally de-*O*-acetylation. Thioglycoside derivatives of acrylamide thus prepared were copolymerized with acrylamide and the gels successfully used as affinity materials for the purification of peanut lectin and in cell-adhesion studies.

Conditions for the gelation of κ-carrageenan, a new matrix for the immobilization of cells and enzymes, have been investigated and a number of cells and enzymes successfully immobilized.[578] Operational stability was found to be enhanced by hardening with glutaraldehyde or 1,6-diaminohexane. *Brevibacterium flavum* cells have also been immobilized by gelation in κ-carrageenan.[579]

Colominic acid, a homopolymer of *N*-acetylneuraminic acid, has been mixed with soluble starch and cross-linked with epichlorohydrin to form a matrix suitable for the affinity chromatographic purification of neuraminidase.[580]

[577] R. T. Lee, S. Cascio, and Y. C. Lee, *Anal. Biochem.*, 1979, **95**, 260.
[578] T. Tosa, T. Sato, T. Mori, K. Yamamoto, I. Takata, Y. Nishida, and I. Chibata, *Biotechnol. Bioeng.*, 1979, **21**, 1697.
[579] I. Takata, K. Yamamoto, T. Tosa, and I. Chibata, *Eur. J. Appl. Microbiol. Biotechnol.*, 1979, **7**, 161.
[580] Y. Uchida, Y. Tsukada, and T. Sugimori, *J. Biochem. (Tokyo)*, 1977, **82**, 1425.

A (1→3)-α-D-glucanase from *Streptomyces* sp KI-8 has been purified on a column of pachyman, a fungal (1→3)-β-D-glucan.[581]

The degradation of proteoglycan by an elastase-enriched neutral protease preparation from leukocytes has been shown to be inhibited by pentosan polysulphate, an anti-inflammatory agent, presumably by formation of a complex between the drug and the proteoglycan.[582]

Reduction with dithiothreitol or alkylation with iodoacetamide has been shown to abolish the antigenicity of species-specific antigenic determinants of proteoglycan preparations from chick epiphyseal cartilage and rat chondrosarcoma, as detected by immunodiffusion and radioimmunoassay.[583] Some, but not all, of the species-common antigenic determinants were also sensitive to the treatment.

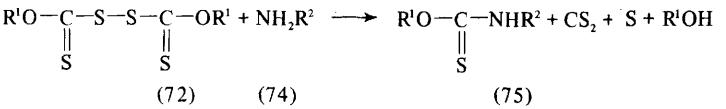
A new dialdose dianhydride, the 1,2′:1′,2-dianhydride of 3,4-di-*O*-acetyl-β-L-rhamnopyranose and methyl 3,4-di-*O*-acetyl-α-D-galactopyranosyluronic acid, has been obtained from an acid hydrolysate of the water-soluble polysaccharide of wobaku wood by successive treatment with methanolic hydrogen chloride and acetic anhydride–pyridine.[584]

The properties of 'pyruvic acid-free' xanthan, *i.e.*, xanthan from which approximately 90% of the pyruvate groups have been removed without removing acetyl groups or causing substantial breakdown of glycosidic bonds, have been reported.[585] Two model xanthate compounds (72) and (73) have been used to evaluate dithiobis(thioformate) and *S*-methyl dithiocarbonate as reactive groups for the immobilization of biological compounds (Scheme 14).[586] Both (72) and (73) react readily with (74), particularly at pH 8.6, to yield (75), indicating the feasibility of the two reactions for coupling enzymes and other biological molecules to polymers.

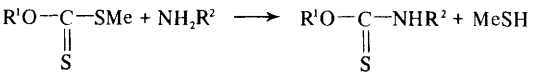
Sodium xylan polysulphate, an artificially sulphated (1→4)-β-D-xylan, has been shown to prolong the partial thromboplastin clotting time of plasma.[587] The differential effects of xylan sulphate on different blood serine proteases were discussed in terms of the antithrombin III-mediated anticoagulant activity of heparin.

The adjuvant and anti-tumour activities of synthetic 6-*O*-corynomycoloyl, 6-*O*-nocardomycoloyl, and 6-*O*-mycoloyl derivatives of *N*-acetylmuramyl-L-alanyl-D-isoglutamine have been evaluated.[588] The preparation of the mycoloyl derivatives by acylation of *N*-acetylmuramyl-L-alanyl-D-isoglutamine was

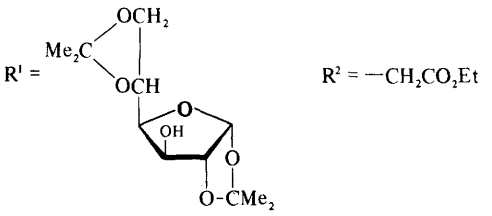
[581] K. Imai, T. Kikuta, M. Kobayashi, and K. Matsuda, *Agric. Biol. Chem. (Jpn.)*, 1977, **41**, 1339.
[582] W. F. Barg, M. E. Englert, C. W. Buermann, A. L. Oronsky, and S. S. Kerwar, *Biochem. Pharmacol.*, 1979, **28**, 2639.
[583] K. Sugahara and A. Dorfman, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 1193.
[584] T. Fujiwara and K. Arai, *Carbohydr. Res.*, 1979, **69**, 67.
[585] G. Holzwarth and J. Ogletree, *Carbohydr. Res.*, 1979, **76**, 277.
[586] V. C. Borlaza, N. W. H. Cheetham, and P. T. Southwell-Keely, *Carbohydr. Res.*, 1979, **68**, 141.
[587] G. Kindness, F. B. Williamson, and W. F. Long, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 1062.
[588] I. Azuma, K. Sugimura, M. Yamakawi, M. Uemiya, S. Kusimoto, S. Okada, T. Shiba, and Y. Yamamura, *Infect. Immun.*, 1978, **20**, 600.



(72) (74) (75)



(73) (74)



Scheme 14

separately reported.[589] The cleavage of *O*-glycosidic bonds to L-serine and L-threonine by trifluoroacetolysis has been reported.[590]

Starch. – The structural modification of various starches by extrusion and the use of modified starches in the food industry have been described.[591]

Starch cross-linked in the presence of L-fucose with epichlorohydrin has provided a matrix for the affinity chromatographic purification of the L-fucose-specific haemagglutinin produced by a *Streptomyces* species.[592]

In a study of the degradation of starch by γ-irradiation, the effect of pH on gelatinization of starch after irradiation has been investigated.[593] Paste viscosities were markedly affected by pH on gelatinization, and the granule structures of irradiated starch easily disintegrated at alkaline pH.

Cationic aminoethyl ether derivatives of potato, tapioca, corn, sweet potato, rice, and wheat starches have been prepared by treatment with *NN*-diethylaminoethyl chloride.[594] The pasting curves of the derivatives were examined, and *X*-ray diffractograms were obtained for the cationic corn and potato starches.

[589] T. Shiba, S. Okada, S. Kusumoto, I. Azuma, and Y. Mamamura, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 3307.
[590] B. Lindberg, B. Nilsson, T. Norberg, and S. Svensson, *Acta Chem. Scand., Ser. B*, 1979, **33**, 230.
[591] 'Polysaccharides in Food', ed. J. M. V. Blanshard and J. R. Mitchell, Butterworths, London, 1979.
[592] T. Kameyama, K. Oishi, and K. Aida, *Biochim. Biophys. Acta*, 1979, **587**, 407.
[593] T. Kume, N. Kobayashi, A. Okuaki, and S. Aoki, *Agric. Biol. Chem. (Jpn.)*, 1979, **43**, 1625.
[594] T. Komiya and S. Nara, *J. Jap. Soc. Starch Sci.*, 1979, **26**, 23.

The kinetics of the oxidation of corn starch granules with sodium hypochlorite have been investigated.[595] There appeared to be two steps involved in the oxidation of the heterogeneous system, *viz*, a diffusion process and a reaction process. It was deduced that the order of the reaction depended upon which of these two steps was the controlling process. The behaviour of dissolved oxygen in the reaction mixture during the sodium hypochlorite oxidation of corn starch granules has also been investigated and its application to process control discussed.[596]

3 Modification of Glycoproteins and Uses of Modified Glycoproteins

The potential of some glycoproteins, and particularly of antibodies, lectins, and albumin, for use as drug carriers has been described.[73]

Albumins. – [^{3}H]Raffinose (76) has been treated with D-galactose oxidase and then coupled to albumin by reductive amination (Scheme 15).[597] The reaction provides a convenient method of labelling albumin for studies of the organ sites of catabolism of proteins in the circulation.

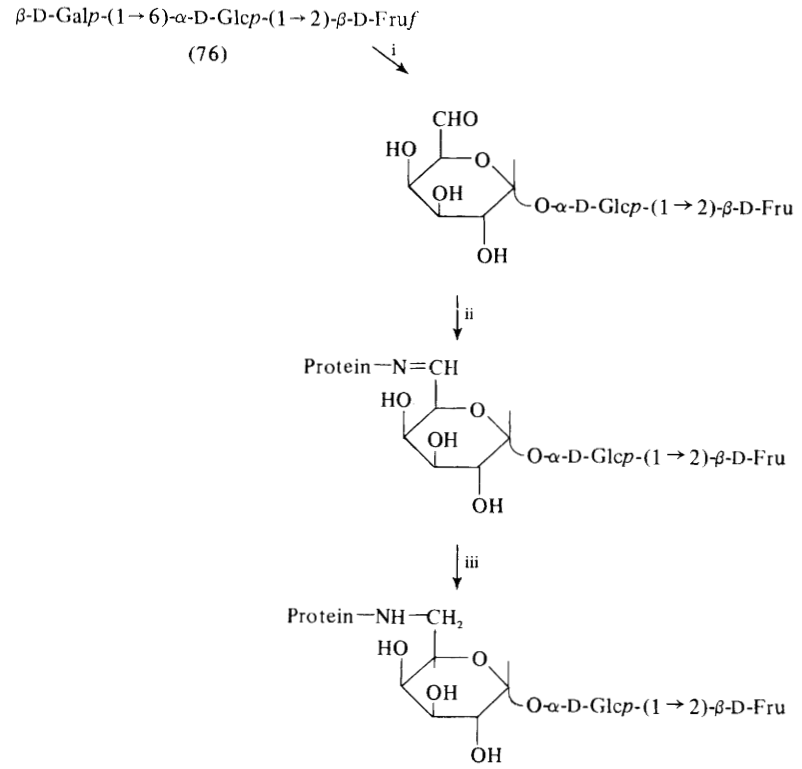
Oligosaccharides derived from baker's yeast D-mannan have been coupled to human serum albumin by reductive amination with sodium cyanoborohydride.[62] [^{14}C]-Labelled derivatives containing two or four D-mannose residues per 10 000 mol. wt. were administered intravenously to rats; selective uptake of these derivatives by the endothelial and Kupffer cells of the liver was observed within ten to fifteen minutes. The extent of hepatic uptake was a function of the number and size of the D-manno-oligosaccharides coupled. Reductive 'mannosamination' may provide a means of directing proteins of potential therapeutic interest towards specific liver cells.

Soluble copolymers of albumin and L-glutamate dehydrogenase have been prepared by glutaraldehyde cross-linking.[598] The kinetic and electron microscopic properties of the soluble derivatives were compared with data available concerning the enzyme immobilized within proteic films.

Crude preparations of gangliosides have been treated with albumin *via* a carbodi-imide mediator.[599] The reaction was stopped by precipitation with ammonium sulphate. Following resuspension of the precipitate, unsubstituted albumin was mixed with the albumin–ganglioside conjugate and the suspension treated with glutaraldehyde and then coated onto cellulose filters. These 'ganglioside affinity filters' could be used for the identification of toxigenic strains of *Clostridium botulinum* types C and D.

Fluoresceinyl derivatives of D-mannose, lactose, 2-acetamido-2-deoxy-α-D-galactopyranose, and L-fucose coupled to bovine serum albumin have been used

[595] H. Suzuki, Y. Hashimoto, H. Kawabata, and T. Ohira, *J. Jap. Soc. Starch Sci.*, 1979, **26**, 211.
[596] H. Suzuki, *J. Jap. Soc. Starch Sci.*, 1979, **26**, 217.
[597] J. Van Zile, L. A. Henderson, J. W. Baynes, and S. R. Thorpe, *J. Biol. Chem.*, 1979, **254**, 3547.
[598] J. N. Barbotin and B. Thomasset, *Biochim. Biophys. Acta*, 1979, **570**, 11.
[599] S. Hayes, *Infect. Immun.*, 1979, **26**, 150.



Reagents: i, D-galactose oxidase; ii, protein-NH_2; iii, $NaBH_3CN$

Scheme 15

as cytochemical markers for the direct visualization of membrane lectins *in situ*.[60]

Antibodies. — A new volume of 'Methods in Enzymology' covers the preparation and use of affinity-labelled immunoglobulins.[600] The specialized procedures required for the preparation of ferritin–antibody conjugates have been reviewed and the use of the conjugates for the localization of enzymes in mitochondrial membranes discussed.[601]

The procedures for the conjugation of rabbit immunoglobulin G and Fab′ antibodies with β-D-galactosidase using *NN*′-2-phenylene-dimaleimide have been improved by performing conjugation under an atmosphere of nitrogen at 4 °C and pH 6.5 for at least fifteen hours.[154] The conjugates were almost completely separated from unreacted molecules by gel filtration and there was no significant impairment of β-D-galactosidase activity or the ability of anti-(human immuno-

[600] *Methods Enzymol.*, 1977, **46**.
[601] C. R. Hackenbrock, R. M. W. Chau, K. Hammon, and L. Hochli, *Methods Enzymol.*, 1979, **56**, 683.

globulin G) antibody to bind to human immunoglobulin G following conjugation. The conjugate preparation, however, was heterogeneous and one-third of each preparation consisted of aggregated conjugates less useful in sandwich enzyme immunoassay than the remaining material.

A new method for the stepwise conjugation of antibodies and marker enzymes has been reported.[602] The amino-groups of both proteins are selectively modified by reaction with 3-hydroxy-3-nitromethyl benzimidate hydrochloride, followed by reduction, and then diazotization of one of the components. The coupling reaction produces azo bridges between the substituent groups and, with the possible exception of exposed thiols, intrinsic functional groups are not involved in the coupling. Activation of one component for selective reaction with the other avoids the formation of homopolymers. Conjugates prepared [anti-(alkaline phosphatase) antibody–lactate dehydrogenase and anti-(aldolase B) antibody–alkaline phosphatase], exhibited high immunoreactivity (80–100%). Optimal conditions for the glutaraldehyde-mediated formation of human immunoglobulin G–peroxidase conjugates have been determined and some properties of the conjugates reported.[603]

The relative merits of use of periodate or glutaraldehyde for the preparation of anti-(human immunoglobulin G)antibody–peroxidase conjugates have been investigated.[604] For application to tissue immunochemistry, glutaraldehyde conjugates were found to be superior, whereas for use in enzyme-linked immunoadsorbent assays, periodate treatment yielded more effective conjugates. Periodate- and glutaraldehyde-conjugates were equally suitable for the determination of surface immunoglobulin G on lymphoid cells.

Sheep erythrocytes sensitized with intact immunoglobulin G or with reduced (dithioerythritol) and alkylated (iodoacetamide) antibody have been lysed by guinea-pig serum, indicating that reduced and alkylated antibody bound and activated complement.[605] Observations suggested that the sole effect of reduction of antibody disulphide bonds was to diminish the co-operativity of antibody–complement interaction.

Glycopeptides prepared from human immunoglobulin G have been used as substrates in an investigation of the specificity of a β-D-2-acetamido-2-deoxyglucosidase from fig latex.[606]

A new phospholipid-containing alkylating reagent, *N*-(N^{α}-iodoacetyl,N^{ϵ}-dansyl-lysyl)phosphatidyl choline ethanolamine, has been synthesized for conjugation to thiol groups of proteins.[607] Model experiments were performed with a Bence–Jones dimer, which had been reduced to generate one thiol group per monomer. After alkylation with the reagent, the modified protein became spontaneously attached to preformed liposomes and red cell ghosts.

[602] J. Muller and G. Pfleiderer, *J. Appl. Biochem.*, 1979, **1**, 301.
[603] B. B. Dzantiev, E. M. Gavrilova, N. Y. Filippova, A. M. Egorov, and I. V. Berezin, *Biochemistry (USSR)*, 1978, **43**, 836.
[604] D. M. Boorma and J. G. Streefkerk, *J. Immunol. Methods*, 1979, **30**, 245.
[605] J. K. Wright, *Biochem. Biophys. Res. Commun.*, 1978, **83**, 1284.
[606] S. F. Chien, R. Weinburg, S. C. Li, and Y. T. Li, *Biochem. Biophys. Res. Commun.*, 1977, **76**, 317.
[607] D. Sinha and F. Karush, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 554.

Sea urchin egg tropomyosin has been isolated from an immunoprecipitate formed between anti-(lantern muscle tropomyosin) antibody and a sea urchin egg tropomyosin fraction.[608] The antibody–antigen complex was heated to denature the antibody and the components were then separated.

Fetuin. – After introduction of aldehyde groups at C-7 of neuraminic acid residues in fetuin, an amine-containing spectroscopic probe has been attached to the molecule by reductive amination.[609] The spin–label has also been introduced into asialofetuin after modification of the glycoprotein with either D-galactose oxidase or sodium periodate.

[^{3}H]Raffinose (76) has been coupled to fetuin as shown in Scheme 15 for use in investigation of the organ sites of catabolism of proteins in the circulation.[597]

Mucins. – Bovine submaxillary mucin and its asialo-derivative have been spin-labelled after introduction of aldehyde groups as described for fetuin.[609]

Phytohaemagglutinins. – Cell fractionation by selective agglutination with lectins has been reviewed.[86] The pH dependence of lectin interaction with sugars has been determined by affinity electrophoresis.[93] Binding studies of native lectins and their ferritin conjugates to dispersed pancreatic acinar cells show that the conjugation procedure does not significantly affect the lectin-binding properties.[298]

When fibroblasts were treated with di- and mono-valent succinylated concanavalin A, α-D-2-acetamido-2-deoxyglucosidase endocytosis was inhibited, but there was no effect on extracellular β-D-2-acetamido-2-deoxyglucosidase accumulation.[610]

Tetravalent concanavalin A and divalent succinylated concanavalin A bind to the surface glycoproteins of macrophages and cause a marked increase in the rate of oxygen consumption owing to activation of the hexose monophosphate shunt.[611] The metabolic change induced could be reversibly inhibited by methyl α-D-glucopyranoside and was temperature-dependent. The effect of polyvalent lectins, including concanavalin A, di- and mono-valent succinylated concanavalin A, and wheat-germ agglutinin, on the secretion and endocytosis of lysosomal enzymes has been investigated.[612]

Concanavalin A has been treated with 3,3′-dimethyldithiobis-propio-imidate and the disulphide bond thus introduced then reduced and treated with ricin to yield a concanavalin A–ricin conjugate, retaining about one third of the inhibitor activity of ricin in a cell-free protein synthesis system.[613] The hybrid conjugate therefore contains a moiety that can bind to cell membrane receptors and an active fragment of a toxic protein. In order to study the mechanisms

[608] T. Ishimodo-Takagi, *J. Biochem. (Tokyo)*, 1978, **83**, 1757.
[609] J. D. Aplin, M. A. Bernstein, C. F. A. Culling, L. D. Hall, and P. E. Reid, *Carbohydr. Res.*, 1979, **70**, C9.
[610] H. Beeck, K. Ullrich, and K. Von Figura, *Biochim. Biophys. Acta*, 1979, **583**, 179.
[611] T. Yasaka and T. Kambara, *Biochim. Biophys. Acta*, 1978, **508**, 306.
[612] H. Beeck, K. Ullrich, and K. Von Figura, *Biochim. Biophys. Acta*, 1979, **583**, 179.
[613] T. Yamaguchi, R. Kato, M. Beppu, T. Terao, Y. Inoue, Y. Ikawa, and T. Osawa, *J. Natl. Cancer Inst.*, 1979, **62**, 1387.

involved in the interaction of cell-surface components and effector proteins, a double-labelled dimeric hybrid molecule, consisting of the toxic A sub-unit of ricin and the cell-specific β-sub-unit of human chorionic gonadotrophin, has been synthesized.[614] The biological properties of the conjugate were reported separately.[615]

Horse-radish peroxidase chemically activated with glutaraldehyde and covalently coupled to wheat-germ agglutinin has been shown to be forty times more sensitive than free horse-radish peroxidase for studies of retrograde axonal transport.[616]

Orosomucoid. – A glycopeptide obtained by pronase digestion of orosomucoid has been obtained and the carboxy-group of its terminal *N*-acetylneuraminic acid esterified with a carbodi-imide and then reduced with sodium borohydride.[617] The reduced glycopeptide was shown to be resistant to hydrolysis by neuraminidase and other glycosidases.

Asialo-orosomucoid inhibits the disappearance of human glucocerebrosidase from the rat circulation following intravenous administration.[618]

Ovalbumin. – Glycopeptides prepared from ovalbumin have been used as substrates for investigation of the specificity of β-D-2-acetamido-2-deoxyglucosidase from fig latex.[606]

The nature of the cross-linking of proteins by glutaraldehyde has been investigated using ovalbumin.[619] Several quaternary pyridinium compounds were isolated from acid hydrolysates of glutaraldehyde cross-linked ovalbumin and their structures were confirmed by synthesis.

Miscellaneous Glycoproteins. – Bovine lutrophin, bovine thyrotrophin, and human chorionic gonadotrophin preparations with carbodi-imide-mediated covalently cross-linked sub-units have been isolated in high yield and characterized by c.d. spectroscopy, radioligand receptor assays, and hormone-specific bioassays.[620] The c.d. spectra of the native and cross-linked hormones did not differ much, suggesting that there is little conformational change on cross-linking.

The reaction of 1-ethyl-3(3-dimethylaminopropyl)carbodi-imide with bovine lutrophin has been studied and the reaction conditions have been investigated.[621] Spectrophotometric measurements, tryptic peptide maps, and sodium dodecyl sulphate–urea polyacrylamide gel electrophoresis banding patterns were obtained and compared for native and cross-linked derivatives of the glycoprotein hormone.[622]

[614] T. O. Oeltmann and E. C. Heath, *J. Biol. Chem.*, 1979, **254**, 1022.
[615] T. N. Oeltmann and E. C. Heath, *J. Biol. Chem.*, 1979, **254**, 1028.
[616] N. K. Gonatas, C. Harper, T. Mizutani, and J. O. Gonatas, *J. Histochem. Cytochem.*, 1979, **27**, 728.
[617] A. Amemura, R. H. Shah, O. P. Bahl, and J. M. Merrick, *Carbohydr. Res.*, 1978, **63**, 241.
[618] F. S. Furbish, C. J. Steer, J. A. Barranger, E. A. Jones, and R. O. Brady, *Biochem. Biophys. Res. Commun.*, 1978, **81**, 1047.
[619] P. M. Hardy, G. J. Hughes, and H. N. Rydon, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2282.
[620] T. F. Parsons and J. G. Pierce, *J. Biol. Chem.*, 1979, **254**, 6010.
[621] J. A. Weare and L. E. Reichert, *J. Biol. Chem.*, 1979, **254**, 6972.
[622] J. A. Weare and L. E. Reichert, *J. Biol. Chem.*, 1979, **254**, 6964.

Plasma transglutaminase (Factor XIIIa) has been shown to catalyse the cross-linking of fibronectin and collagen.[623] The reaction may be of importance in wound healing and tissue repair.

Disuccinimidyl suberate, a cross-linking agent, has been synthesized and used to probe the insulin-responsive D-glucose transport of the rat adipocyte.[624] Incubation of cells with the reagent blocked methyl-α-D-glucopyranoside transport without affecting either basal hexose transport or simple diffusion as indicated by uptake in the presence of cytochalasin B.

α_1-Protease inhibitor reacts with proelastase 2 to form a 1:1 molar complex, which is stable to reduction and denaturation with sodium dodecyl sulphate.[625]

The application of chemical cross-linking for studies on cell membranes and the identification of surface receptors has been reviewed.[626]

Treatment of human erythrocyte membranes with Cu^{2+}-2-phenanthroline at 0 °C results in the cross-linking of the Band 3 polypeptide to a dimer.[627] The cross-linked protein was digested with proteolytic enzymes and the products were resolved by two-dimensional gel electrophoresis. The thiol groups involved in the cross-linking were identified.

The potential use of diazotized [^{125}I]di-iodosulphanilic acid as a label for cell-surface membranes has been investigated.[628] Colorimetric determination of the compound is possible as it reacts at neutral pH with naphth-1-ol to yield a coloured complex (λ_{max} 430 nm). Results obtained suggest that when used under appropriate conditions di-iodo-sulphanilic acid can serve as a highly selective membrane label with minimal incorporation into intracellular soluble protein.

A high molecular weight carbohydrate-rich fragment has been obtained by gel filtration of a collagenase–pronase digest of the bovine glomerular basement membrane.[629] After performic acid oxidation, this fragment became susceptible to further digestion by these proteolytic enzymes and yielded three glycopeptide fractions which were characterized.

Immobilized Derivatives of Glycoproteins. – The modification of glycoproteins to yield biologically active immobilized derivatives continues to attract much attention. These derivatives and their uses are summarized in Table 6.[630–644]

[623] D. F. Mosher, P. E. Schad, and H. K. Kleinman, *J. Clin. Invest.*, 1979, **64**, 781.
[624] P. F. Pilch and M. P. Czech, *J. Biol. Chem.*, 1979, **254**, 3375.
[625] C. Largman, J. W. Brodrick, M. C. Geokas, W. M. Sischo, and J. H. Johnson, *J. Biol. Chem.*, 1979, **254**, 8516.
[626] T. H. Ji, *Biochim. Biophys. Acta.*, 1979, **559**, 39.
[627] R. A. F. Reithmeier and A. Rao, *J. Biol. Chem.*, 1979, **254**, 6151.
[628] R. M. Edwards, S. A. Kempson, G. L. Carlson, and T. P. Dousa, *Biochim. Biophys. Acta*, 1979, **553**, 54.
[629] M. J. Levine and R. G. Spiro, *J. Biol. Chem.*, 1979, **254**, 8121.
[630] S. K. Roy and S. K. Kundu, *Anal. Biochem.*, 1979, **98**, 238.
[631] E. P. Halpern and R. W. Bordens, *Clin. Chem.*, 1979, **25**, 860.
[632] J. H. Fishman and J. Fishman, *Anal. Biochem.*, 1979, **94**, 278.
[633] J. Gabrilovac, K. Pachmann, H. Rodt, G. Jäger, and S. Thierfelder, *J. Immunol. Methods*, 1979, **30**, 161.
[634] W. Hertl and G. Odstrchel, *Mol. Immunol.*, 1979, **16**, 173.
[635] E. P. Halpern and R. W. Bordens, *Clin. Chem.*, 1979, **25**, 1561.

Table 6 *Modification of glycoproteins by coupling to insoluble matrices and other macromolecules*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
Albumin	Reaction with agarose cyclic imidocarbonate	Investigation of binding of fatty acids, steroids, and drugs to albumin	192
	Glutaraldehyde-mediated reaction with aminopropyl-silica gel	Purification of goat anti-(human serum albumin) antibody by immunoadsorption	630
Albumin, glutaraldehyde cross-linked	Carbodi-imide mediated reaction with aminoethyl-agarose	Affinity chromatographic purification of hepatitis B surface antigen from human sera	340
Anti-(A, B, H, blood group antigen) antibody	Reaction with agarose cyclic imidocarbonate	Detection by immunoadsorption of ABO blood group specificity on carcinoembryonic antigen preparations	134
Anti-(AMP-deaminase) antibody		Purification of AMP-deaminases by immunoadsorption	135
Anti-(adenovirus capsid sub-unit) antibody		Preparation of specific antisera against adenoviruses by 'affinity bead immunization'	136
Anti-(alkaline phosphatase) antibody		Development of an immunoadsorbent method for the continuous purification of human placental alkaline phosphatase	137
Anti-[α-amylase inhibitor (*Phaseolus vulgaris*)] antibody		Purification by immunoadsorption of α-amylase inhibitor from *P. vulgaris*	138
Anti-(carboxypeptidase N) antibody		Purification by immunoadsorption of rat serum carboxypeptidase N	142
Anti-cellulase antibody		Purification by immunoadsorption of *Trichoderma viride* cellulase	143

Table 6 *(cont.)*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
Anti-digoxin antibody	Microencapsulation in a semipermeable nylon membrane	Reagent for use in the radioimmunoassay of digoxin	631
Anti-(enterobacterial common antigen) antibody	Carbodi-imide mediated reaction with Affi-gel 10®	Purification by immunoadsorption of enterobacterial common antigen from *Escherichia coli*	428
Anti-(Factor VIII) antibody	Reaction with agarose cyclic imidocarbonate	Immunoadsorption of Factor VIII and fibrinogen from normal plasma; calibration of a one-stage Factor VIII assay	145
Anti-fibronectin antibody		Purification of fibronectin by immunoadsorption	147
Anti-(α-foetoprotein) antibody		Purification by immunoadsorption of human α-foetoprotein	139
Anti-(α-D-glucosidase) antibody		Comparison of α-D-glucosidase activities in urine of normal subjects and patients with Pompe's disease	148
Anti-(β-D-glucuronidase) antibody		Isolation by immunoadsorption of β-D-glucuronidase from human plasma	149
Anti-(glyceraldehyde 3-phosphate dehydrogenase) antibody or Fc fragments thereof		Immobilization by immunoadsorption of glyceraldehyde 3-phosphate dehydrogenase	150
Anti-(β_2-glycoprotein) antibody		Purification by immunoadsorption of an agglutinin in human sera	140
Anti-(hepatitis A virus) antibody		Purification by immunoadsorption of hepatitis A virus particles	151
Anti-(human chorionic gonadotrophin) antibody		Purification by immunoadsorption of human chorionic gonadotrophin-receptor complex	152
Anti-(human immunoglobulin G) antibody		Study of interactions between immobilized antibodies and antigen-peroxidase complex	153
		Isolation by immunoadsorption of human immunoglobulin G for use in enzyme immunoassay	154

Anti-haemocyanin antibody	Reaction with protein A-derivatized agarose	Purification by immunoadsorption of haemocyanin from *Limulus*	185
Anti-(human serum) antibody	Reaction with agarose cyclic imidocarbonate	Isolation by immunoadsorption of C1q (a sub-component of the first component of complement) from human serum	155
Anti-(infectious bovine rhinotracheitis antigen) antibody		Purification by immunoadsorption of infectious bovine rhinotracheitis antigen	156
Anti-interferon antibody		Study of nonspecific adsorption of interferon on immobilized serum immunoglobulin	157
		Purification by immunoadsorption of human interferon	158
Anti-(L-cell colony stimulating factor) antibody		Purification by immunoadsorption of L-cell colony stimulating factor	159
Anti-legumin antibody		Purification by immunoadsorption of legumin from *Pisum* seeds	160
Anti-lysozyme antibody		Assessment of immunological reactivity of synthetic lysozyme peptides	108
Anti-(β_2-microglobulin) antibody		Isolation by immunoadsorption of HLA antigen from human tissue	141
Anti-myosin antibody		Purification by immunoadsorption of rabbit ventricular myosin	161
Anti-oestradiol antibody	Adsorption on to poly(vinylidene fluoride) film	Study of oestradiol-binding proteins	632
Anti-(pregnancy zone protein)-antibody	Reaction with agarose cyclic imidocarbonate	Purification by immunoadsorption of pregnancy zone protein	162
Anti-(rabbit immunoglobulin G) antibody		Immunoadsorption of anti-(human immunoglobulin G) antibody-β-D-galactosidase conjugates prepared for enzyme immunoassay	154
Anti-(rabbit immunoglobulin G) antibody $F(ab)_2$ fragments	Reaction with poly(-L-Lys)-coated polyacrylamide beads	Detection of T-cell surface antigens	633
Anti-(rat cell surface antigen) antibody	Reaction with agarose cyclic imidocarbonate	Study by immunoadsorption of tritiated glycoproteins from the surface of rat lymphoid cells	163

Table 6 *(cont.)*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
Anti-(soluble egg antigen) antibody		Isolation by immunoadsorption of *Schistosoma mansoni* egg antigen	164
Anti-tetanolysin antibody		Purification by immunoadsorption of streptolysin O	165
Anti-(thrombin III) antibody		Separation of heparin into functionally distinct fractions on the basis of immunoreactivity	166
Anti-(L-thyroxine) antibody	Reaction with *N*-hydroxy-succinimide-derivatized controlled pore glass	Study of kinetic and thermodynamic properties of antigen–antibody interactions in heterogeneous reaction phases	634
	Microencapsulation in a semipermeable nylon membrane	Radioimmunoassay determination of free L-thyroxine in serum	635
Anti-(L-tri-iodothyroxine) antibody	Reaction with *N*-hydroxy-succinimide-derivatized controlled pore glass	Study of kinetic and thermodynamic properties of antigen–antibody interactions in heterogeneous reaction phases	636
Arachis hypogaea lectin		Affinity chromatography of antigenic determinants of membranes	223
Bandeiraea simplicifolia lectin		Affinity chromatography of human liver hydrolases	228
		Affinity chromatography of soluble conjugates of *Salmonella* specific oligosaccharides and bovine serum albumin	229
Blood group A substance		Affinity chromatography of *Vicia villosa* lectin	171
Bovine serum albumin		Purification by immunoadsorption of anti-(bovine serum albumin)antibodies; assessment of a modified procedure for preparation of agarose cyclic imidocarbonate	110
3-(*O*-Carboxymethyl)-oximino-5α-testosterone–bovine serum albumin conjugate		Purification by immunoadsorption of antibodies against the steroid antigen	167

7-(*O*-Carboxymethyl)-oximino-5α-dihydrotestosterone–bovine serum albumin conjugate	Purification by immunoadsorption of antibodies against the steroid antigen 167
Carcinoembryonic antigen	Analysis of human sera for carcinoembryonic antigen-binding proteins 168
	Isolation by immunoadsorption of a carcinoembryonic antigen-binding human immunoglobulin G from normal sera 169
Concanavalin A	Purification of bovine sperm forward motility protein 243
	Separation of peroxidase–immunoglobulin G conjugate from unconjugated immunoglobulin G for use in enzyme immunoassay 244
	Purification of β-D-2-acetamido-2-deoxyhexosidases 189
	Purification of α-L-fucosidase 245
	Purification of 2-dansylamido-2-deoxy-D-glucose 246
	Affinity chromatography of soluble membrane receptors from mouse submaxillary glands 247
	Affinity chromatographic adsorption of activating factor of lymphocyte adenylate cyclase with consequent reversible deactivation of the enzyme 248
	Purification of mammalian serum haptoglobin 249
	Purification of *Schistosoma mansoni* soluble egg antigens 250
	Affinity chromatography of human alkaline phosphatases 251
	Purification of carcinoembryonic antigen 252
	Purification of D-glucocerebrosidase 253
	Purification of *Phaseolus vulgaris* phytohaemagglutinin 254
	Affinity chromatography of human liver acid hydrolases 228

Table 6 *(cont.)*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
		Purification of human liver 'acid' β-D-galactosidases	218
		Separation of cellulase and 'cellobiase' from *Trichoderma viride*	255
		Purification of a myeloma immunoglobulin M and its tryptic Fab and $(Fc)_5$ fragments	256
		Fractionation of soluble brain glycoproteins	257
		Purification of rat kidney glycoproteins for production of heterologous antisera which induce congenital abnormalities	258
		Affinity chromatographic separation of integral membrane glycoproteins E1 and E2 of Semliki Forest virus	259
		Separation of A- and B-type glycopeptides of Semliki Forest virus	260
		Purification of human placental α-D-galactosidase	261
		Affinity chromatography of antigenic fractions of human seminal plasma	262
		Affinity chromatography of glycopeptides obtained by pronase digestion of immunoglobulin G from a patient with multiple myeloma	263
	Reaction with agarose cyclic imidocarbonate	Purification of 2-deoxy-2-sulphamido-D-glucose sulphohydrolase	264
		Affinity chromatography of glycoproteins in purified myelin	265
		Affinity chromatographic purification of a D-galacto-D-mannan antigen from *Aspergillus fumigatus*	266
		Studies of the interaction of concanavalin A with tryptic fragments from plasma fibronectin	267

	Affinity chromatography of acid ribonuclease from HeLa cell lysosomes; demonstration that the enzyme is a glycoprotein	268
	Purification of human liver 'acid' α-D-glucosidase	148
	Purification of two forms of α-D-2-acetamido-2-deoxygalactosidase	269
	Study of specific binding of [^{35}S]-labelled proteoglycans from secretions of human skin fibroblasts	270
	Separation of two molecular variants of α-foetoprotein	271
	Purification of bull seminal plasma hyaluronidase	272
	Purification of ribonuclease B	59
	Purification of biliary glycoprotein I	273
	Separation of human amylase isoenzymes	274
	Purification of cathepsin D from rat spleen	275
	Purification of a cathepsin E-like acid proteinase from rat spleen	276
	Study of inhibition of binding of immobilized concanavalin A to insulin receptors on isolated intact rat fat cells by inhibitors of concanavalin A	388
	'Anchoring units' for enzymes reversibly immobilized on the support for use in an analytical flow system	389
	Comparison of 4-methylumbelliferyl-β-D-galactosidase and G_{M_1} β-D-galactosidase levels in normals and patients with I-cell disease	390
	Adsorption of *N*-[^{14}C]methyl ribonuclease B for use as substrate in a semi-quantitative assay of *endo*-β-D-2-acetamido-2-deoxyglucanase	391
	Isolation of antibodies specific for the carbohydrate-binding site of concanavalin A	637
Glutaraldehyde-cross-linked onto Sephadex®	Investigation of the tertiary and quaternary protein structure of concanavalin A	638

Table 6 *(cont.)*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
	Reaction with *N*-hydroxysuccinimide ester of cross-linked agarose	Support matrix for the immobilization of enzymes	117
Concanavalin A, succinylated	Reaction with agarose cyclic imidocarbonate	Adsorption of β-D-fructofuranosidase for use in enzyme immunoassay	392, 393
Cowper's gland mucous, desialylised		Purification of β-D-2-acetamido-2-deoxyhexosidase from rabbit sperm cytoplasmic droplets	277
2,4-Dinitrophenylated albumin		Isolation by immunoadsorption of anti-(2,4-dinitrophenyl) antibody preparations	170
Factor VIII		Purification of low molecular weight Factor VIII	284
Factor X		Separation of heparin into functionally distinct fractions; investigation of mechanism of action of heparin	166
Fetuin	Reaction with agarose cyclic imidocarbonate	Purification of neuraminidase from *Streptomyces griseus*	285
Fetuin, desialylised		Purification of β-D-2-acetamido-2-deoxyhexosidase from rabbit sperm cytoplasmic droplets	277
Haptoglobin		Purification of the lymphocytosis-promoting factor haemagglutinin of *Bordetella pertussis*	288
Hog sulphated gastric mucin glycopeptides		Purification of lectins by a general affinity chromatographic method	298
Immunoglobulin A	Adsorption onto polystyrene	Reagent for enzyme-linked immunoassay	639
Immunoglobulin E		Immunological study of surface glycoproteins and the immunoglobulin E receptor from rat basophilic leukaemia cells	174
		Purification by immunoadsorption of a cell-surface receptor for immunoglobulin E from rat basophilic leukaemia cells	175

Immunoglobulin G	Reaction with agarose cyclic imidocarbonate	Reagent for enzyme-linked immunoassay	639
		Immunoadsorption of anti-(human immunoglobulin G) antibody–β-D-galactosidase conjugate prepared for enzyme-linked immunoassay	154
		Purification by immunoadsorption of C1r, an activated component of the first component of the complement system	176
	Adsorption onto polystyrene latex beads	Investigation of the interaction between antibody and immobilized antigen using density-gradient centrifugation	640
	Adsorption onto polystyrene	Reagent for enzyme-linked immunoassay	639
Immunoglobulin G, Fc fragments	Reaction with agarose cyclic imidocarbonate	Isolation by immunoadsorption of immunoglobulin G Fc fragment-binding glycoproteins from human blood platelets	177
Immunoglobulin M	Adsorption onto polystyrene	Reagent for enzyme immunoassay	639
Influenza A virus	Adsorption onto polystyrene	Reagent for enzyme immunoassay	639
	Reaction with agarose cyclic imidocarbonate	Purification by immunoadsorption of anti-carbohydrate antibodies	178
α-Lactalbumin		Purification of lactose synthase from pig thyroid	299
Lens culinaris lectin		Investigation of mitogenic response of lymphocytes	180
		Immunoadsorption of surface proteins and glycoproteins of rat basophilic leukaemia cells	174
		Purification of a hyaluronidase from *Streptococcus pyogenes*	300
		Purification of an organ-specific tumour-associated antigen from rat mammary carcinoma	301
		Affinity chromatography of surface glycoproteins and immunoglobulin E receptors from rat basophilic leukaemia cells	174
	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of membrane immunoglobulins M and D from murine lymphocytes; study of their affinities for the lectin	302

Table 6 *(cont.)*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
		Affinity chromatography of glycoprotein fractions from long-term cultured human lymphocytes	180
		Purification of *Trypanosoma cruzi* cell-surface protein	303
		Affinity chromatography of tritiated glycoproteins from the surface of rat lymphoid cells	163
		Purification of the major surface coat glycoprotein of *Trypanosoma brucei brucei*	304
		'Anchoring units' for enzymes reversibly immobilized on the support for use in an analytical flow system	389
	Carbodi-imide-mediated reaction with gelatin-coated tubes and petri dishes	Affinity fractionation of human peripheral blood lymphocytes	641
Lotus tetragonolobus lectin		Affinity chromatography of glycoproteins in purified myelin	265
Melanoma cell membranes		Isolation by immunoadsorption of immunoglobulins from sera of patients with melanoma, sarcoma, and carcinoma	182
Ovomucoid	Reaction with agarose cyclic imidocarbonate	Purification of a lectin from rice bran	309
Prealbumin		Investigation of interaction between prealbumin and retinol-binding proteins	316
Prothrombin		Purification of staphylocoagulase	317
Ricinus communis lectin		Affinity chromatography of human liver hydrolases	228
		Purification of a myeloma immunoglobulin M and its tryptic Fab and $(Fc)_5$ fragments	256
		Affinity chromatography of glycoproteins in purified myelin	265

		Affinity chromatography of major cell-surface glycoproteins from normal and diabetic rat liver membranes	319
		Affinity chromatography of tyrosine tRNA's	320
	Reaction with epichlorohydrin-activated agarose	Affinity chromatographic purification of porcine thyroglobulin	424
	Reaction with dextran cyclic imidocarbonate	Isolation of serum inhibitors of desialylised glycoprotein-binding to hepatocyte membranes	642
	Glutaraldehyde-mediated reaction with agarose	Support for lysozyme immobilization	643
Rubella virus	Adsorption onto polystyrene	Reagent for enzyme-linked immunoassay	639
Soybean agglutinin	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of human liver hydrolases	228
Soybean trypsin inhibitor	Carbodi-imide-mediated reaction with an alkyl spacer arm previously treated with 1,1'-carbonyldi-imidazole-activated agarose	Affinity chromatographic purification of trypsin	109
	Glutaraldehyde-mediated reaction with sub-micron ferrite particles	Recovery of trypsin from product liquors by bioaffinity adsorption	644
Tamm–Horsfall glycoprotein		Affinity chromatographic separation of *Phaseolus vulgaris* leucoagglutinin and haemagglutinin lectins	322
Tetragonolobus purpurea lectin		Affinity chromatography of human liver hydrolases	228
Thrombin		Fractionation of low molecular weight porcine heparin into 'active' and 'relatively inactive' forms	297
		Affinity chromatography of heparin	129
Thyroglobulin		Affinity chromatography of rabbit anti-thyroglobulin antibodies	323
		Purification of *Phaseolus vulgaris* isolectins	324
Transplantation antigens		Purification of *in vivo* activity of an H-Z allo-antiserum	326

Table 6 *(cont.)*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*	*Use of product*	*Ref.*
Tridacna maxima lectin	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of an L-arabino-D-galactan protein from *Gladiolus*	327
Ulex europaeus I lectin		Affinity chromatography of human liver hydrolases	228
Urinary glycoprotein		Purification of human urinary glycoprotein by subunit exchange chromatography	403
Vicia faba lectin		Affinity chromatography of human serum proteins	328
Vicia villosa lectin		Selective immunoaffinity fractionation of murine cytotoxic T lymphocytes	171
Viscum album lectin		Affinity chromatography of human serum proteins	328
Wheat-germ agglutinin		Affinity chromatography of human liver hydrolases	228
		Affinity chromatographic investigation of interactions between wheat-germ agglutinin and sialoglycoproteins	329
		Affinity chromatography of glycoproteins; development of improved coupling procedure for wheat-germ agglutinin	330

A mechanical device for the continuous purification of biological material using an immunoadsorbent has been described.[137] The use of immobilized lectins in affinity chromatography has been discussed,[645] and the purification of cell-membrane glycoproteins on columns of immobilized lectins reviewed.[85] New techniques of cell fractionation involving use of immobilized lectins or selective agglutination with lectins have been described.[86]

Covalent coupling of hog sulphated gastric mucin glycopeptides to agarose has provided a 'universal' affinity chromatographic material for purification of lectins and their ferritin conjugates.[298]

The 'spreading-out' of a protein on an affinity matrix at a critically low density, below which intermolecular bridge formation does not occur, prior to reaction with a chemical cross-linker (*e.g.*, glutaraldehyde) has provided an approach which has yielded valuable information about the tertiary and quaternary structure of concanavalin A.[638] Results were in agreement with evidence available from crystallography and other chemico- and bio-physical techniques. This matrix approach promises to be particularly useful in the structural study of poorly soluble membrane-bound proteins and other proteins which are difficult to crystallize.

The immunoglobulin G fraction of rabbit anti-haemocyanin has been specifically adsorbed onto protein A–agarose and then covalently coupled to the matrix using dimethyl suberimidate.[185] The antibody molecules are thus in the correct orientation for combination with antigen since the association with protein A is *via* the Fc portion of the immunoglobulin G molecules, leaving the Fab combining sites free to interact with antigen.

Development of a simple technique has been reported for coating glass cover slips and plastic Petri dishes with various immune complexes.[646] The supports were coated with poly(L-Lys), which was then treated with 2,4-dinitrobenzene sulphonate followed by glutaraldehyde-mediated reaction with the appropriate substance.

Immobilization of Cells. – The immobilization of cellular material for use both in affinity chromatography and for the production of enzymatically active preparations continues to receive considerable attention, as has been described

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in a general review[647] and in a discussion of the transformation of organic compounds by immobilized microbial cells.[648]

Achromobacter butyri cells entrapped in polyacrylamide gel have been used in column form for the continuous high yield production of D-glucose 6-phosphate from D-glucose and metaphosphate.[649] The potential of active immobilized cells as catalysts for the production of hydrogen from water as an energy source has been investigated using *Anabaena cylindrica* cells adsorbed onto glass beads.[650]

Arthrobacter simplex cells coprecipitated with calcium alginate gel have been used for the production of prednisolone from cortisol.[651] The conversion of hydrocortisone into prednisolone has been carried out using *Arthrobacter simplex* cells entrapped in a polypropylene glycol–polyethyleneglycol copolymer,[652] in a maleic polybutadiene gel,[653] or in urethane prepolymers.[654,655] Mass-transfer effects on the rate of isomerization of D-glucose into D-fructose catalysed by commercially available immobilized *Arthrobacter* cells have been investigated and a theoretical treatment proposed.[656]

The nitrogenase activity of *Azotobacter vinelandii* cells immobilized by adsorption onto anionic exchange cellulose ('Cellex E') has been investigated under batch and continuous flow conditions.[657] *Bacillus* cells copolymerized in acrylamide have been used for the production of bacitracin.[658]

Brevibacterium ammoniagenes cells immobilized by copolymerization with acrylamide have been used in column form for the continuous production in high yield of pure nicotinamide adenine dinucleotide phosphate,[659] for the continuous production of L-malic acid from fumaric acid,[660] and for study of the NAD-kinase activity of the immobilized cells.[661] The continuous production of L-malic acid has also been achieved with *Brevibacterium flavum* cells immobilized by gelation in κ-carrageenan.[579]

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Catharanthus roseus, Digitata lanata, and *Morinda citrifolia* plant cells have been immobilized in calcium alginate gels and applied to the production and transformation of natural products.[662] Chloroplasts of *Brassica campestris* L copolymerized with acrylamide have been used to investigate carbon dioxide fixation by the immobilized cells, whose fixation activity was 65% that of free cells but which were more stable than free cells to alkaline conditions and high temperature.[663]

The properties of spinach chloroplasts adsorbed onto diethylaminoethylcellulose have been reported and the immobilized chloroplasts used for the continuous photoreduction of 2,6-dichloroindophenol as an indicator of photosystem II activity in the chloroplasts.[664] The formation of ATP from ADP and orthophosphate *via* the cyclic photophosphorylation system has been achieved using chloroplasts copolymerized with polyvinylalcohol onto an electrode.[665] *Citrobacter freundii* cells immobilized by co-casting with collagen have been used in a flow type sensor for cephalosporins.[666]

The properties of *Escherichia coli* cells entrapped in κ-carrageenan and then glutaraldehyde-cross-linked have been investigated and used for the production of L-aspartic acid.[667] L-Tryptophan has been synthesized by *E. coli* cells entrapped in polyacrylamide.[668] A correction to a previously published paper on the synthesis of L-tryptophan by immobilized *E. coli* cells has been noted.[669]

Steroid conversions have been carried out with *Nocardia rhodocrous* cells immobilized by entrapment in hydrophobic poly(propylene glycol)[653] and poly(urethane)[654] polymers, in hydrophilic poly(urethane) polymers,[655] and in hydrophilic or lipophilic gels.[670]

Penicillium cells immobilized by copolymerization with acrylamide, by co-casting with collagen followed by glutaraldehyde-cross-linking, and by coprecipitation with calcium alginate have been used for the production of penicillin G from D-glucose.[671]

The analytical determination of D-glucose in molasses has been achieved using a microbial electrode consisting of *Pseudomonas fluorescens* cells co-cast with collagen onto an oxygen electrode.[672]

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[672] I. Karube, S. Mitsuda, and S. Suzuki, *Eur. J. Appl. Microbiol. Biotechnol.*, 1979, **7**, 343.

Immobilized *Saccharomyces cerevisiae* cells have been used for the continuous production of glutathione,[673] and for the continuous regeneration of ATP from adenosine.[674] *S. paradoxus* cells have been immobilized onto glutaraldehyde-activated glycyl, β-alanyl, and 6-aminohexanoyl derivatives of 1,6-diaminohexane-derivatized hydroxyalkyl methacrylate gel.[675] The effect of the length of the spacer arm and the manner of its activation was investigated.

The physical properties and characteristics of calcium alginate gels for cell immobilization have been investigated using *Saccharomyces uvarum* cells.[457]

The properties of *Streptomyces phaeochromogenes* cells immobilized by copolymerization with 2-hydroxyethyl methacrylate gels have been reported.[676-678] The radiation-induced polymerization was performed at low temperatures to avoid radiation damage to the cellular enzymes.

Immobilized *Trichosporon brassicae* cells have been used for the amperometric determination of acetic acid.[679] Some properties of *Zygosaccharomyces lactis* cells immobilized on a modified hydroxyalkyl methacrylate gel have been reported.[680]

Rat-liver mitochondria have been adsorbed onto alkylsilylated Porasil® beads for the simulation of dynamic cellular environments in flow experiments.[681] The immobilized mitochondria were functionally indistinguishable from mitochondria in suspension.

4 Modification of Enzymes and Uses of Modified Enzymes

β-D-Galactosidase. – Sedimentation analyses of an activatable mutant β-D-galactosidase and the products of its reaction with Fab fragments of activating antibody have shown that the enzyme exists mainly as 10S dimers, while the native enzyme is a 16S tetramer.[682] Activation of the mutant enzyme by purified antibody resulted in formation of 16S tetramers. Conditions were described under which the mutant enzyme loses the specific antigenic determinant(s) responsible for binding activating antibody, allowing its subsequent use as an absorbent to obtain immunologically purified activating antibody.

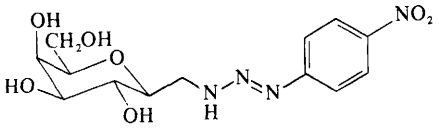
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β-D-Galactosidase has been modified with active Brilliant Orange KKh and immobilized by adsorption on diethylaminoethyl-Sephadex®.[683]

Carboxymethylated β-D-galactosidase dissociates at 100 °C to form carboxymethylated fragments A and B, whose characteristics have been investigated.[684] When a crude extract of *Escherichia coli* M15 was chromatographed on Sepharose® 6B, carboxymethylated fragment B restored β-D-galactosidase activity to fractions of mol. wt. 1.23×10^5, 2.62×10^5, and 5.06×10^5, whereas carboxymethylated fragment A did the same to fractions of mol. wt. 0.63×10^5, 2.53×10^5, and 5.06×10^5. The results suggest that multiple forms of complemented β-D-galactosidase can exist.

Citraconylated β-D-galactosidase has been subjected to tryptic hydrolysis.[685] All eighty theoretically possible tryptic fragments have been isolated and sequenced.

The site of attachment to β-D-galactosidase of the active site-directed inhibitor β-D-galactopyranosyl methyl 4-nitrophenyl triazene (77) has been determined to be L-methionine-500.[686] When the enzyme is completely inactivated one mole of the inhibitor is bound per mole of monomer, with retention of the tetrameric structure.



(77)

Two 3-maleimidobenzoyl derivatives of cortisol have been synthesized and conjugated to β-D-galactosidase through the thiol groups of the enzyme.[687] The conjugation procedure was compared with a mixed-anhydride method for conjugate synthesis and proved superior for use in enzyme immunoassay of cortisol.

A 3-maleimidobenzoyl derivative of L-thyroxine methyl ester has also been coupled to β-D-galactosidase and used in an enzyme immunoassay of L-thyroxine.[688] The procedures for the conjugation of rabbit immunoglobulin G and Fab antibodies with β-D-galactosidase using *NN'*-2-phenylene-dimaleimide have been improved by altering the reaction conditions.[154]

α-Amylase. – Tryptic digests of α-amylase from several strains of *Bacillus amyloliquefaciens* have been chromatographed on cation-exchange resins and

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found to yield identical elution patterns, suggesting that they may have the same primary structures.[689]

Glucoamylase. – Glucoamylase I (mol. wt. 9×10^4) from *Aspergillus awamori* has been converted by the action of acid protease into a smaller active enzyme (mol. wt. 8.3×10^4) whose properties resemble those of glucoamylase I′, a second enzyme from cultures of *A. awamori*.[690] Treatment of glucoamylase I with α-mannosidase yielded an enzyme (mol. wt. 8.6×10^4) identical to glucoamylase I in its mode of action on various substrates but of lower stability at pH 9.

β-D-Xylosidase. – β-D-Xylosidase from *Bacillus pumilus* contains one intramolecular disulphide bridge and four L-cysteine residues per monomer (mol. wt. 6.8×10^4).[691] Under denaturing conditions, two of these thiol groups react with 5,5′-dithiobis(2-nitrobenzoic acid) with concomitant formation of a second L-cystine. Under native conditions one L-cysteine residue per polypeptide chain reacts with 5,5′-dithiobis(2-nitrobenzoic acid) or 4-mercuribenzoate with complete loss of enzyme activity; this loss can be reversed by dithiothreitol.

Lysozyme. – Natural abundance ^{13}C n.m.r. spectroscopic studies have been reported for native lysozyme, lysozyme in solution with Gd^{3+}, La^{3+}, Eu^{3+}, Pr^{3+}, and Co^{2+}, and lysozyme modified by treatment with iodine at pH 5.5.[692] It was possible in the case of the chemically modified enzyme to assign certain resonances to specific residues in the molecule.

The self-associations of lysozyme and of L-glutamic acid-35-L-tryptophan-108-lysozyme prepared by iodine oxidation, in the absence and presence of saccharides which block the self-association, have been studied using ^1H-n.m.r. spectroscopy.[693]

The pH difference spectra of intact hen lysozyme, L-aspartic acid-52-esterified lysozyme and L-glutamic acid-35-L-tryptophan-108-esterified lysozyme have been measured at various ionic strengths and temperatures.[694] The pH dependence of the extinction difference at 301.5 nm was well interpreted in terms of the ionization of the catalytic groups L-aspartic acid-52 and L-glutamic acid-35 which interact electrostatically.

β-(3-Oxindolyl)-L-alanine-108 lysozyme and δ_1-hydroxy-L-tryptophan-108 lysozyme esterified to the carboxy group of L-glutamic acid-35 (*i.e.* L-tryptophan-108 coupled to L-glutamic acid-35) have been prepared.[695] The effect of these chemical modifications at L-tryptophan-108 on the binding of lanthanide ions to hen egg-white lysozyme was studied using natural abundance ^{13}C n.m.r. spectroscopy. The binding of substrate analogues methyl 2-acetamido-2-deoxy-β-D-glucopyranoside, *NN′*-diacetylchitobiose, and *NN′N″*-triacetyl chitotriose to lysozyme modified with an ester linkage between L-glutamic acid-35 and L-

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tryptophan-108 has been studied.[696] Results indicated that sub-sites B and C were not changed but that there was an increase in affinity for 2-acetamido-2-deoxy-D-glucose residues at sub-site D, which is near the esterified L-glutamic acid residue.

The gross conformation of acetylated lysozyme is similar to that of the native enzyme, as has been shown by measurement of enzymic activity and fluorescence properties.[697] However, protease digestion, t-butyl hypochlorite modification, and thermal denaturation experiments on native, acetylated, and guanidinated lysozymes have demonstrated that acetylation causes a small but significant shift of the (native → denatured) transition to the right. It was concluded that the charge balance in the protein plays an important part in maintaining conformation.

A study of the effect of protease ('Nagarse') digestion of native, acetylated, and guanidinated lysozymes in the absence of *NN'N''*-triacetylchitotriose has shown that only the denatured forms of the enzymes are digested.[698]

Various modification products have been isolated by chromatography on Amberlite® CG-50 of a mixture of *N*-carboxymethylated derivatives of lysozyme.[699] In each carboxymethyl derivative the position of the modified amino-acid was established. It was found that L-histidine-15, L-lysines-1, -33, -96, or -97 are blocked, whereas L-lysines-13 and -116 remain unaffected. Possible causes of this selectivity were discussed. Salt activation of carboxymethyl derivatives of lysozyme proceeds more readily the higher the degree of modification, and the enzymatic activity of carboxymethyl derivatives of lysozyme is higher than that of the native enzyme owing to their increased affinity for substrate (chitin).

The peptide map of a tryptic hydrolysate of lysozyme oxidized at the S–S bonds has been obtained and compared with a map of the same hydrolysate of lysozyme reduced at the disulphide bonds.[700] It was established that the mobility of the peptides containing oxidized amino-acid residues decreases during electrophoresis and in chromatography.

Reduced partially carboxymethylated hen egg-white lysozyme has been air oxidized to yield four isomers containing three native disulphide bonds and one open disulphide bond.[701] In each of these isomers the formation of one of the four native disulphide bonds is permanently blocked, indicating that no one of the four disulphide bonds of native lysozyme is obligatory in the formation of the other three native disulphide bonds.

Lysozyme has been ozonized and deformylated to yield kynurenine 62-lysozyme.[501] The modified enzyme, in which Try-62 is replaced by kynurenine, was purified by affinity chromatography. It was shown to retain affinity for chitin and to remain essentially an active enzyme with a slightly weakened but

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distinct catalytic activity. Conditions for the selective cleavage of the kynurenyl bond in oxidized lysozyme in aqueous hydrazine solution have been established and the resultant peptides analysed.[702]

The effects of dimethyl sulphoxide, lithium bromide, guanidinium chloride, sodium dodecyl sulphate, and urea on lysozyme have been studied using Raman spectroscopy.[703] The spectrum observed was found to depend on the denaturant used, suggesting there is not a unique denatured state for lysozyme. An analysis of the interaction of sodium dodecyl sulphate with lysozyme has been published.[704] A kinetic study of the denaturation and subsequent reduction of disulphide bonds in lysozyme has been made using rapid ultrasonic absorption measurements.[705]

Ricinus communis II lectin immobilized on agarose has been monoconjugated to lysozyme.[643] Lysozyme conjugated with β-D-galactopyranose near its active site has been used to develop a rapid and sensitive assay method for the determination of nanogram quantities of *R. communis* lectins.[706] When the lectin is incubated with sugar-substituted lysozyme, the enzyme is inhibited owing to binding of lectin to sugar, thus allowing quantitative measurement.

Miscellaneous Glycoenzymes – The uptake of native and desialylized human D-glucocerebrosidase by rat hepatocytes and Kupffer cells has been studied.[618] The asialo-derivative retained 85% of the activity of the native enzyme but only one-tenth of its half-life in the circulation.

A glycopeptide prepared from stem bromelain has been *N*-[^{14}C]acetylated.[707] The peptide–carbohydrate linkage was cleaved with an enzyme preparation from almond emulsin to yield an intact oligosaccharide unit containing two moles of 2-acetamido-2-deoxy-D-glucose and a pentapeptide.

Horse-radish peroxidase has been treated with acetic, butyric, valeric, enanthic, and succinic anhydrides of mono- and di-carboxylic acids, and with picrylsulphonic acid.[708] The effects of these modifications on catalytic activity, rates of irreversible thermal inactivation, and absorption and c.d. spectra were studied. The degree of modification rather than the nature of the modifier was found to be responsible for the major effect on the macromolecular conformation and thermostability of the enzyme after modification.

The relative merits of periodate oxidation and glutaraldehyde cross-linking for the preparation of peroxidase conjugates have been described.[604]

The isolation and properties of human immunoglobulin G–peroxidase conjugates have been reported.[603] Peroxidase covalently coupled to wheat-germ agglutinin is forty times more sensitive than free peroxidase in studies of retrograde axonal transport.[616]

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A 'mannosaminated' ribonuclease dimer has been prepared by reductive amination of a cross-linked dimer of bovine pancreatic ribonuclease and oligosaccharides obtained by acetolysis of bakers' yeast D-mannan.[62] Its selective uptake by endothelial and Kupffer cells of the liver was investigated.

Immobilized Derivatives of Enzymes. – The continuing interest in the immobilization of enzymes has been highlighted in a number of general reviews of the subject.[709-715] Analytical applications of immobilized enzymes have been discussed.[714,716] Biomedical applications of immobilized enzymes have been reviewed with particular reference to experimental applications in therapy,[717] and for diagnostic purposes in such methods as immunosorbent assays and enzyme electrode analyses.[718] The future prospects for applying immobilized enzymes in medicine have been described.[719] Encapsulation in liposomes for biological and medical applications has been the subject of a conference.[720] A discussion of the future of enzyme technology has included a brief review of the use of immobilized enzymes, particularly industrially.[721] A comprehensive review concerning the alteration of enzyme stability by immobilization has been published.[722] Stabilization against microbial and proteolytic digestion, autolysis and aggregation, heating, denaturing agents, pH inactivation, and the action of hydrogen peroxide, oxygen, and other 'poisons' was discussed.

The use of polymeric carriers as immobilizing media has been described.[723] Spherical macroporous reactive carriers capable of forming covalent bonds with amino-acids and proteins have been prepared by suspension copolymerization of 2-hydroxyethyl methacrylate, ethylene dimethacrylate, and 4-nitrophenyl esters of methacrylic acid, and by suspension copolymerization of methacryloyl derivatives of glycine, β-alanine, and 6-aminohexanoic acid.[724] The carriers were reported to be suitable for enzyme immobilization.

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A new method of enzyme immobilization involving adsorption of enzyme and stabilizer onto an inert carrier and subsequent coating of particles with a semi-permeable film has been suggested for the immobilization of enzymes catalysing the splitting of small molecular weight substrates.[725]

The application of immobilized model systems for investigation of the kinetics of sequential enzyme reactions has been reviewed.[726]

A series of papers concerning the use of immobilized enzymes in industrial reactors has been published.[727] The operational effectiveness factors of immobilized enzyme systems have been described.[728] Analytical expressions have been developed that allow the generation of effectiveness graphs for immobilized whole-cell hollow-fibre reactors.[729] A theoretical method of determining the kinetic constants of immobilized enzymes in continuous stirred tank and plug-flow reactors by transformation of rate-equation variables has been presented.[730] A new method for accurately determining kinetic constants of immobilized enzymes in a packed bed reactor requires only the graphical analysis of data obtained from experiments with a column reactor containing immobilized enzyme for calculation of the true kinetic constants without external diffusion resistance.[731] A mathematical theory has been developed to describe proton diffusion through an immobilized protein membrane.[732]

Use of immobilized enzymes in various types of process streams has been compared with use of soluble enzymes 'immobilized' by ultrafiltration or recovered from the media by affinity chromatography.[733]

The effects of external diffusion and design geometry on the performance of an immobilized D-glucose isomerase reactor system have been investigated and a theoretical treatment proposed.[734] It was suggested that prediction of optimal design and operating conditions for this and similar systems should be possible. A detailed theoretical analysis of the coupled transport, reaction, and deactivation phenomena in the immobilized D-glucose oxidase and catalase system has been presented.[735]

Many preparations of immobilized enzymes and of enzymes coupled to other macromolecules to give water-soluble or -insoluble forms have been reported during the past year. These derivatives and their uses are summarized in Table 7.[736–825] Immobilized enzymes are referred to in this Table, whether they are enzymatically active or not.

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Table 7 *Modification of enzymes by coupling to insoluble matrices and other macromolecules*

Enzyme	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
β-D-2-Acetamido-2-deoxy-hexosidase	3.2.1.52	Glutaraldehyde cross-linking with bovine serum albumin	Study of the properties of the active immobilized enzyme	736
Acetylcholinesterase	3.1.1.7	Glutaraldehyde-mediated reaction with derivatized polyethylene tubing	Study of the kinetics of the active immobilized enzyme	737
Achromobacter butyri cells	–	Entrapment in polyacrylamide gel	Continuous production of D-glucose 6-phosphate in a column of immobilized cells	649
Alcohol dehydrogenase	1.1.1.1	Entrapment in polyacrylamide gel over a platinum grid matrix	Enzyme electrode for analysis of substrate concentrations	738
		Reaction with agarose cyclic imido-carbonate	Investigation of substitution of zinc(II) ions by cobalt(II) ions in the active immobilized enzyme	112
			Study of sub-unit interactions of the active immobilized enzyme	111
Aldehyde dehydrogenase	1.2.1.3	Carbodi-imide-mediated reaction with alkylamine glass; reaction with diazotized arylamine glass; glutaraldehyde cross-linking onto alkylamine glass	Active immobilized enzyme	739
Alkaline phosphatase	3.1.3.1	Reaction with agarose cyclic imido-carbonate	Immunoadsorbent purification of anti-(human placental alkaline phosphatase) antibody	113
		Reaction with hydrophobic esters of cellulose	Active immobilized enzyme	504
		Glutaraldehyde- or acrolein-mediated cross-linking with chitosan gel	Investigation of a novel matrix for the preparation of active immobilized enzymes	521

		Reaction with 5,5′-dithiobis(2-nitrobenzoic acid)-activated thiolagarose or with dithiobis(succinimidyl propionate)-agarose	Investigation of the quaternary structure of the reversibly immobilized enzyme subunits produced by dissociation of the matrix-bound dimer	441
D-Amino-acid oxidase	1.4.3.3	Glutaraldehyde-mediated reaction with silanized attapulgite support particles	Active immobilized enzyme; investigation of a new method of enzyme immobilization	740
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme for the preparation of pure flavin adenine dinucleotide	114
Aminoacylase	3.5.1.14	Adsorption onto tannin coupled to aminohexyl-cellulose	Active immobilized enzyme; investigation of the suitability of the preparation for industrial production of L-amino-acids	492
		Adsorption onto tannin coupled to aminohexyl-cellulose	Active immobilized enzyme	493
		Entrapment in κ-carrageenan	Active immobilized enzyme	578
Aminopeptidase	3.4.11.11	Unspecified reaction with agarose, dextran, aminoethyl-cellulose, and several inorganic carriers	Active immobilized enzyme	741
α-Amylase	3.2.1.1	Reaction with polyphenolic resins	Active immobilized enzyme	742
		Adsorption onto tannin coupled to aminohexyl-cellulose	Active immobilized enzyme	493
		Radiation-induced copolymerization with 2-hydroxyethyl methacrylate at low temperatures	Active immobilized enzyme; investigation of method of immobilization	743
		Radiation-induced copolymerization of hydrophobic glass-forming monomers at low temperatures	Active immobilized enzyme; investigation of method of immobilization	744
		Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of proteinaceous α-amylase inhibitors from wheat and rye	115
		Glutaraldehyde cross-linking onto phenol–formaldehyde resin	Study of the action of the active immobilized enzyme on cornstarch	745

Table 7 *(cont.)*

Enzyme	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Carbodi-imide-mediated reaction with epichlorohydrin-activated agarose previously treated with glycine	Active immobilized enzyme	503
		Reaction with a *sym*-trichlorotriazine-activated polystyrene anion exchanger; reaction with 1,4-benzoquinone-activated aminopropyl silica; reaction with an *N*-hydroxysuccinimide ester derivative of 3-succinimidopropyl silica	Investigation of the influence of charged matrix surfaces on the thermostabilizing effect of Ca^{2+} ions on the active immobilized enzyme	746
		Carbodi-imide- or 2,4-toluenedi-isocyanate-mediated reaction with amino- or carboxy-aerosil	Comparison of properties of active immobilized enzyme preparations	747
	–	Reaction with dextran cyclic imido-carbonate	Investigation of utility of soluble enzyme–dextran conjugates for therapeutic administration to animals	549
β-Amylase	3.2.1.2	Glutaraldehyde-mediated reaction with alkylamine-silica	Active immobilized enzyme for conversion of maize dextrins into limit dextrins	748
Amylosubtilin	–	Reaction with 2,4-toluene di-isocyanate- or cyanuric chloride-activated Sylochrome®	Active immobilized enzyme	749
Anabaena cylindrica cells	–	Adsorption onto glass beads	Investigation of potential of active immobilized cells as catalysts for the production of hydrogen from water as an energy source	650
Arginase	3.5.3.1	Reaction with agarose cyclic imido-carbonate	Investigation of the influence of immobilization on the conformation of the enzyme	116
Arthrobacter simplex cells	–	Coprecipitation with calcium alginate	Production of prednisolone from cortisol by the living immobilized cells	651

		Entrapment in polypropyleneglycol and polyethyleneglycol polymers	Conversion of hydrocortisone into prednisolone by the living immobilized cells	652
		Entrapment in a maleic polybutadiene gel	Conversion of hydrocortisone into prednisolone by the living immobilized cells	653
		Entrapment in a hydrophobic urethane polymer	Conversion of hydrocortisone into prednisolone by the living immobilized cells	654
		Entrapment in a hydrophilic urethane polymer	Conversion of hydrocortisone into prednisolone by the living immobilized cells	655
		Commercially available preparation	Investigation of the rate of isomerization of D-glucose to D-fructose, catalysed by the living immobilized cells	656
L-Asparaginase	3.5.1.1	Reaction with cellulose fibres activated with cyanogen bromide	Active immobilized enzyme for use in an extracorporeal reactor dialyser for enzyme therapy	500
		Adsorption onto tannin coupled to aminohexyl-cellulose	Active immobilized enzyme	493
		Reaction with nylon imidate	Active immobilized enzyme	750
Aspartate ammonia-lyase	4.3.1.1	Entrapment in κ-carrageenan	Active immobilized enzyme	578
		Entrapment in cellulose acetate porous fibres	Active immobilized enzyme	751
Aspartate aminotransferase	2.6.1.1	Reaction with water-insoluble collagen films	Study of the properties of the immobilized sub-units of holoenzyme obtained after alkaline dissociation of the immobilized enzyme	752
Azotobacter vinelandii cells	–	Adsorption onto an anionic exchange cellulose (Cellex E®)	Investigation of the nitrogenase activity of the living immobilized cells	657
Bacillus sp. cells	–	Copolymerization with acrylamide	Production of bacitracin by the living immobilized cells	658
Brevibacterium ammoniagenes cells	–	Entrapment in polyacrylamide gel	Immobilized living cells; used in column form for the continuous production of pure NADP	659

Table 7 *(cont.)*

Enzyme	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
			Active immobilized cells; investigation of kinetics and decay of fumarase activity of the immobilized cells	660
			Study of the stability and kinetics of the NAD kinase activity of the immobilized cells	661
		Entrapment in κ-carrageenan	Living immobilized cells	578
			Continuous production of L-malic acid by the immobilized cells	579
Carbonate dehydratase	4.2.1.1	Entrapment in liposomes	Inhibition of the hyperglycaemic effect of cyclic AMP by intravenous injection into mice	753
Carboxypeptidase Y	–	Reaction with agarose cyclic imidocarbonate	Deblocking of protected groups during peptide syntheses	118
		Reaction with agarose cyclic imidocarbonate or with an aminohexanoic derivative of cross-linked agarose after modification of the enzyme with periodate and 1,2-diaminoethane or glycyl-L-tyrosine; glutaraldehyde cross-linking onto concanavalin A-agarose	Investigation of methods for the immobilization of glycoproteinaceous enzymes through their carbohydrate side-chains	117
Catalase	1.11.1.6	Adsorption onto a glutaraldehyde–diaminobenzene mixture copolymerized in the presence of pumice or titania	Study of the enzymatic oxidation of D-glucose in a differential-bed loop reactor	754
		Co-immobilized with D-glucose oxidase by glutaraldehyde cross-linking onto controlled pore glass	Development of an enzyme thermistor for continuous measurement of D-glucose	123
Catharanthus roseus cells	–	Coprecipitation with calcium alginate gel	Formation of indole alkaloids from distant precursors	662

Cellulase	3.2.1.4	Radiation-induced copolymerization with 2-hydroxyethyl methacrylate at low temperatures	Investigation of method of immobilization and properties of the active immobilized enzyme	743
		Radiation-induced copolymerization with hydrophobic glass-forming monomers at low temperatures	Investigation of method of immolization	744
		Glutaraldehyde-mediated coupling to collagen fibrils	Active immobilized enzyme for hydrolysis of cellulose in a fluidized bed reactor	755
Chloridazondihydrodiol dehydrogenase	–	Adsorption onto diethylaminoethyl-cellulose; carbodi-imide-mediated reaction with carboxyhexyl-agarose	Active immobilized enzyme for preparation of a chloridazon metabolite	756
Chloroplasts	–	Entrapment in polyacrylamide gel	Study of carbon dioxide fixation by the living immobilized cells	663
		Adsorption onto diethylaminoethyl-cellulose	Study of properties of active immobilized chloroplasts	664
		Copolymerization into a film with vinyl alcohol	Formation of ATP from ADP *via* the cyclic photophosphorylation system	665
Chlorophyllase	3.1.1.14	Adsorption onto methyl-aerosil	Active immobilized enzyme	757
		Adsorption onto aminohexadecyl-, and heptyl-agarose	Investigation of the role of the molecular environment on chlorophyllase functioning during its immobilization	99
Cholesterol oxidase	1.1.3.6	Adsorption onto Amberlite® IRC-50; glutaraldehyde cross-linking onto Amberlite® XAD-7; reaction with agarose cyclic imidocarbonate	Active immobilized enzyme	119
Chymotrypsin	3.4.21.1	Glutaraldehyde-mediated reaction with a silanized ferrite support	Active immobilized enzyme	758
		Reaction with thioester-containing glass beads	Investigation of a procedure for immobilization which permits subsequent release of the enzyme	759
		Reaction with water-soluble periodate-oxidized dextran or alginate	Investigation of the effect of charged and neutral matrices on the stability of the modified enzyme	120

Table 7 *(cont.)*

Enzyme	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Glutaraldehyde-mediated reaction with aminopropyl silica gel	Resolution of *N*-acetyl-DL-phenylalanine by the active immobilized enzyme	597
		Co-casting with spiropyran propionic anhydride and collagen followed by glutaraldehyde cross-linking	Light irradiation-controlled esterification of *N*-acetyl-L-tyrosine by the active immobilized enzyme	760
		Reaction with trichloro-*sym*-triazine-derivatized cross-linked agarose	Isolation by affinity chromatography of human urinary proteinase inhibitor	454
		Adsorption or glutaraldehyde cross-linking onto nickel powders coated by controlled oxidation to nickel oxide or by deposition of titanium oxide	Development of improved non-porous magnetic supports for enzyme immobilization; investigation of stabilities of immobilized enzyme preparations	761
		Adsorption onto stretched nylon fibre	Active immobilized enzyme	762
		Carbodi-imide-mediated reaction with heparin	Active immobilized enzyme of potential therapeutic importance	565
Citrate synthase	4.1.3.28	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme	763
Citrobacter freundii cells	–	Co-casting with collagen	Immobilized whole cell-based flow type sensor for cephalosporins	666
Creatinase Creatine kinase	3.5.3.3 2.7.3.2	Adsorption onto nylon activated with triethyloxonium tetrafluoroborate; glutaraldehyde cross-linking onto a nylon–polyethyleneimine copolymer	Development of a nylon tube reactor for the analytical determination of creatinine and creatine	764
Cyclic nucleotide phosphodiesterase	3.1.4.17	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of an inhibitor of the enzyme	121
CMP-acylneuraminate synthase	–	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; synthesis of CMP-*N*-acetylneuraminic and CMP-*N*-glycoloylneuraminic acids	122
Dextranase	3.2.1.11	Reaction with 1,3-diaminobenzene-coated porous titanium(IV) oxide	Active immobilized enzyme; study of the kinetics of the immobilized enzyme	765

Digitalis lanata cells	–	Coprecipitation with calcium alginate gel	Position- and stereospecific-hydroxylation of digitoxin	662
Elastase	3.4.21.11	Glutaraldehyde cross-linking onto polyacrylamide beads	E.s.r. spectroscopic investigation of conformational changes undergone by the enzyme during immobilization	766
Escherichia coli cells	–	Entrapment in κ-carrageenan followed by glutaraldehyde cross-linking	Living immobilized cells; used in column form for the continuous conversion of ammonium fumarate into L-aspartic acid	667
		Entrapment in κ-carrageenan	Living immobilized cells	578
		Entrapment in polyacrylamide gel	Investigation of enzymatic production of L-tryptophan by the living immobilized cells	668
β-D-Fructofuranosidase	3.2.1.26	Adsorption onto Amberlite® IRA-93	Development of a new recirculating reactor system for kinetic studies of immobilized enzymes	767
		Adsorption onto hide and nylon powders	Active immobilized enzyme	768
Fumarate hydratase	4.2.1.2	Entrapment in κ-carrageenan	Living immobilized cells	578
β-D-Galactosidase	3.2.1.23	Reaction with agarose cyclic imidocarbonate	Continuous hydrolysis of lactose in a column reactor	123
		Adsorption onto tannin coupled to aminohexyl-cellulose	Active immobilized enzyme	493
		Glutaraldehyde-mediated reaction with sub-micron ferrite particles	Hydrolysis of lactose in whole milk followed by magnetic recovery of the immobilized enzyme	769
		Reaction with mercaptosuccinylated insulin using *NN'*-2-phenylene-dimaleimide	Active immobilized enzyme; study of competition in binding in an insulin-(anti-insulin antibody) reaction; enzyme-linked immunoassay of insulin	770
		Entrapment in cellulose fibres	Active immobilized enzyme	497
Glucoamylase	3.2.1.3	Adsorption onto tropocollagen followed by glutaraldehyde cross-linking	Active immobilized enzyme	771

Table 7 *(cont.)*

Enzyme	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Glutaraldehyde cross-linking onto Duolite® S761 or onto Spherosil® beads	Saccharification of liquefied barley starch	772
		Glutaraldehyde-mediated reaction with silanized alumina or alumina activated with titanium(IV) chloride	Investigation of industrial potential for conversion of cornstarch into D-glucose in a fluidized-bed immobilized enzyme reactor	773
		Adsorption or glutaraldehyde cross-linking onto activated carbon; reaction with diazotized activated carbon derivatives; carbodi-imide mediated reaction with activated carbon	Investigation of effects of pre-treatments on the structure and subsequent behaviour of the active carbon matrix as a support material for enzyme immobilization	774
		Reaction with agarose cyclic imidocarbonate or with an aminohexanoic derivative of cross-linked agarose after modification of the enzyme with periodate and 1,2-diaminoethane or glycyl-L-tyrosine	Investigation of methods for the immobilization of glycoproteinaceous enzymes through their carbohydrate side-chains	117
		Radiation-induced copolymerization with 2-hydroxyethyl methacrylate at low temperatures	Investigation of method of immobilization and properties of the active immobilized enzyme	743
		Radiation-induced copolymerization with hydrophobic glass-forming monomers at low temperatures	Investigation of method of immobilization	744
		Reaction with polyphenolic resins	Active immobilized enzyme	742
		Carbodi-imide-mediated reaction with epichlorohydrin-activated agarose previously treated with glycine	Active immobilized enzyme	503
		Carbodi-imide-mediated reaction with chitosan	Active immobilized enzyme	520

D-Glucose dehydrogenase	1.1.1.47	Entrapment in polyacrylamide gel over a platinum grid matrix	Enzyme electrode for analysis of substrate concentrations	738
		Glutaraldehyde-mediated reaction with a nylon–polyethyleneimine copolymer	Immobilized enzyme nylon tube reactor for the routine analysis of serum D-glucose	775
D-Glucose isomerase	5.3.1.18	Reaction with macroporous aminated silochrome coated with an acrolein–vinylpyridine copolymer	Investigation of properties of active immobilized enzyme	776
		Adsorption onto tannin coupled to aminohexyl-cellulose	Active immobilized enzyme	493
		Entrapment in cellulose fibres	Active immobilized enzyme	497
		Carbodi-imide-mediated reaction with chitosan	Active immobilized enzyme	520
		Reaction with polyphenolic resins	Active immobilized enzyme	742
		Glutaraldehyde cross-linked in cellulose beads	Active immobilized enzyme for conversion of high fructose syrups in a packed bed reactor	772
D-Glucose oxidase	1.1.3.4	Carbodi-imide-mediated reaction with glassy carbon electrodes	Immobilized enzyme electrode for the determination of D-glucose	777
		Glutaraldehyde-mediated reaction with alkylamine glass	Active immobilized enzyme for clinical analysis of D-glucose; used in microcolumn form as part of an Autoanalyzer continuous flow system	778
		Commercially available, immobilized on nylon tubing	Immobilized enzyme reactor for determination of D-glucose in a continuous flow analyzer	779
		Paraformaldehyde-mediated reaction in the presence of bovine serum albumin with the surface of a Teflon membrane	Enzyme electrode detector for the continuous monitoring of D-glucose	780
		Glutaraldehyde cross-linking in the presence of ovalbumin onto mycelium pellets of *Aspergillus* species	Active immobilized enzyme; used in batch and column reactors for the conversion of D-glucose into D-gluconic acid	781
		Periodate-oxidized enzyme treated with amino-organosylochrome	Active immobilized enzyme	782

Table 7 *(cont.)*

Enzyme	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Reaction with agarose cyclic imidocarbonate or with an aminohexanoyl derivative of cross-linked agarose after modification of the enzyme with periodate and 1,2-diaminoethane or glycyl-L-tyrosine	Investigation of methods for the immobilization of glycoproteinaceous enzymes through their carbohydrate side-chains	117
		Co-immobilized with catalase on glutaraldehyde-activated controlled pore glass	Active immobilized enzyme for continuous determination of D-glucose using an enzyme thermistor	123
		Carbodi-imide-mediated reaction with chitosan	Active immobilized enzyme	520
		Adsorption onto a glutaraldehyde-diaminobenzene mixture copolymerized in the presence of pumice or titania	Study of the enzymatic oxidation of D-glucose in a differential-bed loop reactor	754
D-Glucose 6-phosphate dehydrogenase	1.1.1.49	Reaction with activated glass rod	Analytical determination of D-glucose 6-phosphate using the active immobilized enzyme	783
		Commercially available co-immobilized with hexokinase inside a nylon coil	Evaluation of an analytical procedure for D-glucose determination	784
β-D-Glucuronidase	3.2.1.31	Reaction with agarose cyclic imidocarbonate	Demonstration of the existence of a rat-liver microsomal binding protein specific for β-D-glucuronidase	124
L-Glutamate decarboxylase	4.1.1.15	Reaction with a photocross-linkable prepolymer	Active immobilized enzyme for the automated analysis of L-glutamate	785
D-Glyceraldehyde 3-phosphate dehydrogenase	1.2.1.12	Adsorption onto anti-(D-glyceraldehyde 3-phosphate dehydrogenase) antibody or Fab fragments previously treated with agarose cyclic imidocarbonate	Active immobilized enzyme; dissociation of immobilized tetrameric enzyme into dimers	150

Hexokinase	2.7.1.1	Commercially available co-immobilized with D-glucose 6-phosphate dehydrogenase inside a nylon coil	Evaluation of an analytical procedure for D-glucose determination	784
		Reaction with activated glass rod	Analytical determination of D-glucose 6-phosphate using the active immobilized enzyme	783
Hydroxysteroid dehydrogenases (3α-, β-, and 20β-)	1.1.1.50, 1.1.1.51, 1.1.1.53	Reaction with agarose cyclic imidocarbonate	Active immobilized enzymes for the transformation of steroid in water–organic solvent systems	125
Kallikrein	3.4.21.8	Carbodi-imide-mediated reaction with carboxyhexyl-agarose	Study of properties of the active immobilized enzyme	126
Lactate dehydrogenase	1.1.1.28	Reaction with nylon imidate	Active immobilized enzyme	750
		Adsorption onto nylon activated with triethyloxonium tetrafluoroborate; glutaraldehyde cross-linking onto a nylon–polyethylene-imine copolymer	Development of a nylon tube reactor for the analytical determination of creatinine and creatine	764
		Reaction with nylon imidate	Enzyme nylon tube reactor for routine determination of lactate in serum	786
Lactoperoxidase	–	Reaction with phenoxyacetyl-cellulose	Active immobilized enzyme; evaluation of potential of immobilized enzyme in the iodination of small molecules	505
Lactose synthase	2.4.1.22	Reaction with glutathion-2-(5-nitropyridyl) disulphide derivate of agarose	Investigation of the active site of the immobilized enzyme	435
Luciferase	–	Reaction with activated glass rod	Analytical determination of NADH using the active immobilized enzyme	783
		No details given	Active immobilized enzyme for quantitative determination of ATP and enzymes that synthesize and destroy ATP	787
Lysozyme	3.2.1.17	Glutaraldehyde-mediated reaction with a silanized ferrite support	Active immobilized enzyme	758
		Reaction with agarose cyclic imidocarbonate; reaction with benzoquinone-activated agarose	Purification by immunoadsorption of antilysozyme antibody	108,181

Table 7 *(cont.)*

Enzyme	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
Malate dehydrogenase (halophilic and non-halophilic)	1.1.1.37	Reaction with agarose cyclic imido-carbonate	Active immobilized enzyme; comparison of the stability of the immobilized halophilic and non-halophilic enzymes at various ionic strengths	127
Monoamine oxidase	1.4.3.4	Glutaraldehyde-mediated reaction with amino-substituted glass; reaction with agarose cyclic imidocarbonate	Immobilized enzyme for determination of primary amines with an enzyme thermistor	788
Morinda citrifolia cells	–	Coprecipitation with calcium alginate	Living immobilized cells; *de novo* synthesis of anthraquinones	662
Naringinase	–	Adsorption onto tannin immobilized on aminohexyl-cellulose	Active immobilized enzyme	493
Neuraminidase	3.2.1.18	Glutaraldehyde-mediated reaction with nylon tubing	Active immobilized enzyme; investigation of its potential for the desialylation of cell surfaces	789
Nocardia rhodocrous cells	–	Co-immobilization with a hydrophobic prepolymer	Living immobilized cells	653
		Entrapment in a hydrophilic urethane polymer	Steroid transformations by the living immobilized cells	655
		Entrapment in hydrophilic and lipophilic gels	Steroid bio-conversion in water-insoluble organic solvents by the living immobilized cells	670
Papain	3.4.22.2	Reaction with polymers obtained by suspension copolymerization of methacrylate and methacryloyl type monomers	Active immobilized enzyme; comparison of the proteolytic activities of free and immobilized papain	790
		Adsorption onto tannin immobilized on aminohexyl-cellulose	Active immobilized enzyme	493

		Reaction with titanium(IV)-activated derivatives of polypropylene netting, zirconia fibre sheet, and glass fibre paper	Comparison of the properties of the active immobilized enzymes	791
Parathion hydrolase	–	Reaction with azide derivatives of controlled pore glass and silica	Active immobilized enzyme; detoxification of pesticides in production waste-waters	792
Penicillin acylase	–	Unspecified method of covalent binding to Amberlite® XAD-7	Active immobilized enzyme; deacylation of benzylpenicillin in a continuous four-stage stirred-tank reactor	793
Penicillin amidase	3.5.1.11	Copolymerization with acrylamide after maleylation of the enzyme	Investigation of properties of the active immobilized enzyme	794
		Glutaraldehyde- or carbodi-imide-mediated reaction with glycidyl methacrylate–ethylene dimethacrylate copolymers or amino-derivatives thereof	Investigation of new carriers for enzyme immobilization	795
Penicillinase	3.5.2.6	Entrapment between a glass electrode and a membrane	Investigation of design and response characteristics of an enzyme electrode for the determination of penicillin	796
		Glutaraldehyde-mediated reaction with aminoethyl-cellulose	Investigation of the kinetic properties and stability of the active immobilized enzyme	491
Penicillium chrysogenes cells	–	Copolymerization with acrylamide; cocasting with collagen followed by glutaraldehyde cross-linking; coprecipitation with calcium alginate	Living immobilized cells for the production of penicillin G from D-glucose	671
Pepsin	3.4.23.1	Glutaraldehyde or acrolein cross-linking with chitosan	Investigation of a novel matrix for enzyme immobilization	521
Peroxidase	1.11.1.7	Reaction with agarose cyclic imidocarbonate or with an aminohexanoic derivative of cross-linked agarose after modification of the enzyme with periodate and 1,2-diaminoethane or glycyl-L-tyrosine	Investigation of methods for the immobilization of glycoenzymes through their carbohydrate side-chains	117
		Reaction with diazotized poly(acryloylmorpholine)	Study of the properties of the active immobilized enzyme in organic–aqueous solvent mixtures	797

Table 7 *(cont.)*

Enzyme	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
Phenol mono-oxygenase	1.14.13.7	Entrapment in polyacrylamide gel; carbodi-imide-mediated coupling to aminohexyl-agarose or to derivatized nylon nets; adsorption onto diethyl-aminoethyl-dextran	Active immobilized enzyme; comparison of different methods of immobilization of the enzyme	798
Plasminogen	–	Glutaraldehyde-mediated reaction with aminoethyl-cellulose	Demonstration of the activation of plasminogen on immobilization by this method; the immobilized molecule has plasmin activity and is not further activated by streptokinase	799
Pseudomonas fluorescens cells	–	Co-casting with collagen followed by glutaraldehyde cross-linking	Living immobilized cells in a microbial electrode for the determination of D-glucose	672
Pseudomonas primary amine dehydrogenase	–	Reaction with agarose cyclic imido-carbonate	Active immobilized enzyme; development of a new method for spectrophotometric assay of immobilized enzymes	128
Pyruvate decarboxylase	4.1.1.1	Reaction with activated derivatives of polystyrene	Investigation of the influence of the chemical surface structures of function-alized polystyrenes on the kinetic properties of the active immobilized enzyme	800
Pyruvate kinase	2.7.1.40	Adsorption onto nylon activated with triethyloxonium tetrafluoroborate; glutaraldehyde cross-linking onto a nylon–polyethyleneimine copolymer	Development of a nylon tube reactor for the analytical determination of creatinine and creatine	764
Riboflavin kinase	2.7.1.26	Carbodi-imide-mediated reaction with aminoalkyl-agarose	Active immobilized enzyme; used in a continuous plug-flow reactor for the conversion of riboflavin into riboflavin 5′-phosphate	98

Ribonuclease	–	Glutaraldehyde-mediated reaction with a silanized ferrite support	Active immobilized enzyme	758
		Glutaraldehyde-mediated reaction with porous ceramic beads derivatized with titanium oxide	Active immobilized enzyme	801
Saccharomyces cerevisiae cells	–	Entrapment in polyacrylamide gel	Continuous production of glutathione by the living immobilized cells	673
		Entrapment in a derivative of poly(ethylene glycol hydroxyethylacrylate)	Living immobilized cells; continuous production of ATP from adenosine in a reactor employing sheets of the immobilized cells	674
Saccharomyces paradoxus cells	–	Glutaraldehyde-mediated reaction with glycyl-, β-alanyl-, and 6-aminohexanoyl-derivatives of 1,6-diaminohexane-derivatized hydroxyalkylmethacrylate gel	Living immobilized cells; investigation of the potential of the gels with particular reference to spacer arm length for cell immobilization	675
Saccharomyces uvarum cells	–	Co-precipitation with calcium alginate	Investigation of potential of alginate gels for cell immobilization	457
Streptokinase	–	Reaction with partially acid-hydrolyzed nylon	Investigation of the fibrinolytic effects *in vivo* (in rabbits) after subcutaneous implantation of the active immobilized enzyme	802
Streptomyces griseus neutral proteinase (Pronase)	3.4.24.4	Reaction with silylated sylochrome activated with cyanuric chloride, 2,4-toluene di-isocyanate, or glutaraldehyde; reaction with sylochrome activated with titanium(IV) chloride	Active immobilized enzyme; comparison of the stabilities and activities *versus* high and low molecular weight substrates of the different immobilized preparations	803
Streptomyces phaeochromogenes cells	–	Entrapment in κ-carrageenan	Living immobilized cells	578
		Copolymerization with 2-hydroxyethyl-methacrylate at low temperatures	Investigation of properties of the living immobilized cells	676, 677, 678
Thrombin	3.4.21.5	Reaction with agarose cyclic imidocarbonate	Affinity chromatographic fractionation of heparin	129

Table 7 *(cont.)*

Enzyme	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
Thylakoids		Glutaraldehyde cross-linking with albumin at sub-zero temperatures	Attempted stabilization of chloroplast membrane functions (*i.e. in vitro* photochemical activity of thylakoids) by immobilization	804
Trichosporon brassicae cells	–	Adsorption onto a porous cellulose acetate membrane	Living immobilized cells; used in a microbial sensor for amperometric determination of acetic acid	679
Trypsin	3.4.21.4	Carbodi-imide-mediated reaction with chitosan	Active immobilized enzyme	520
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; activation of plasminogen	130
		Reaction with agarose cyclic imidocarbonate and then incorporated into agarose gel slabs	Electrophoretic method for the quantitation of trypsin inhibitors	131
		Reaction with dextran cyclic imidocarbonate; reaction with glass; reaction with 4-aminobenzylcellulose	Investigation of effect of immobilization on the conformation and folding of trypsin	132
		Adsorption onto tannin coupled to aminohexyl-cellulose	Active immobilized enzyme	493
		Glutaraldehyde-mediated reaction with a silanized ferrite support	Active immobilized enzyme	758
		Adsorption onto stretched nylon fibre	Active immobilized enzyme	762
		Reaction with agarose cyclic imidocarbonate	Affinity chromatographic purification of trypsin inhibitors from *Psophocarpus tetragonolobus*	805
		Adsorption onto polyurethane	Investigation of effect of immobilized enzyme on the growth of rat fibroblasts *in vitro*	806

		Cross-linking onto agarose using ethylene glycolyl bis(succinimidyl succinate)	Active immobilized enzyme; assessment of a new bifunctional reagent for the cross-linking and reversible immobilization of proteins through their amine groups	807
		Reaction with an organo-silica support previously activated with cyanuric chloride or maleic anhydride	Active immobilized enzyme	808
		Reaction with an activated polyurethane matrix	Active immobilized enzyme	809
		Reaction with periodate-oxidized glucose previously coupled to a hydroxyalkyl-methacrylate gel	Active immobilized enzyme	810
		Immobilization on Spherosil®	Active immobilized enzyme; study of the influence of coupling conditions on coupling efficiency	811
Urate oxidase	1.7.3.3	Glutaraldehyde-mediated reaction with alkylamine glass	Active immobilized enzyme; used in column form for the automated clinical analysis of uric acid	778
		Commercially available immobilized on nylon tubes in a reactor system	Determination of uric acid in a continuous flow analyser	779
		Glutaraldehyde cross-linked	Model immobilized enzyme for mathematical study of certain effects occurring in realistic immobilized enzyme systems	812
		Commercially available immobilized on activated polyamide tubing	Continuous-flow analysis of uric acid	813
Urease	3.5.1.5	Glutaraldehyde-mediated reaction with chitin	Active immobilized enzyme	513
		Carbodi-imide-mediated reaction with chitosan	Active immobilized enzyme	520
		Reaction with nylon imidate	Active immobilized enzyme	750
		Commercially available immobilized on nylon tubes in a reactor system	Determination of urea in a continuous flow analyser	779

Table 7 *(cont.)*

Enzyme	*EC No.*	*Matrix or macromolecule coupled and mode of coupling*	*Use of product*	*Ref.*
		Commercially available immobilized on activated polyamide tubing	Continuous-flow analysis of urea	813
		Reaction with inner surface of disposable pipette tips made of alkylated nylon tubing	Routine determination of blood and urine urea	814, 815
		Microencapsulation by interfacial polymerization with 1,6-diaminohexane and sebacoyl chloride	Active immobilized enzyme; investigation of optimal conditions for microencapsulation	816
		Entrapment in polyacrylamide gel	Active immobilized enzyme; study of the characteristics and overall reaction rate of unbuffered gel-immobilized urease particles	817
		Entrapment in polyacrylamide gel	Active immobilized enzyme; theoretical treatment of and experimental results for the use of the immobilized enzyme in a packed-bed differential recycle reactor	818
		Co-casting with gelatin onto a polyethylene film	Elucidation of the effects of mass transfer and immobilization on the apparent kinetics of urea hydrolysis by the active immobilized enzyme	819
		Glutaraldehyde cross-linking with albumin onto a nylon grating	Investigation of the effects of an electric field on the activity of the immobilized enzyme membrane	820
		Adsorption onto aluminium hydroxide	Investigation of this immobilization technique as a possible method for determination of enzyme structures	821
		Reaction with diazotized derivatized nylon filter fabric disks	Multilayer immobilized enzyme filter reactor; study of kinetic and other reactor properties	822

Xanthine dehydrogenase	1.2.1.37	Adsorption onto *n*-octylamine-agarose	Active immobilized enzyme; investigation of kinetics and stability of the preparation	823
Yeast cells	–	Glutaraldehyde-mediated reaction with a derivatized hydroxyalkylmethacrylate gel	Living immobilized cells; comparison of behaviour of permeabilized and non-permeabilized cells on immobilization	680
		Entrapment in calcium alginate gel	Physical studies on the mechanical stability of columns of calcium alginate gel pellets containing living microbial cells	824
Yeast microbodies		Entrapment in resins, and in glutaraldehyde cross-linked albumin gels	Study of properties of immobilized yeast microbody enzymes	825

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The following information on immobilized glycoside and polysaccharide hydrolases and carbohydrate isomerases and oxidases is worthy of note. α-Amylase, glucoamylase, and D-glucose isomerase have been immobilized by adsorption onto new matrices synthesized by copolymerization of hydroquinone and other phenolic compounds with formaldehyde or glutaraldehyde.[742] The immobilized enzymes retain their activity in continuous use and high flow rates may be achieved in packed bed reactors, as the resin matrices are highly porous and hydrophilic.

The immobilization of α-amylase, β-D-galactosidase, and D-glucose isomerase by adsorption onto gallotannin coupled to aminohexyl-cellulose has been described.[493] Conditions of the adsorption process studied included time of contact, protein concentration, salt concentration, pH, temperature, and buffer used.

The influence of charged matrix surfaces (*sym*-trichlorotriazine-activated anion-exchange polystyrene, 1,4-benzoquinone-activated aminopropyl-silica, and *N*-hydroxysuccinimide-derivatized 3-succinimido-propyl-silica) on the thermostabilizing effect of Ca^{2+} ions on immobilized α-amylase has been investigated.[746] The immobilized enzyme preparations had lower thermostability than the soluble enzyme but with increasing Ca^{2+} concentration the immobilized preparation lost activity more slowly than the soluble preparations.

The immobilization of α-amylase and glucoamylase on epichlorohydrin-activated cellulose has been investigated.[503]

α-Amylase, cellulase, glucoamylase, D-glucose oxidase, and α-D-glucosidase have been immobilized by radiation-induced polymerization of hydrophilic[743] and hydrophobic[744] glass-forming monomers at low temperatures. The methods of immobilization were investigated and the properties of the active immobilized enzymes studied. By performing the polymerization at temperatures less than 0 °C retention of enzymic activity was considerably improved, 100% retention of activity being observed at −78 and −196 °C.

Cellulase has been stabilized to an unusual extent by reaction with collagen fibrils.[755] No leakage of cellulase from the matrix was observed.

Dextranase has been immobilized by adsorption onto porous titanium(IV) oxide particles coated with diazotized 1,3-diaminobenzene.[765] Investigations of the effects of changes in dextran concentration, pH, temperature, and flow rate upon a continuously operated column of the immobilized dextranase permitted assessment of the kinetic aspects of the system *via* Lineweaver–Burk plots.

Silanized and glutaraldehyde-activated sub-micron ferrite particles have been used to immobilize β-D-galactosidase.[769] The immobilized enzyme was used for the hydrolysis of lactose in whole milk, from which the enzyme is readily recovered magnetically. The immobilized β-D-galactosidase is of potential industrial importance: hydrolysis improves some processed dairy products and there are also nutritional products requiring modification for people with lactose intolerance.

Glucoamylase immobilized with a glutaraldehyde-mediator on either phenol–formaldehyde resin or on silanized silica has been applied successfully to the saccharification of liquefied barley starch to obtain glucose syrups of up to DE 96.[772] D-Glucose isomerase entrapped in cellulose beads and then glutaralde-

hyde cross-linked was also prepared for conversion of the D-glucose syrup into a high D-fructose-content syrup in a packed bed reactor.

After initial periodate oxidation of the carbohydrate portions of glucoamylase and D-glucose oxidase, 1,2-diaminoethane, or glycyl-L-tyrosine groups have been introduced into the glycoproteinaceous enzymes by reductive amination.[117] The modified enzymes were then coupled either to an aminohexanoate adduct of cross-linked agarose *via* an *N*-hydroxysuccinimide ester or to agarose cyclic imidocarbonate, both with good retention of enzymic activity.

Active derivatives of glucoamylase immobilized on activated carbon have been prepared by adsorption, glutaraldehyde cross-linking, or covalent attachment by reaction with carbodi-imide-activated or diazotized activated carbon.[774] The effects of a number of pretreatments of the carbon matrix on the efficacy of immobilization were investigated.

Glucoamylase covalently immobilized by reaction with glutaraldehyde-activated silanized alumina or by glutaraldehyde cross-linking onto alumina activated with titanium(IV) chloride has been used in a fluidized bed reactor for the economical conversion of cornstarch into D-glucose.[773] Higher yields of D-glucose were obtained than with a comparable fixed bed reactor as fluidization permitted use of very small catalytic particles (down to 50 μm), thus overcoming yield-limiting diffusion-associated problems in conventional fixed bed reactors.

The thermostability of glucoamylase is increased by immobilization on inorganic supports.[826] The thermostability that may be achieved is restricted by an upper limit independent of the method of immobilization and is chiefly determined by the character of the enzyme–substrate complex formed. Glucoamylase is the only enzyme for which such a limit has thus far been demonstrated, but its existence has important implications both theoretically and industrially.

D-Glucose oxidase has been immobilized for use in a number of analytical systems. Immobilization of the enzyme onto nonporous glassy carbon electrodes by carbodi-imide-mediated coupling to superficial oxides generated by anodic oxidation has afforded an immobilized enzyme electrode with which hydrogen peroxide released enzymically from D-glucose may be measured amperometrically.[777] Properties of the enzyme immobilized on amino-organo-sylochrome have been studied.[782]

By glutaraldehyde cross-linking of the enzyme in the presence of ovalbumin, D-glucose oxidase has been immobilized on mycelium pellets of *Aspergillus* species.[781] The preparation was successfully used in batch and column reactors for conversion of D-glucose to D-gluconic acid.

D-Glucose in serum has been determined (a) using microcolumns of D-glucose oxidase immobilized by glutaraldehyde-mediated reaction with alkylamine glass in an autoanalyser continuous flow system,[778] (b) using D-glucose oxidase immobilized in commercially available reactors consisting of plastic cartridges containing spirally wound nylon tubes, on the inner walls of which the enzyme is immobilized by covalent binding,[779] and (c) using an oxygen electrode coated with a Teflon membrane on which D-glucose oxidase is immobilized by

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covalent paraformaldehyde-mediated bonding in the presence of bovine serum albumin.[780]

D-Glucose oxidase co-immobilized with catalase by glutaraldehyde-mediated reaction with alkylamine-glass has been used in an enzyme thermistor for continuous measurement of D-glucose in the effluent from a column reactor.[123] The two enzymes have also been co-immobilized by adsorption onto a mixture of glutaraldehyde and diaminobenzene copolymerized in the presence of pumice and titanium oxide carrier particles.[754] The enzymatic oxidation of D-glucose was then studied in a differential-bed loop reactor. Deactivation of D-glucose oxidase was found to remain small in the presence of excess immobilized catalase. A mathematical model was developed to describe the experimental results.

The evaluation of a procedure for D-glucose determination using hexokinase and D-glucose 6-phosphate dehydrogenase co-immobilized inside a nylon tube has been reported.[784] The practical and economic feasibility of the system for use in a small laboratory was assessed.

Lysozyme has been immobilized by reaction with a glutaraldehyde-activated silanized ferrite support to yield an active immobilized enzyme readily recoverable magnetically from reaction solutions.[758]

The inner surface of nylon tubing has been activated with dimethylsulphate and then treated with L-lysine.[789] After activation of the derivatized surface with glutaraldehyde or dimethyl adipimidate, neuraminidase was covalently linked to the surface of the tubing. The stability of the active immobilized enzyme was investigated and its potential application for release of neuraminic acid from surfaces of leukaemic AKR mouse thymus and spleen lymphocytes evaluated.

Errata

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Page 243. Overhead arrows should not appear in structures (2)–(5).

Page 294. Structure (1)
Page 346. Structures (33) and (35)
Page 356. Structures (46) and (47)
Page 369. Structure (65)
Page 370. Line 14

for Neu*f* *read* Neu*p*.

Page 368. In top line of structure *for* Man*f* *read* Man*p*.

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