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Volume 6

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Senior Reporter

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Organic formulae composed by Wright's Symbolset method

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Preface

This Report, the sixth in the series, covers the literature available to us between mid-January 1972 and mid-January 1973.

A notable feature of these Reports over the past two years has been the substantial expansion of the material covered in Part II. Opinions solicited from a number of readers suggest that the present format, dealing with monosaccharides *etc.* and macromolecules under one cover, is generally the most acceptable and useful. The Reporters would, however, welcome any suggestions aimed at the improvement of these Reports.

As has been our policy in previous years, *Abstracts of the American Chemical Society Meetings*, *Dissertation Abstracts*, and the patent literature have not been abstracted. The abbreviation 'Bn' is again used throughout to denote the benzyl group.

We are indebted once again to Professor N. K. Kochetkov for providing us with English abstracts of a large number of Russian papers and to Drs. L. W. Doner, N. A. Hughes, and L. C. N. Tucker for reading and commenting on the whole of Part I. Miss Moira Endersby typed considerable proportions of this Report.

This is the last Report for which we will have the benefit of the expert reporting of Dr. T. D. Inch. Thanks are due to him for his excellent contributions to these Reports over the past four years.

April 1973 J. S. B.

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Abbreviations

The following abbreviations have been used:

ATP	adenosine triphosphate
Bn	benzyl
c.d.	circular dichroism
CDP	cytidine diphosphate
CMP	cytidine monophosphate
DCC	dicyclohexylcarbodi-imide
DEAE	diethylaminoethyl
DMF	*NN*-dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dpm	dipivaloylmethane
e.s.r.	electron spin resonance
g.l.c.	gas–liquid chromatography
HMPT	hexamethylphosphortriamide
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
t.l.c.	thin-layer chromatography
TMS	trimethylsilyl
UDP	uridine diphosphate

Part I

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

By
J .S. Brimacombe
R. J. Ferrier
R. D. Guthrie
T. D. Inch

1
Introduction

The general terms of reference remain those set out in the Introduction to Volume 1 (Vol. 1, p. 3) and the arrangement of subject matter follows that of previous Reports.

It is not easy to pick out any significant trends in a year that has seen much satisfactory progress and activity on a number of fronts. As has been pointed out to us, it is unwise to generalize about the state of a subject over a period as short as a year. There have been a number of notable achievements during the year in the area of synthetic carbohydrate chemistry, particularly among the antibiotic substances, where full details of the total syntheses of gougerotin, blasticidin S, and kasugamycin have been made available (Chapter 20). The nucleosides, covered in Chapter 21, generally remain an area of intense activity and there has also been a marked increase in the number of papers dealing with the synthesis of branched-chain sugars (Chapter 15). The functionalization of branched chains has interested a number of groups. Another interesting feature to emerge this year is the influence that metal ions can exert on the reactions of carbohydrates. The way in which Angyal and his colleagues have harnessed this information to useful synthetic ends is reported in Chapters 3 and 18. Exciting developments can be anticipated in this area.

Recent advances in the applications of n.m.r. spectroscopy to the study of carbohydrate chemistry are covered in Chapter 23, where a number of useful and extensive reviews in this area are mentioned. It is also interesting to note the increasing frequency with which reference is made in Part II to n.m.r. methods. The application of *X*-ray crystallography in carbohydrate chemistry shows a spasmodic, but nonetheless increasing, growth and the types of structure that can now be resolved by direct methods become increasingly more complex.

The International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB) have jointly published[1] tentative rules dealing with the nomenclature of carbohydrates (*cf.* Vol. 5, p. 3).

[1] *Biochem. J.*, 1971, **125**, 673.

Several books of general interest have appeared [2] and the life and scientific works of Michael Tswett – the founder of chromatography – have been reviewed to mark the centenary of his birth.[3]

An obituary in Volume 27 (1972) of *Advances in Carbohydrate Chemistry and Biochemistry* has paid tribute to Professor W. W. Zorbach (1916—1970).

The April [4] and October [5] issues of *Carbohydrate Research* were dedicated to Professors M. Stacey and J. E. Courtois, respectively, in honour of their sixty-fifth birthdays.

[2] R. J. Ferrier and P. M. Collins, 'Monosaccharide Chemistry', Penguin Library of Physical Sciences, 1972; J. Yudin, J. Edelman, and L. Hough, 'Sugar–Chemical, Biological, and Nutritional Aspects of Sucrose,' Butterworths, London, 1971; 'The Carbohydrates. Chemistry and Biochemistry', ed. W. Pigman and D. Horton, Academic Press, 1972, 2nd edn., vol. 1A; 'Methods in Carbohydrate Chemistry', ed. R. L. Whistler and J. N. BeMiller, Academic Press, 1972, vol. 6.

[3] *J. Chromatog.*, 1972, **73**, 303.

[4] *Carbohydrate Res.*, 1972, vol. 22.

[5] *Carbohydrate Res.*, 1972, vol. 24.

2
Free Sugars

The effects of ionizing radiation on carbohydrates have been reviewed.[6]

An automated method has been developed for the separation of neutral monosaccharides, which is based on ion-exchange chromatography of sugar borate complexes at pH 7. Mixtures of trehalose, cellobiose, L-rhamnose, D-ribose, D-mannose, L-arabinose, D-galactose, D-xylose, and D-glucose were resolved easily.[7] The separation of sugars on polyamide layers was shown to depend on the number of and the distance between peptide groups in the macromolecule. It is possible to compare and determine the nature of the polyamide used from the sugar R_f values.[8]

Isolation and Synthesis

L-Rhamnose has been identified as the sugar component in a new microbial metabolite of phosphoramidon.[9] Fructose, sorbose, glucose, sucrose, inositol, and mannitol were among the products identified by g.l.c. of silylated derivatives of cocoa-bean extracts from many sources.[10]

Most of the arabinose obtained by acid treatment of soil has the L-configuration, indicating that it probably originates in plants.[11]

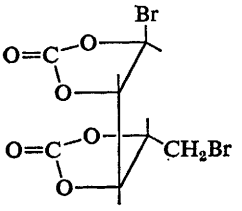
Methanolysis has been assessed as a procedure for releasing free sugars, acetamidohexoses, and uronic acids from sugar-containing natural products.[12] Monosaccharides were found to be stable for 24 h in 2M-methanolic hydrogen chloride at 100 °C, but underwent decomposition under these conditions in 4M-methanolic hydrogen chloride. Glycopeptides and oligosaccharides released their carbohydrates within 3 h in 1M-methanolic hydrogen chloride at 85 °C. Various factors relating to analytical determination of the released sugars were discussed.

Total syntheses of DL-erythrose and DL-threose,[13] dihydroxyacetone and

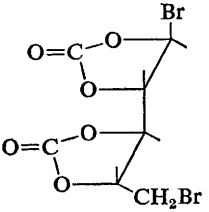
[6] G. O. Phillips, *Radiation Res. Rev.*, 1972, **3**, 335.
[7] L. Hough, J. V. S. Jones, and P. Wusteman, *Carbohydrate Res.*, 1972, **21**, 9.
[8] I. Grafe and H. Engelhardt, *Chromatographia*, 1972, **5**, 307.
[9] H. Umezawa, *Tetrahedron Letters*, 1972, 97.
[10] G. A. Reineccius, D. A. Andersen, T. E. Kavanagh, and P. G. Keeney, *J. Agric. Food Chem.*, 1972, **20**, 199.
[11] M. V. Cheshire and S. J. Thompson, *Biochem. J.*, 1972, **129**, 19P.
[12] R. E. Chambers and J. R. Clamp, *Biochem. J.*, 1971, **125**, 1009.
[13] K. Sonogashira and M. Nakagawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 2616.

DL-erythrulose,[13a] DL-glyceraldehyde,[14] and 2-deoxy-DL-, L-, and D-*erythro*-pentoses[14, 15] have been reported. The effects of monosaccharides[16] and lead oxide[17] as catalysts on carbohydrate-forming condensations of formaldehyde have been studied.

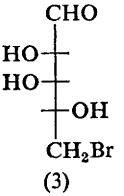
Cyclic carbonates such as (1) and (2), which were formed by telomerization of vinylene carbonate with polyhalogenomethanes, have been converted by treatment with acid into derivatives of DL-lyxose (3) and DL-xylose (4).[18]



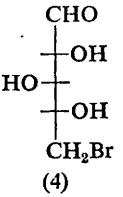
(1)



(2)



(3)



(4)

D-Talose has been obtained, in 90% yield, by hydroxylation of D-galactal with hydrogen peroxide in the presence of sodium molybdate.[19] Deamination of 2-amino-2-deoxy-D-mannopyranose with sodium nitrite in aqueous acetic acid at 0 °C afforded D-glucose in 72% yield.[20]

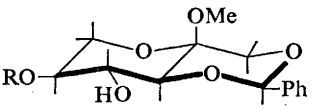
Acetolysis of methyl D-riboside derivatives afforded mixtures of D-ribose and D-arabinose acetates in a ratio of 4 : 1. Similar acetolysis of methyl D-lyxoside derivatives afforded mixtures of acetylated D-lyxose and D-xylose in a ratio of 5 : 2. Epimerization of D-glucose, D-xylose, and D-arabinose derivatives did not occur.[21]

The enzymic synthesis of lactose labelled with ^{14}C in the D-galactose residue and with ^{3}H in the D-glucose residue has been described.[22] '2-Deoxy-D-[1-^{14}C]ribose' has been synthesized by standard procedures.[23]

[13a] T. Ando, S. Shioi, and M. Nakagawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 2611.
[14] K. Mimaki, M. Masunari, G. Nakaminami, and M. Nakagawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 2620.
[15] G. Nakaminami, S. Shioi, Y. Sugiyama, S. Isemura, M. Shibuya, and M. Nakagawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 2624.
[16] V. A. Uspenskaya, O. V. Krylov, and Yu. E. Sinyak, *Kosm. Biol. Med.*, 1971, **5**, 9.
[17] A. N. Surzhenko and T. P. Kornienko, *Katal. Katal.*, 1971, 81 (*Chem. Abs.*, 1972, **76**, 25494).
[18] T. Tamura, T. Kunieda, and T. Takizawa, *Tetrahedron Letters*, 1972, 2219.
[19] V. Bilik, *Chem. Zvesti.*, 1972, **26**, 76.
[20] D. Horton, K. D. Philips, and J. Defaye, *Carbohydrate Res.*, 1972, **21**, 417.
[21] G. J. F. Chittenden, *Carbohydrate Res.*, 1972, **22**, 491.
[22] B. G. Hudson, L. McKenzie, and K. E. Ebner, *Analyt. Biochem.*, 1972, **48**, 524.
[23] V. Soukupova and K. Veres, *J. Labelled Compounds*, 1971, **7**, 213.

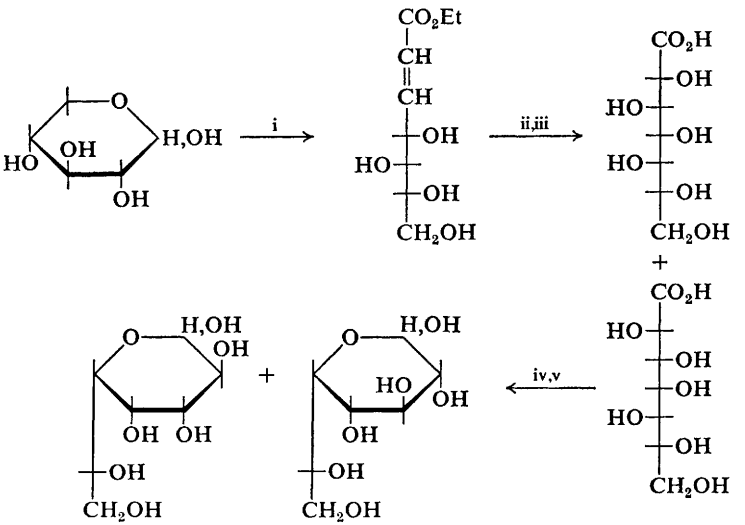
A large-scale preparation of D-allose has been described.[24] The key steps involving an oxidation–reduction sequence on 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose were investigated in detail. The oxidant of choice was ruthenium dioxide–potassium periodate, while sodium borohydride, lithium aluminium hydride, and sodium bis-(2-methoxyethoxy)aluminium hydride all gave essentially pure D-*allo*-isomer in the reduction stage.

L-Psicose has been prepared by selective tosylation of methyl 1,3-*O*-benzylidene-α-L-sorbopyranoside (5) to give (6), which was then oxidized



(5) R = H
(6) R = Ts

and reduced at C-4, desulphonylated with lithium aluminium hydride, and acid hydrolysed.[25] A new synthesis of D-tagatose has been developed[26] (see Chapter 13).



Reagents: i, $Ph_3P{=}CHCO_2Et$; ii, OsO_4–$HClO_4$; iii, KOH; iv, $(CF_3CO)_2O$; v, Na–Hg.

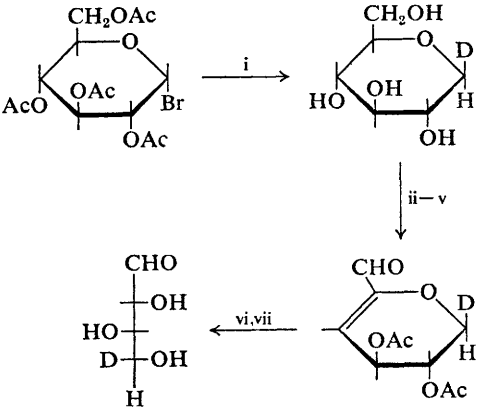
Scheme 1

[24] D. C. Baker, D. Horton, and C. G. Tindall, jun., *Carbohydrate Res.*, 1972, **24**, 192.
[25] A. Armenakian, M. Mahmood, and D. Murphy, *J.C.S. Perkin I*, 1972, 63.
[26] K. James and S. J. Angyal, *Austral. J. Chem.*, 1972, **25**, 1967.

D-*Glycero*-L-*galacto*- and D-*glycero*-L-*ido*-heptoses have been synthesized from D-xylose as illustrated in Scheme 1.[27] D-*Glycero*-D-*allo*-heptose has also been prepared.[28]

Following an earlier report (Vol. 5, p. 156) on the preparation of 2-*O*-methyl-D-arabinose by degradation of 3-*O*-methyl-D-glucose with silver carbonate on Celite, it has now been shown that tetroses can be similarly prepared from readily available pentoses.[29] L-Threose has been prepared, in 40% yield, as its isopropylidene derivative following oxidation of L-sorbose with silver carbonate on Celite.[30]

L-(4*S*)[4-^{2}H]Threose has been synthesized stereospecifically as illustrated in Scheme 2.[31]



Reagents: i, $LiAlD_4$; ii, TrCl–py; iii, Ac_2O–py; iv, H^+; v, DMSO–SO_3–py–Et_3N; vi, O_3; vii, HCO_3^-.

Scheme 2

Acetolysis of 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose with acetic acid, acetic anhydride, and sulphuric acid occurred with partial inversion of configuration at C-2 and provided a means of obtaining D-altrose (D-altrose : D-allose ratio 1.4 : 1) following deacetylation of the products. The method was essentially confirmed as a small-scale procedure, since a chromatographic separation of the sugars was necessary.[32] It had been shown previously that certain aldoses undergo epimerization under these conditions, but that D-allose only gives a trace of D-altrose. Presumably, the reaction is a further example of an epimerization in a vicinal, cyclic trihydroxy-system in which the steric relation is *cis*, *trans*.

[27] B. A. Dmitriev, A. Ya. Chernyak, and N. K. Kochetkov, *Zhur. obshchei Khim.*, 1971, **41**, 2754.
[28] B. A. Dmitriev, A. Ya. Chernyak, O. S. Chizhov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1972, 2298.
[29] S. Morgenlie, *Acta Chem. Scand.*, 1972, **26**, 1709.
[30] S. Morgenlie, *Acta Chem. Scand.*, 1972, **26**, 2146.
[31] A. Maradufu, D. M. Mackie, and A. S. Perlin, *Canad. J. Chem.*, 1972, **50**, 2617.
[32] W. Sowa, *Canad. J. Chem.*, 1972, **50**, 1092.

Mutarotation and Epimerization

The mutarotation of sugars has been reviewed (in Polish).[33]

Both the α- and β-furanose forms have been identified in aqueous solutions of L-arabinose. The equilibrium composition in water at 25 °C is α-pyranose 57%, β-pyranose 30.5%, α-furanose 8%, and β-furanose 4.5%. The rate constants for the approach to equilibrium appeared to be the same for all components in the tautomerization of β-L-arabinopyranose in water. Thus, the complex mutarotation of β-L-arabinopyranose remains unexplained.[34]

Catalysis of the mutarotation of β-D-mannopyranose by Cu^{2+}, Co^{2+}, Fe^{2+}, Zn^{2+}, and Mn^{2+} ions has been studied.[35, 36] Evidence for metal complexes was adduced and it was suggested that the complexes mutarotate faster than the free sugar.

Ribose, arabinose, xylose, and lyxose were epimerized on heating in aqueous molybdic acid solution at 90 °C for 6 h to give an equilibrium mixture containing the aldoses, erythrose, threose, and anhydropentoses. Transitory molybdate complexes with the C-1 and C-3, or with the C-2 and C-4, hydroxy-groups underwent epimerization at C-2 and C-3, respectively. Arabinose and xylose with *trans*-related C-2 and C-3 hydroxy-groups were formed preferentially.[37] D-Glucose and D-mannose were epimerized by catalytic amounts of aqueous molybdic acid to a 3 : 1 mixture of the two sugars.[38] A mechanism involving inversion of configuration of the carbanion formed by loss of a proton from a 1,3-molybdate complex with the pyranose ring was proposed.

L-Glucose has been prepared by epimerization of either L-mannose or its phenylhydrazone in a reaction promoted by molybdate ion.[39]

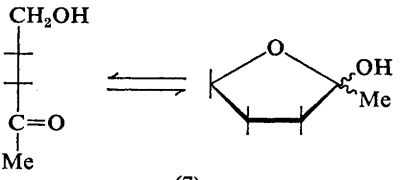
The specific reaction rate for inversion of sucrose in aqueous hydrochloric acid was determined with a reproducibility of 5% by a complexometric method designed to eliminate mutarotational lag.[39a]

A full report has appeared on a study of the mutarotation of 2,3,4,6-tetra-*O*-methyl-D-glucose, catalysed by reversed micelles in non-polar solvents. Following n.m.r. spectroscopic studies, it was suggested that strong interaction occurs between the sugar and the hydrophilic groups of the micellar surfactants (*e.g.* dodecylammonium propionate). Catalytic factors of several hundreds were observed and the relevance of these findings to enzyme studies was discussed.[40]

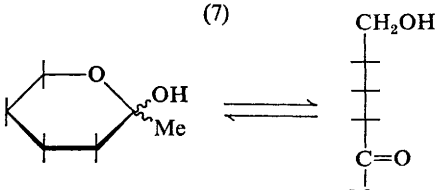
[33] K. Smiataczowa, *Wiad. Chem.*, 1971, **25**, 513.
[34] A. H. Connor and L. Anderson, *Carbohydrate Res.*, 1972, **25**, 107.
[35] R. Mitzner and E. Behrenwald, *Z. phys. Chem.* (*Leipzig*), 1972, **249**, 236.
[36] R. Mitzner and E. Behrenwald, *Z. phys. Chem.* (*Leipzig*), 1972, **249**, 56.
[37] V. Bilik, *Chem. Zvesti*, 1972, **26**, 372.
[38] V. Bilik, *Chem. Zvesti*, 1972, **26**, 183.
[39] V. Bilik, *Chem. Zvesti*, 1972, **26**, 187.
[39a] T. S. B. Narasaraju and V. L. N. Rao, *Current Sci.*, 1972, **41**, 61.
[40] J. H. Fendler, E. J. Fendler, R. T. Medary, and V. A. Woods, *J. Amer. Chem. Soc.*, 1972, **94**, 7288.

The proportions of anomeric furanoses and pyranoses present at equilibrium in deuterium oxide solutions of all the aldopentoses and aldohexoses have been determined. A valuable, detailed discussion of each of the aldohexoses marks a very significant point of sophistication in the development of sugar chemistry.[41]

5-Hydroxypentan-2-one (7) and 6-hydroxyhexan-2-one (8) were shown to be acyclic in water, but there was only a slight preference for this form in



(7)



(8)

organic solvents. Increases in temperature and solvent polarity favoured the acyclic forms of these compounds, which are models for 2-ketoses.[42]

Replacement of the C-3 hydroxy-group of L-idose by a fluorine atom had no effect on the mutarotational equilibrium.[43] The use of ^{19}F n.m.r. spectroscopy in the study of this equilibrium is described in Chapter 23. 5-Thio-α-D-glucopyranose mutarotated more rapidly than α-D-glucopyranose [44] (see Chapter 12).

Physical Measurements

A very simple kinetic model for the homogeneous oxidation of D-glucose and D-fructose in aqueous alkaline solution has been developed.[45] It involves the influence of the type and concentration of the hexose, the hydroxide ion concentration, the oxygen concentration in the liquid phase, and the temperature on the rate of formation of the acidic reaction products. In furthering this work,[46] the kinetic model was extended to cover the distribution of products. The kinetics and mechanism of cupric-ion oxidation of D-glucose in alkaline solution have been studied.[47]

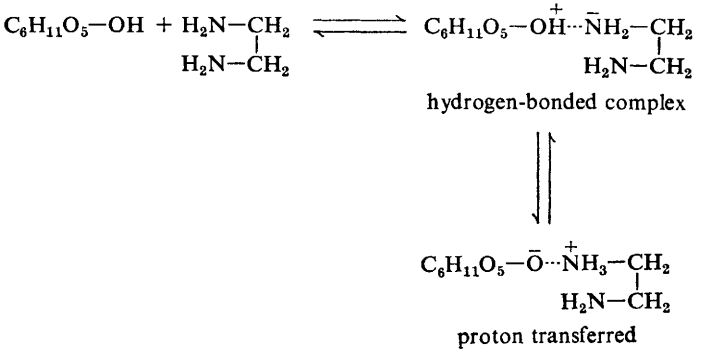
[41] S. J. Angyal and V. A. Pickles, *Austral. J. Chem.*, 1972, **25**, 1695.
[42] J. E. Whiting and J. T. Edward, *Canad. J. Chem.*, 1971, **49**, 3799.
[43] A. B. Foster, R. Hems, J. H. Westwood, and J. S. Brimacombe, *Carbohydrate Res.*, 1972, **25**, 217.
[44] M. Suzuki and R. L. Whistler, *Carbohydrate Res.*, 1972, **22**, 473.
[45] H. G. J. de Wilt and I. Lindhout, *Carbohydrate Res.*, 1972, **23**, 333.
[46] H. G. J. de Wilt and B. F. M. Kuster, *Carbohydrate Res.*, 1972, **23**, 343.
[47] U. Shanker, *Z. phys. Chem.* (*Leipzig*), 1972, **249**, 426.

The decomposition of D-glucose and D-xylose in dilute sulphuric acid solution at 160—200 °C has been studied kinetically.[48]

The electronic charge distributions in mono-, di-, and poly-saccharides have been calculated [49] following the LCAO–MO method of Del Re, and a theoretical study of the vibrational spectra of α-D-glucose has been made by normal-co-ordinate analysis.[50] The positional vibrational frequencies were compared with those observed in the i.r. and Raman spectra of α-D-glucose both as a crystalline solid and in aqueous solution. The overall agreement between the observed and calculated spectra was considered to be satisfactory.

A study of the fluorescence of 'sweet sensitive' protein and its sugar complexes has shown that sucrose gives a different fluorescence pattern to that of D-glucose. It was suggested that mono- and di-saccharides interact with the protein at different sites.[51]

Ethylenediamine forms hydrogen-bonded complexes with D-glucose, D-mannose, D-galactose, 2-amino-2-deoxy-D-glucose, and maltose [52] (see Scheme 3). The formation of complexes was established from physico-



Scheme 3

chemical studies such as paper chromatography, pH titration, and u.v. spectroscopy. The spectral properties confirmed a 1 : 1 complex in each case and, where possible, the hydroxy-group at C-1 is the presumed site of amine bonding.

Aldopentoses exhibited a very weak c.d. absorption band at 290 nm in aqueous solution. The wavelength of this band is characteristic of the $n \rightarrow \pi^*$ transition of a carbonyl group, and the band was attributed to the presence of the *aldehydo*-form of the pentose in solution. The 290 nm band was not observed with aqueous solutions of D-glucose and sucrose.[53]

[48] B. N. Ershov and V. G. Kul'nevich, *Zhur. priklad. Khim.*, 1971, **44**, 2085.
[49] N. Yathindra and V. S. Rao, *Carbohydrate Res.*, 1972, **25**, 256.
[50] P. D. Vasko, J. Blackwell, and J. L. Koenig, *Carbohydrate Res.*, 1972, **23**, 407.
[51] F. R. Dastoli, *Experientia*, 1972, **28**, 387.
[52] S. P. Moulik and A. K. Mitra, *Carbohydrate Res.*, 1972, **23**, 65.
[53] R. N. Totty, J. Hudec, and L. D. Hayward, *Carbohydrate Res.*, 1972, **23**, 152.

A study has been made of the low-pressure ultrafiltration of solutions of sucrose and raffinose using anisotropic membranes.[54]

X-Ray structures of hydrated complexes of lactose, D-galactose, and *myo*-inositol with calcium bromide have been determined.[54a]

E.s.r. spectroscopy has been used to investigate the γ-radiolysis of pentoses and hexoses;[55, 56] the results indicated that C—H bond cleavage occurs at positions 1—4 of the sugar molecule.

Reactions

The mechanism of the cyanohydrin reaction of D-arabinose has been investigated and the sequence of reactions depicted in Scheme 4 was proposed.[57]

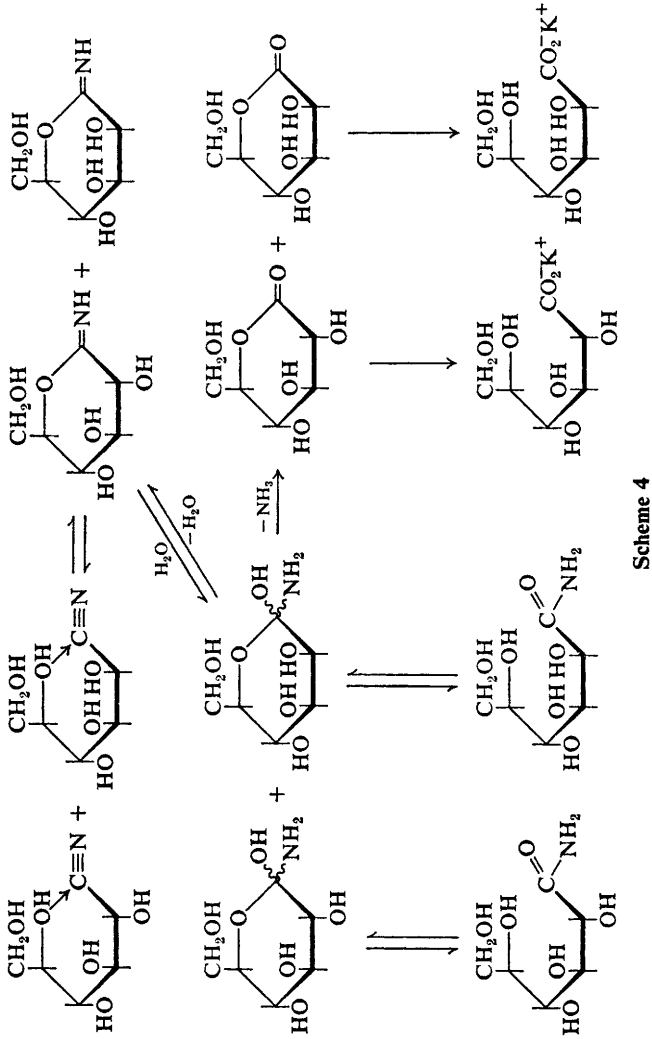
Standard procedures for the preparation of 1 : 1 adducts of D-glucose and maltose with the hydroxides of barium, calcium, and strontium have been established. It was suggested that the adducts may be useful in the isolation of oligosaccharides from industrial wastes.[58] Similar 1 : 1 adducts were formed with cellobiose, lactose, maltitol, maltose, melibiose, sucrose, and $\alpha\alpha$-trehalose.[59] One metal atom was associated with every two D-glucose (or related) residues in the adducts formed with amylose and amylopectin.

D-Fructose was converted into 2-(hydroxyacetyl)furan, in low yield, on treatment with mineral acids at elevated temperatures. D-Glucose also gave a low yield of the furan derivative, presumably by a similar reaction mechanism following its conversion into D-fructose by way of the 1,2-enediol. Experiments with D-[2-^{3}H]glucose and in tritiated, acidified water have shown that intramolecular transfer of the proton from C-2 in D-glucose to C-1 in D-fructose occurred.[60]

The mechanism of conversion of various carbohydrates (including D-xylose, D-glucuronic acid, and L-ascorbic acid) into 2-furaldehyde has been studied by measuring tritium incorporation from ^3H_2O. The 2-furaldehyde from D-xylose contained no tritium, whereas that from L-ascorbic acid contained 60% of the radiochemical activity of the solvent at the aldehyde group.[61]

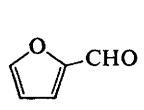
Treatment of D-glucose in dilute sulphuric acid at 100 °C for 2 h afforded less than 1% of D-fructose and D-arabinose;[62] other sugars were studied under similar conditions.

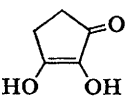
[54] R. W. Baker, F. R. Eirich, and H. Strathmann, *J. Phys. Chem.*, 1972, **76**, 238.
[54a] C. E. Bugg and W. J. Cook, *J.C.S. Chem. Comm.*, 1972, 727.
[55] I. V. Nikitin, I. V. Miroshnichenko, L. I. Kudrjashov, and M. E. Djatkina, *Doklady Akad. Nauk S.S.S.R.*, 1972, **204**, 387.
[56] I. V. Nikitin, I. V. Miroshnichenko, L. I. Kudrjashov, M. E. Djatkina, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1972, **206**, 880.
[57] R. Varma and D. French, *Carbohydrate Res.*, 1972, **25**, 71.
[58] N. Roy and A. K. Mitra, *Carbohydrate Res.*, 1972, **24**, 175.
[59] N. Roy and A. K. Mitra, *Carbohydrate Res.*, 1972, **24**, 180.
[60] D. W. Harris and M. S. Feather, *Tetrahedron Letters*, 1972, 4813.
[61] M. S. Feather, D. W. Harris, and S. B. Nichols, *J. Org. Chem.*, 1972, **37**, 1606.
[62] K. Koizumi and K. Hashimoto, *J. Pharm. Soc. Japan*, 1972, **92**, 1133.

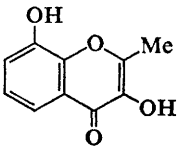


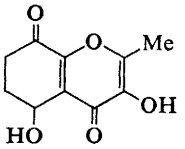
Scheme 4

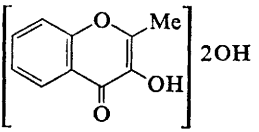
On heating at 96 °C in aqueous solution at pH 3.5 or 4.5, D-glucuronic acid afforded compounds (9)—(20) and 3-acetyl-1-oxocyclohexane-2,3,6-triol. Under these conditions, D-galacturonic acid gave similar compounds, but in lower yields. D-Xylose afforded (9), (11), (12), (14), (18),

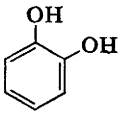

(9)

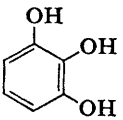

(10)

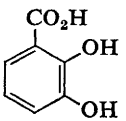

(11)

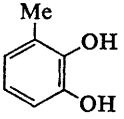

(12)

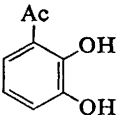

(13), (14)

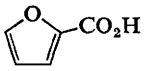

(15)


(16)


(17)


(18)

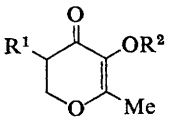

(19)


(20)

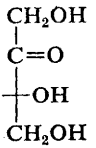
and (19), and D-arabinose afforded the same products apart from (12). It was considered likely that many of the compounds isolated from the treatment of pentoses and uronic acids under these slightly acidic conditions are intermediates in colour formation during heating and ageing of cellulosic material containing xylans and other polysaccharides possessing uronic acids and pentoses as structural units.[63]

Syntheses of acetoxydihydromaltol acetate (21) and dihydromaltol (22) have been described. These compounds were required to establish the structures of some pyrolysis products from amine–hexose systems and for studies of the non-enzymic browning of sugars.[64]

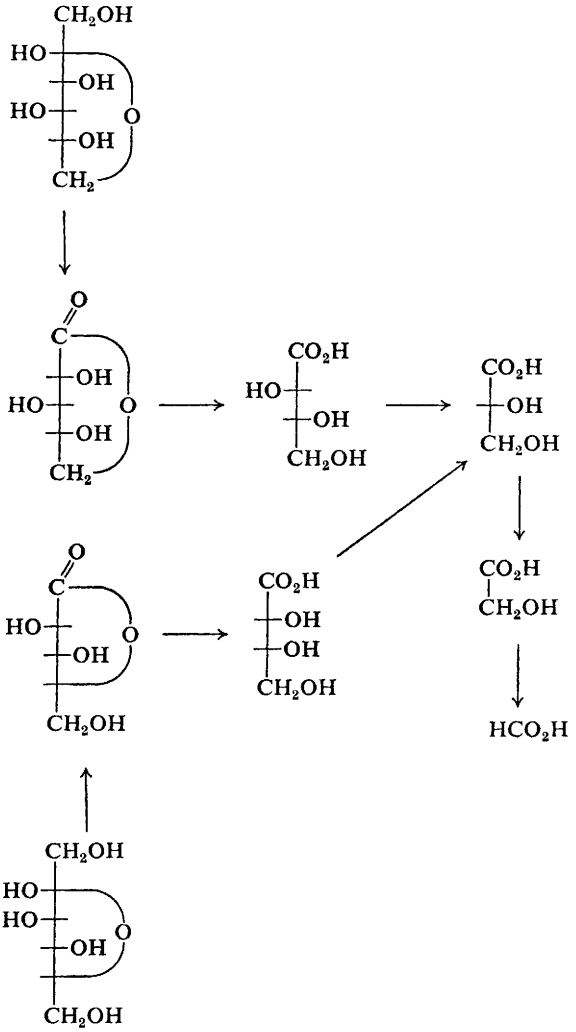
[63] T. Popoff and O. Theander, *Carbohydrate Res.*, 1972, **22**, 135.
[64] F. D. Mills, *Carbohydrate Res.*, 1972, **23**, 433.



(21) $R^1 = OAc$, $R^2 = Ac$
(22) $R^1 = R^2 = H$



(23)



Scheme 5

D-Erythrose and D-threose were both transformed into D-*glycero*-tetrulose (23) in boiling pyridine. Evidence was presented to show that only the 1,2-enediol, but not the 2,3-enediol, was formed during the rearrangement.[65]

The alkaline decompositions of D-[1-^{14}C]xylose, D-[1-^{14}C]glucose, and D-[6-^{14}C]glucose have been studied and the distribution of radioactivity in such products as the three- and four-carbon saccharinic acids, lactic acid, and 2,4-dihydroxybutyric acid was measured. The relative importance of various mechanisms for the formation of 2,4-dihydroxybutyric acid was determined. At the high alkalinity and temperature employed, recombination of two-carbon fragments was found to be an important mechanism.[66]

Details of the oxidative metabolism of D-fucose by a pseudomonad have been described in a series of four papers.[67–70] The electrochemical oxidation of sugars has been reviewed,[71] and the oxidation of hexoses and ketoses by vanadium(V) in sulphuric acid has been described.[72] For the vanadium oxidation of D-sorbose and D-fructose, the pathways illustrated in Scheme 5 were suggested.

Various pyrazine and other N-heterocycles have been detected by g.l.c. and mass spectrometry among the volatile products formed by heating D-glucose with L-alanine [73] and DL-alanine.[73a]

Transesterification of alkylborates by sugars was demonstrated to proceed without opening of sugar rings.[74]

[65] K. Linek, M. Fedoroňko, and H. S. Isbell, *Carbohydrate Res.*, 1972, **21**, 326.
[66] J. F. Harris, *Carbohydrate Res.*, 1972, **23**, 207.
[67] A. S. Dahms and R. L. Anderson, *J. Biol. Chem.*, 1972, **247**, 2222.
[68] A. S. Dahms and R. L. Anderson, *J. Biol. Chem.*, 1972, **247**, 2228.
[69] A. S. Dahms and R. L. Anderson, *J. Biol. Chem.*, 1972, **247**, 2233.
[70] A. S. Dahms and R. L. Anderson, *J. Biol. Chem.*, 1972, **247**, 2238.
[71] I. A. Avrutskaya, M. Ya. Fioshin, and A. I. Borisov, *Itogi Nauki Elektrokhim.*, 1971, **6**, 239.
[72] P. Malangeau and M. Guernet, *Carbohydrate Res.*, 1972, **24**, 499.
[73] M. Fujimaki, M. Tajima, and H. Kato, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 663.
[73a] H. Shigematsu, T. Kurata, H. Kato, and M. Fujimaki, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 1631.
[74] V. V. Gertsev and L. A. Frolova, *Doklady Akad. Nauk S.S.S.R.*, 1971, **200**, 1107.

3
Glycosides

O-Glycosides

Reviews on the chemistry of cyanogenic glycosides [75] and of sucrose [76] have appeared.

Synthesis.—Evans and Angyal have developed an interesting modification of the Fischer glycosidation process. By introducing calcium chloride, or preferably strontium chloride, into the methanolyses it was possible to prepare all four methyl D-allosides. The metal ions complexed with the products and favoured the formation of α-glycosides. Under optimal conditions, the yields of the hitherto less readily obtainable glycosides were: methyl β-D-allofuranoside, 61%; methyl α-D-allofuranoside, 68%; and methyl α-D-allopyranoside, 47%.[77]

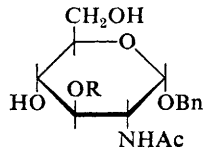
Wulff and his colleagues have continued their work on the use of silver carboxylates in the alcoholysis of acetobromo-sugars. Studies have been reported on the use of 2-, 3-, and 4-hydroxycarboxylates and 1,3- and 1,4-dicarboxylates using ether as the solvent of choice.[78] Silver 4-hydroxyvalerate was then compared with silver oxide and silver carbonate. β-Glycosides were produced as well as smaller proportions of the 1,2-orthoesters, 3,4,6-tri-*O*-acetyl-α- and -β-D-glucopyranosides, and the alcohol acetates.[79] The kinetics of the reaction between 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and alcohols in ether solution in the presence of silver carbonate and other silver salts were also reported. A notable feature of the study is the claim that the bromide and alcohol both take part in the rate-determining step of the glycosidation, whereas most previous studies have suggested that pre-ionization of the halide is the slow step in the reaction.[80] In an independent study, various 6-esterified 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromides were methanolysed with a view to assessing their use as glycosylating agents in solid-phase oligosaccharide synthesis. Whereas the 4-methoxybenzoate reacted almost

[75] R. Eyjolfsson, *Fortschr. Chem. org. Naturstoffe*, 1970, **28**, 74.
[76] L. Hough, in 'Sugar: Chemistry, Biological Nutritional Aspects of Sucrose', ed. J. Yudkin, Butterworth, 1969, p. 49.
[77] M. E. Evans and S. J. Angyal, *Carbohydrate Res.*, 1972, **25**, 43.
[78] G. Wulff, G. Röhle, and W. Krüger, *Chem. Ber.*, 1972, **105**, 1097.
[79] G. Wulff, G. Röhle, and U. Schmidt, *Chem. Ber.*, 1972, **105**, 1111.
[80] G. Wulff and G. Röhle, *Chem. Ber.*, 1972, **105**, 1122.

exclusively with inversion at C-1 to give greater than 90% of the β-glycoside, the 4-nitrobenzoyl derivative gave greater than 90% of the α-anomer. It was suggested that, in the latter case, the ester does not participate so that the carbonium ion at C-1 is solvolysed from the α-side. Alternatively, with the 4-methoxy ester, delocalization of charge occurs between C-1 and the ester carbonyl group so that the solvolysing agent is directed from the upper β-face of the molecule. It would normally be expected, however, that such an influence would protect the β-face and give rise to the 1,6-orthoester. A second explanation for this interesting observation is that specific collapse of an orthoester could take place in such a way that delivery of the alkoxy-group occurs on the β-side of C-1.[81] Other work on glycosyl halides is discussed in Chapter 7.

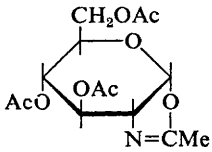
In an extension of the above work, 6-*O*-(4-nitrobenzoyl)- and 6-*O*-(4-methoxybenzoyl)-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl bromides were linked to a resin carrying allylic hydroxy-groups. High coupling yields were obtained by use of high monomer : resin ratios in the presence of small amounts of 2,6-dimethylpyridine as an acid acceptor. Sequential de-esterification and re-treatment with the glycosyl bromide gave the polymer-bound disaccharide; trisaccharide linkages were formed similarly. The completed oligomers were cleaved from the resin by ozonolysis and were obtained as glycosides of 2-hydroxyethanol.[82]

A solid-phase synthesis of 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose has been achieved by linking one glucosamine unit to a cross-linked styrene–divinylbenzene polymer to give (24) and condensing to it the second unit by use of the oxazoline (25).[83]



R = polymer—CO—

(24)



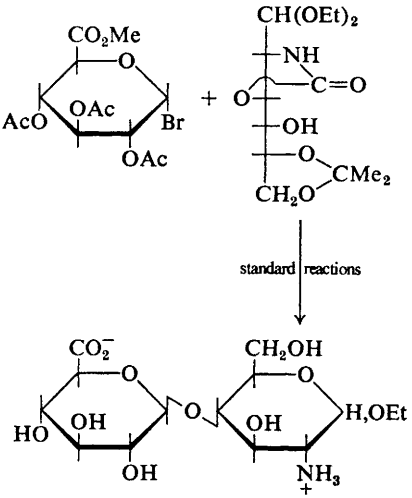
(25)

Specific applications of the Koenigs–Knorr reaction have led to the synthesis of 2-nitro-4-azidophenyl glycosides of β-D-galactopyranose, β-D-glucopyranose, β-cellobiose, and 1-thio-β-D-galactopyranose (for use as affinity labelling agents),[83a] various β-D-glucosyl derivatives of quercetin,[84] mono- and di-acetylenic alcohols,[85] and salicylanilide.[86]

[81] J. M. Fréchet and C. Schuerch, *J. Amer. Chem. Soc.*, 1972, **94**, 604.
[82] J. M. Fréchet and C. Schuerch, *Carbohydrate Res.*, 1972, **22**, 399.
[83] G. Exoffier, D. Gagnaire, J. P. Utille, and M. Vignon, *Tetrahedron Letters*, 1972, 5065.
[83a] M. B. Perry and L. L. W. Heung, *Canad. J. Biochem.*, 1972, **50**, 510.
[84] L. Farkas, B. Vermes, and M. Nógrádi, *Chem. Ber.*, 1972, **105**, 3505.
[85] R. Mestres, A. Gonzalez, and C. Santamaria, *Carbohydrate Res.*, 1972, **22**, 457.
[86] B. N. Stepanenko and L. V. Prohorova, *Doklady Akad. Nauk S.S.S.R.*, 1972, **202**, 611.

Similarly, 17α-oestradiol-17-β-D-galactopyranoside and oestriol-16α-β-D-glucopyranoside have been prepared as reference materials for characterizing biochemically produced steroidal glycosides.[87] The tetragalactoside of gossipol has been obtained.[88]

Considerable attention was still given to the application of the Koenigs–Knorr reaction to oligosaccharide synthesis. Disaccharides and derivatives that have been prepared by standard use of the method include: 6-*O*-(4-*O*-methyl-β-D-glucopyranosyl)-D-galactose,[89] 4-*O*-β-D-glucopyranosyl-L-rhamnose,[90] 2-*O*-α-L-rhamnopyranosyl-D-galactose (some flavonoid glycosidic derivatives were prepared from this compound),[91] 4-nitrophenyl 6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranoside,[92] and D-fructofuranosyl D-fructofuranoside octabenzoate.[93] Amino-sugar disaccharides continue to command attention: 2-acetamido-2-deoxy-6-*O*-α-L-fucopyranosyl-D-glucose[94] and methyl 2-amino-2-deoxy-6-*O*-α- and β-L-mycarosyl-β-D-glucopyranosides[95] have been reported. Ethyl 2-amino-2-deoxy-4-*O*-β-D-glucuronopyranosyl-αβ-D-glucopyranoside was prepared as shown in Scheme 6,[96] and 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyluronic

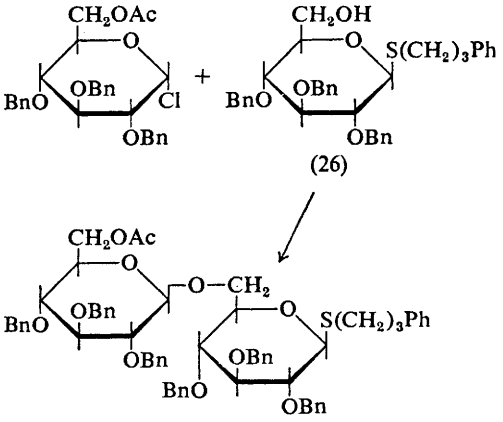


Scheme 6

[87] D. G. Williamson, D. S. Layne, and D. C. Collins, *J. Biol. Chem.*, 1972, **247**, 3286.
[88] N. I. Baram, M. N. Uldasheva, A. I. Ismailov, and A. S. Sadykov, *Khim. prirod. Soedinenii*, 1972, 126.
[89] J. M. Berry and G. G. S. Dutton, *Canad. J. Chem.*, 1972, **50**, 1424.
[90] G. M. Bebault and G. G. S. Dutton, *Canad. J. Chem.*, 1972, **50**, 3373.
[91] D. M. van Niekerk and B. H. Koeppen, *Experientia*, 1972, **28**, 123.
[92] R. Bourbouze, F. Pratviel-Sosa, and F. Percheron, *Carbohydrate Res.*, 1972, **24**, 496.
[93] A. Klemer and U. Buntrock, *Tetrahedron Letters*, 1972, 2315.
[94] M. Dejter-Juszyniski and H. M. Flowers, *Carbohydrate Res.*, 1972, **23**, 41.
[95] S. Koto, K. Yago, S. Zen, S. Omura, and T. Hata, *Chem. Letters*, 1972, 731.
[96] A. Klemer and U. Kraska, *Tetrahedron Letters*, 1972, 431.

acid)-D-glucose has also been synthesized.[97] Other disaccharides containing uronic acid residues are noted in Chapter 17.

Interest in the synthesis of higher oligosaccharides is noticeably developing and, apart from the solid-support syntheses of trisaccharides mentioned above, initial studies have been conducted on thioglycosides having benzyl ether protecting groups [*e.g.* (26)] as illustrated in Scheme 7. Clearly, the



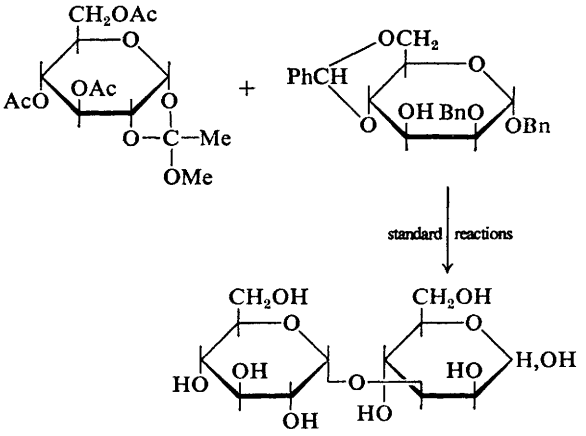
Scheme 7

product illustrated can be used for further condensations since the ester group, the benzyl ether groups, and the aglycone can be removed selectively.[98] Gentianose [*O*-β-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl β-D-fructofuranoside] has been prepared by condensation of 2,3,4,1′,3′,4′,6′-hepta-*O*-acetylsucrose with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide followed by deacetylation,[99] and the series gentiotriose through to gentiohexaose has been synthesized by reactions involving condensation of acetobromo-derivatives of appropriate mono-, di-, and tri-saccharides with di- and tri-saccharide derivatives having an unprotected hydroxy-group at C-6.[100]

Applications of the orthoester glycoside synthesis have continued to grow in number. Sterol β-D-glucosides have been prepared by this procedure and their formation was accompanied by that of the acetates of the steroidal alcohols.[101] Application of the method to 1-*O*-β-D-galactofuranosyl-D-glycerol, 2-*O*-β-D-galactofuranosyl-D-arabinitol, 3-*O*-β-D-glucopyranosyl-D-mannose, and methyl 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-α-D-

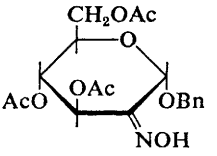
[97] R. Iwamoto and Y. Imanaga, *Carbohydrate Res.*, 1972, **24**, 133.
[98] P. J. Pfäffli, S. H. Hixson, and L. Anderson, *Carbohydrate Res.*, 1972, **23**, 195.
[99] T. Suami, T. Otake, N. Kato, T. Nishimura, and T. Ikeida, *Carbohydrate Res.*, 1972, **21**, 451.
[100] K. Takiura, S. Honda, T. Endo, and K. Kakehi, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 438.
[101] G. B. Elyakov, N. I. Uvarova, and G. I. Oshitok, *Khim.-Farm. Zhur.*, 1971, **5**, 7.

mannopyranoside has been reviewed,[102] and 3-*O*-β-D-glucopyranosyl-1,2-di-*O*-palmitylglycerol and the corresponding α-D-mannopyranosyl compound were also prepared by this method.[103, 104] In a disaccharide synthesis, 3-*O*-α-D-glucopyranosyl-D-mannose was prepared as shown in Scheme 8. It is noteworthy that, in this case, the α-linked disaccharide was

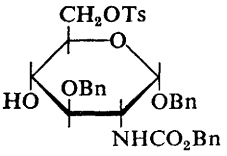


Scheme 8

obtained in addition to the expected β-linked product.[105] A better known method for obtaining α-glucosides utilized the dimeric form of 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride, which was condensed with benzyl alcohol to give the α-oximo-compound (27). However, the use of benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside inexplicably gave a mixture of anomers.[106] 2-Amino-2-deoxy-4-*O*-α-D-glucopyranuronosyl-D-glucose was obtained in related fashion by treatment of the 4-hydroxy-compound (28) with either methyl (3,4-di-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucuronosyl chloride) or 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose.[107]



(27)



(28)

[102] H. Boren, *Chem. Comm. Univ. Stockholm*, 1972, **1**, 22pp.
[103] A. I. Bashkatova, G. V. Smirnova, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1971, **7**, 1644.
[104] A. I. Bashkatova, V. N. Volynskaya, G. V. Smirnova, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1972, **8**, 543.
[105] G. Alfredsson, H. B. Borén, and P. J. Garegg, *Acta Chem. Scand.*, 1972, **26**, 2531.
[106] K. Miyai and R. W. Jeanloz, *Carbohydrate Res.*, 1972, **21**, 45.
[107] I. Kiss and P. C. Wyss, *Tetrahedron Letters*, 1972, 3055.

By the action of an α-mannosidase on a mixture of D-ribose and phenyl α-D-mannopyranoside, 2-*O*-, 3-*O*-, and 5-*O*-α-D-mannopyranosyl-D-ribose have been obtained.[108]

Several glycosides with complex aglycones have been obtained by chemical modification of the aglycones of available compounds. Into this category can be placed 2,3-epoxypropyl β-D-xylopyranoside (from the allyl glycoside)[109] and the corresponding D-glucosides,[110] a series of substituted 2-phenoxyethyl β-D-glucosides (from 2-bromoethyl compounds treated with the appropriate phenoxide),[111] and several mono- and di-saccharide 2-deoxyglycer-2-yl glycosides (from the corresponding 1,3-dichloro-compounds).[112] Prop-2-ynyl glycosides have been subjected to oxidative dimerization and other reactions.[113]

6,6′-Di-*O*-tritylsucrose has been proved to be a useful intermediate for the preparation of a variety of sucrose derivatives,[113a] and other compounds have been synthesized from 6,6′-di-*O*-toluene-*p*-sulphonylsucrose; these include various derivatives containing substituted groups at the primary carbon centres, 3,6:3′,6′-dianhydrosucrose, and a diene (see p. 98).[113b]

Hydrolysis and Related Reactions.—Acid-catalysed hydrolysis of natural glycosides can be carried out in t-butyl alcohol–water mixtures, since it has been shown that, under conditions of Fischer glycosidation, D-glucose does not react with t-butyl alcohol to give glycosides.[114] 1-Adamantyl β-D-glucopyranoside was hydrolysed about fifty times faster than the methyl analogue in acid solution, but only eleven times faster than the t-butyl compound; glycosyl–oxygen fission is the dominant type of bond cleavage. It was concluded that steric acceleration is the main driving force for the rapid hydrolysis of tertiary glycosides.[115]

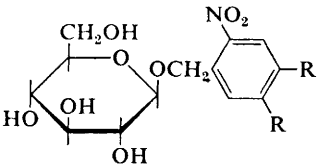
Studies have continued on the acidic hydrolysis of alkyl glycosides. Investigation of the hydrolysis of twenty-one alkyl β-D-galactopyranosides has indicated, in agreement with earlier results, that an *A*1 mechanism is involved. The results implied that the rate differences between glycosides are mainly due to entropy factors.[116] Aryl β-D-galactopyranosides have also been investigated with particular attention to the influence of the acid concentration on the reaction, but application of Hammett–Zucker and Bunnett criteria led to contradictory conclusions on the mechanism of the reaction.[117]

[108] A. Zurowska, E. Villarroya, and F. Peter, *Carbohydrate Res.*, 1972, **24**, 319.
[109] E. Saman, M. Claeyssens, and C. K. De Bruyne, *Carbohydrate Res.*, 1972, **24**, 173.
[110] E. M. Bessell and J. H. Westwood, *Carbohydrate Res.*, 1972, **25**, 11.
[111] F. Márquez and A. Mesquida, *Carbohydrate Res.*, 1972, **22**, 446.
[112] M. Horisberger, B. A. Lewis, and F. Smith, *Carbohydrate Res.*, 1972, **23**, 175.
[113] M. Horisberger, B. A. Lewis, and F. Smith, *Carbohydrate Res.*, 1972, **23**, 144.
[113a] L. Hough, K. S. Mufti, and R. Khan, *Carbohydrate Res.*, 1972, **21**, 144.
[113b] C. H. Bolton, L. Hough, and R. Khan, *Carbohydrate Res.*, 1972, **21**, 133.
[114] L. Szekeres and K. Tettamanti, *Microchem. J.*, 1972, **17**, 148.
[115] D. Cocker and M. L. Sinnott, *J.C.S. Chem. Comm.*, 1972, 414.
[116] C. K. de Bruyne and G. van Der Groen, *Carbohydrate Res.*, 1972, **25**, 59.
[117] C. K. de Bruyne and J. Wouters-Leysen, *Carbohydrate Res.*, 1972, **23**, 189.

Specific compounds of particular biochemical interest have been studied. The 2-aminoethyl α-, and 2-pyridyl α- and β-glycosides of *N*-acetyl-D-neuraminic acid were prepared in order to study the action of neuraminidase. The pyridyl glycosides were particularly sensitive and at pH 8 the α-anomer was cleaved within only a few minutes.[118] The acid stabilities of the glycosidic linkages of 3-*O*-β-D-xylopyranosyl-L-serine and -threonine have been investigated in relation to the graded hydrolysis of glycopeptides,[119] and the kinetics of hydrolysis of cellobiose in acid solution were also examined.[120]

A general kinetic expression has been derived for the rate of alkaline cleavage of glycosides in terms of the formation of anionic species as reactive intermediates that undergo slow intramolecular displacement of the aglycone. It is claimed that the reactivities of the anomers are primarily dependent upon the relative acidities of the hydroxy-groups involved. The kinetic data confirmed that the reactions of α- and β-D-glucopyranosides are facilitated by anchimeric assistance from the hydroxy-groups at C-6 and C-2, respectively (see Scheme 9).[121] A related study has been reported on the alkaline hydrolysis of 4-nitrophenyl α- and β-D-glucopyranoside. Previous knowledge of these reactions has been surveyed and amplified by the claim that radicals may be involved to some extent. As noted previously by Horton and Leutzow (Vol. 5, p. 21), the α-compound reacted by migration of the aglycone.[122]

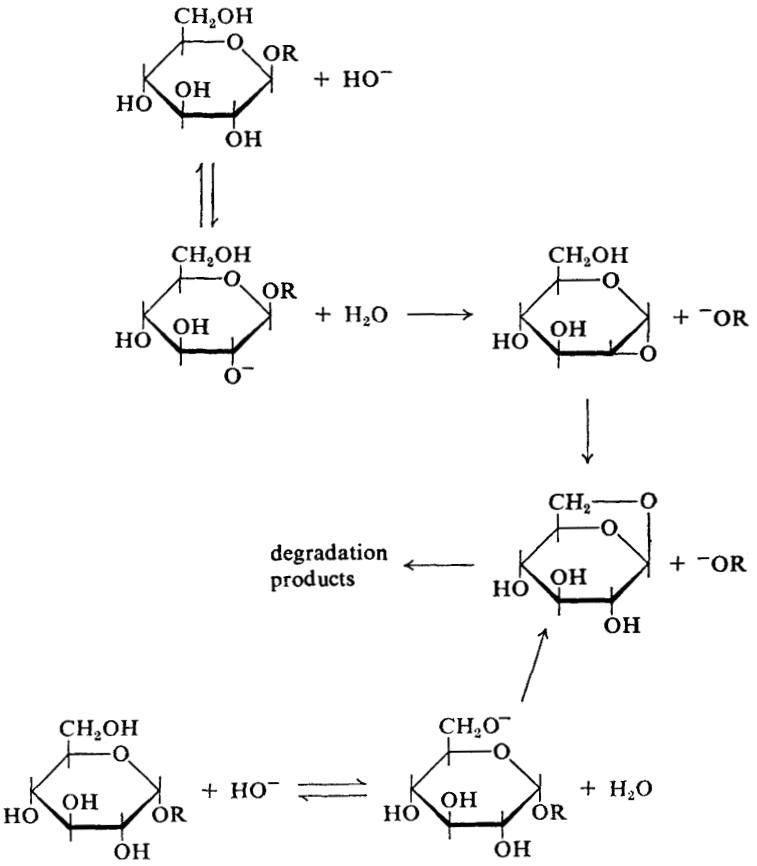
A particularly noteworthy report relates to the development of a glycosidic system that is capable of hydrolysis by a photo-induced process. Compounds (29) and (30) (prepared by standard Koenigs–Knorr synthesis)



(29) R = H
(30) R = OMe

were photolysed to give D-glucose on irradiation at 320 nm by a process presumed to involve the formation of hemiacetals by photochemical oxidation at the benzylic centre.[123] In the course of work with the α-L-rhamnosyl and β-L-fucosyl analogues, which were likewise photosensitive,

[118] L. Holmquist and T. Brossmer, *Z. physiol. Chem.*, 1972, **353**, 1346.
[119] L. P. Egan, J. R. Vercelloti, and W. T. Lowry, *Carbohydrate Res.*, 1972, **23**, 261.
[120] N. A. Halturynskii, Yu. V. Moiseev, M. I. Vinnik, and G. E. Zaikov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1971, 2664.
[121] Y. Z. Lai, *Carbohydrate Res.*, 1972, **24**, 57.
[122] C. S. Tsai and C. Reyes-Zamora, *J. Org. Chem.*, 1972, **37**, 2725.
[123] U. Zehavi, B. Amit, and A. Patchornik, *J. Org. Chem.*, 1972, **37**, 2281.



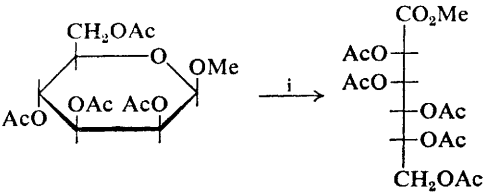
Scheme 9

it was shown that isopropylidene groups can be removed with acid without cleavage of the nitrobenzyl glycosidic bonds.[124]

Other Reactions and Features of Glycosides.—The acetolysis of fifteen oligosaccharides has been examined[125] and acetolyses of some methyl glycosides, which resulted in inversion of configuration at C-2, are noted[21] in Chapter 2. A new oxidation reaction (Vol. 5, p. 114) of potential importance (*e.g.* in the analysis of polysaccharides) showed a specificity for glycosides with equatorially oriented aglycones reminiscent of that shown by chromium trioxide in acetic acid (Vol. 4, p. 115). Methyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside and -galactopyranosides were inert towards

[124] U. Zehavi and A. Patchornik, *J. Org. Chem.*, 1972, **37**, 2285.
[125] V. A. Govorchenko and Yu. S. Ovodov, *Khim. prirod. Soedinenii*, 1972, **3**, 256.

ozone in acetic anhydride containing sodium acetate, but the β-anomers were oxidized as shown for the D-*manno*-compound in Scheme 10.[126]



Reagents: i, O_3–Ac_2O–NaOAc, 10 h, room temperature.

Scheme 10

Pyrolytic reactions of substituted phenyl β-D-glucopyranosides and related 2-deoxy-compounds have been investigated by thermal analysis and e.s.r. methods. The pyrolytic decompositions of these sugars were initiated by cleavage of the glycosidic bond. Furthermore, the thermal stabilities of the glycosides and the kinetics of the pyrolysis processes appeared to be related to the electron density in the region of the glycosidic bond.[127] Studies have been carried out on aryl β-D-glucopyranosides as models for lignin.[128]

1,2-*O*-Ethylene-β-D-glucopyranose and the α-D-glucofuranose isomer, which are intramolecular glycosides formed during hydrolysis of *O*-(2-hydroxyethyl)starch, have been characterized by mass and n.m.r. spectral examination of their tri-*O*-acetyl derivatives.[129]

The transport of methyl α-D-glucopyranoside by *Pseudomonas aeruginosa* has been studied using ^{14}C-tracer methods[130] and, in a similar type of biological study, it was established that the inflow of β-galactosides into the cells of *Escherichia coli* is accompanied by a pH increase in the medium and an accompanying proton migration into the cells.[131]

Natural Products.—As in previous volumes, no attempt has been made to produce full coverage in this section and only a few compounds of special interest are noted.

Of particular note are biosynthetic studies of the virescenosides (Vol. 4, p. 19); these mushroom metabolites were the first compounds containing

[126] P. Deslongchamps, C. Moreau, D. Fréhel, and P. Atlani, *Canad. J. Chem.*, 1972, **50**, 3402.
[127] F. Shafizadeh, R. A. Susott, and G. D. McGinnis, *Carbohydrate Res.*, 1972, **22**, 63.
[128] G. V. Davydova, A. V. Losanova, and N. N. Shorygina, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1972, 1837.
[129] H. C. Srivastava, A. S. Chaudhari, N. M. Doshi, D. Horton, and J. D. Wander, *Carbohydrate Res.*, 1972, **24**, 188.
[130] M. Midgley, *Biochem. J.*, 1972, **127**, 50P.
[131] I. C. West and P. Mitchell, *Biochem. J.*, 1972, **127**, 56P.

D-altrose to be found in Nature.[132] Studies on iridoid glycosides,[133–135] phenolic glycosides from *Rhizoma rhei*,[136] macrocyclic flavonoid glycosides from *Leucothoe keiskei* Miq.,[137] mono- and di-glucosides from bacteriorubin (the characteristic carotenoid of halophilic bacteria),[138] and gibberellin-A_1 β-D-glucopyranoside [139] have been described.

Holarosine B [3β-(4-amino-2,4,6-trideoxy-3-*O*-methyl-β-D-*ribo*-hexopyranosyl)oxy-14β-hydroxy-5α,17β*H*-card-20(22)-enolide] and holantosines E and F have been isolated from *Holarrhena antidysenterica* leaves. The latter compounds are β-glycosides of a new amino-sugar (D-holacosamine or 4-amino-4-deoxysarmentose) and holantogenine or anhydroholantogenine.[139a]

Of biochemical interest is the evidence (including detailed 220 MHz n.m.r. studies) for the presence of a terminal α-D-galactopyranosyl residue in the galactosylgalactosylglucosyl ceramide obtained from human kidney [140] and the finding of a new hexasaccharide 'lacto-*N*-*neo*hexaose' (31) in human milk.[141]

A new apiosyl glycoside is mentioned in Chapter 15.

β-D-Gal-(1→4)-β-D-Glc*N*Ac
1
↑
6

β-D-Gal-(1→4)-β-D-Glc*N*Ac-(1→3)-β-D-Gal-(1→4)-D-Glc

(31)

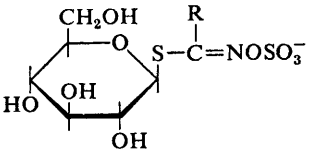
S-Glycosides

A procedure has been described for the preparative isolation of the main glucosinolates [142] and reports continued to appear from Kjaer's group on new naturally occurring members of this series. Compounds (32) and (33) have now been isolated from the seeds of a Crucifer.[143]

The biosynthesis of thio-β-D-glucuronides and -glucosides has been found to involve transfer from UDP-D-GlcUA and UDP-D-Glc to thiol

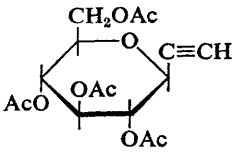
[132] J. Polonsky, Z. Baskevitch, N. Cagnoli-Bellavita, P. Ceccherelli, B. Buckwalter, and E. Wenkert, *J. Amer. Chem. Soc.*, 1972, **94**, 4369.
[133] R. Guarnaccia, K. M. Madyastha, E. Tegtmeyer, and C. J. Coscia, *Tetrahedron Letters*, 1972, 5125.
[134] H. Inouye and T. Nishioka, *Tetrahedron*, 1972, **28**, 4231.
[135] I. Kitagawa, T. Tani, K. Akita, and I. Yosioka, *Tetrahedron Letters*, 1972, 419.
[136] T. Murakami and K. Tanaka, *Tetrahedron Letters*, 1972, 2965.
[137] A. Ogiso, A. Sato, S. Sato, and C. Tamura, *Tetrahedron Letters*, 1972, 3071.
[138] N. Arpin, J.-L. Fiasson, and S. Liaaen-Jensen, *Acta Chem. Scand.*, 1972, **26**, 2526.
[139] G. Schneider, *Tetrahedron Letters*, 1972, 4053.
[139a] R. Goutarel, C. Monneret, P. Choay, I. Kaboré, and Q. Khuong-Huu, *Carbohydrate Res.*, 1972, **24**, 297.
[140] J. T. R. Clarke, L. S. Wolfe, and A. S. Perlin, *J. Biol. Chem.*, 1971, **246**, 5563.
[141] A. Kobata and V. Ginsburg, *Arch. Biochem. Biophys.*, 1972, **150**, 273.
[142] R. Björkman, *Acta Chem. Scand.*, 1972, **26**, 1111.
[143] A. Kjaer and A. Schuster, *Acta Chem. Scand.*, 1972, **26**, 8.

and, in the same paper, the relative susceptibilities of *S*- and *O*-glycosides to enzymic hydrolysis were discussed.[144]

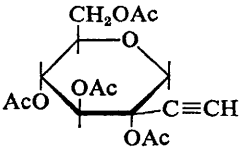


(32) R = $\cdot CH_2CH(OH)C_6H_4OMe(p)$
(33) R = $\cdot CH_2CH_2C(CH_2)_5SOMe$
$\quad\quad\quad

The second paper has described the reaction between 2,3,4,6-tetra-*O*-acetyl-D-glucosyl bromide and ethynylmagnesium bromide with the formation (after acetylation) of the *C*-glycoside (34) and the branched-chain derivative (35).[146]

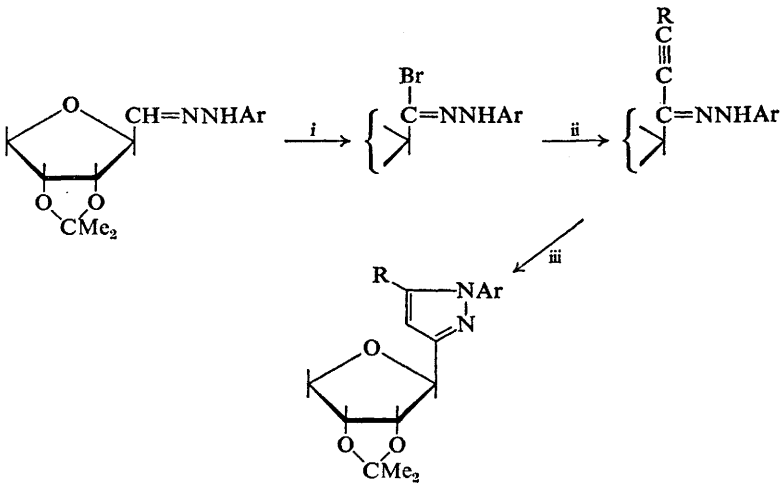


(34)



(35)

An interesting series of *C*-linked nucleoside analogues has been produced by the reaction of acetylenes and bromophenylhydrazones derived from a 2,5-anhydroaldose derivative (Scheme 12). Various analogues and deriva-



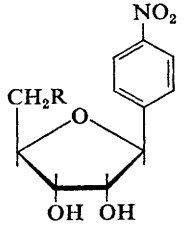
Reagents: i, Br_2–AcOH; ii, RC≡CH; iii, EtOH–NaOH.

Scheme 12

tives were prepared[147] (see also Chapter 4). Also in the biochemical area, compounds (36) and (37) were prepared as analogues of chloramphenicol and, consequently, as potential inhibitors of protein synthesis.[148]

Reaction of either α- or β-D-glucopyranose penta-acetate with Δ^8-tetrahydrocannabinol in benzene containing boron trifluoride gave a novel

[146] H. Ogura and M. Ogiwara, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 848.
[147] J. M. J. Tronchet and F. Perret, *Helv. Chim. Acta*, 1972, **55**, 2121.
[148] R. S. Klein, M. P. Kotick, K. A. Watanabe, and J. J. Fox, *J. Org. Chem.*, 1971, **36**, 4113.



(36) R = $\cdot OP(OH)_2$ (O)

(37) R = $\cdot NHCOCHCl_2$

C-glycoside considered to be 2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-Δ^8-tetrahydrocannabinol.[149]

C-Glycosides produced by additions to glycals are discussed in Chapter 14.

[149] K. Bailey and D. Verner, *J.C.S. Chem. Comm.*, 1972, 89.

4
Ethers and Anhydro-sugars

Ethers

Methyl Ethers.—Alternative syntheses of 5-*O*-, 2,5-di-*O*-, 3,5-di-*O*-, and 2,3,5-tri-*O*-methyl-D-xylofuranose have been described,[150] and all the possible methyl ethers of methyl β-D-arabino-pyranoside and -furanoside have been obtained by partial methylation procedures.[151] 2-*O*-Methyl-D-ribose has been produced by Ruff degradation of 3-*O*-methyl-D-allose, and 3-*O*-methyl-D-ribose from 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-allofuranose by oxidation with periodate ion, reduction, and hydrolysis with acid.[152]

In the D-glucose series, alternative syntheses of the 3,4-, 4,6-,[153] and 2,4-di-*O*-methyl ethers [154] have been reported, and the 6-*O*- and 2,6-di-*O*-methyl ethers of the methyl D-glucofuranosides have been prepared.[155] During the methylation of ethyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside with methyl iodide and silver oxide in DMF, acetyl migration from O-4 to O-6 and from O-3 to O-4 has been noted (see Chapter 6). Selective methylations of the sodium salts and copper chelates of methyl 4,6-*O*-benzylidene-α- and -β-D-glucopyranoside have been examined. With the copper derivatives, selective ether formation occurred at O-3, whereas the sodio-compounds reacted less selectively.[156] Similarly, with the methyl 2,3-di-*O*-methyl-D-glucopyranosides, the sodium salts gave less selectivity than did the copper derivatives, which led mainly to etherification at the primary position.[157] Methyl iodide in the presence of the dimethylsulphinyl carbanion has been used for the complete methylation of 2-acetamido-2-deoxyglycosides.[158]

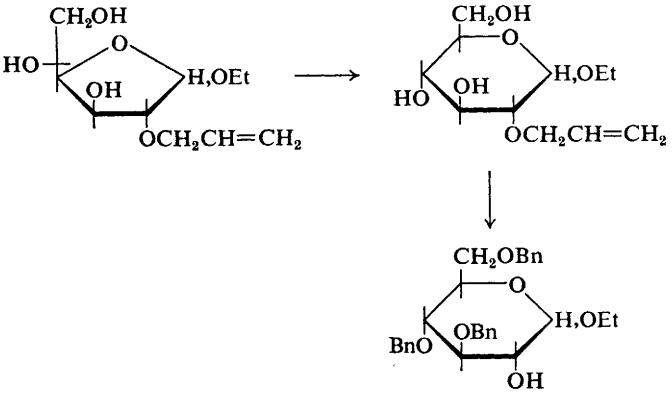
4,6-Di-*O*-methyl-D-mannose has been prepared by methylation of methyl 2,3-di-*O*-(ethoxycarbonyl)-6-*O*-methyl-α-D-mannopyranoside using diazomethane–boron trifluoride followed by removal of the protecting groups.[159] A synthesis of 3,4-di-*O*-methyl-D-galactose from 1,6-anhydro-β-D-galactopyranose is referred to later in this chapter, and methyl

[150] P. Kováč, *Chem. Zvesti*, 1971, **25**, 460.
[151] P. A. Mied and Y. C. Lee, *Analyt. Biochem.*, 1972, **49**, 534.
[152] M. Haga, M. Takano, and S. Tejima, *Carbohydrate Res.*, 1972, **21**, 440.
[153] P. Kováč and Z. Longauerová, *Chem. Zvesti*, 1972, **26**, 179.
[154] P. Kováč and Z. Longauerová, *Carbohydrate Res.*, 1972, **25**, 253.
[155] P. Kováč and M. Petriková, *Chem. Zvesti*, 1972, **26**, 71.
[156] E. Avela and B. Holmbom, *Acta Acad. Aboensis, Ser. B*, 1971, **31**, 14.
[157] E. Avela, B. Melander, and B. Holmbom, *Acta Acad. Aboensis, Ser. B*, 1971, **31**, 15.
[158] A. Stoffyn, P. Stoffyn, and J. C. Orr, *Carbohydrate Res.*, 1972, **23**, 251.
[159] E. G. Gros and E. M. Gruñeiro, *Carbohydrate Res.*, 1972, **23**, 148.

3,4-di-*O*-methyl-α-D-fructofuranoside has been prepared from the 1,6-di-*O*-trityl ether in relation to studies on the alkaline degradation of sucrose.[160] In the disaccharide field, 1′,6,6′-tri-*O*-, 2,3,3′,4′-tetra-*O*-, and 2,3,3′,4,4′-penta-*O*-methylsucrose have been synthesized in connection with the same studies.[161] Methyl (methyl 3,4-di-*O*-methyl-α-D-galactopyranosid)uronate has been obtained from the 3,4-*O*-isopropylidene derivative by sequential benzylation, hydrolysis of the acetal, methylation, and catalytic debenzylation.[162]

The results obtained from a study of the ^{1}H n.m.r. spectra of partially and fully methylated sugars are referred to in Chapter 23.

Substituted Alkyl Ethers.—A synthesis of methyl 2,4,6-tri-*O*-benzyl-α-D-glucopyranoside has been achieved, in 62% yield, by partial benzylation of the glycoside;[163] in a somewhat related reaction, the 2′- and 3′-*O*-benzyl ethers of adenosine, inosine, guanosine, cytidine, and uridine were obtained by direct treatment of the nucleosides with phenyldiazomethane in cellosolve in the presence of stannous chloride. A novel spectrophotometric method was used to distinguish between the isomers produced.[164] Ethyl 3,4,6-tri-*O*-benzyl-D-glucopyranoside and -galactopyranoside, isomers of the pharmacologically active ethyl 3,5,6-tri-*O*-benzyl-D-glucofuranoside, were synthesized as shown in Schemes 13 and 14, respectively.[165]

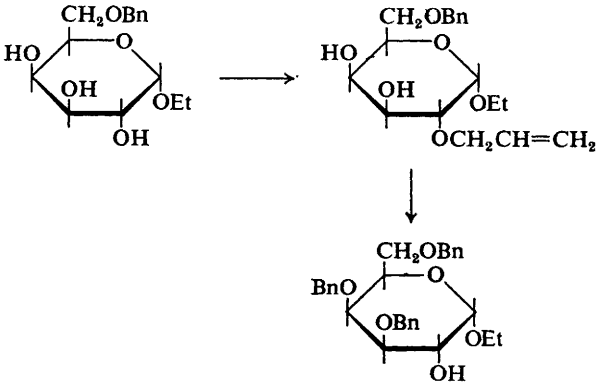


Scheme 13

Ethylene carbonate in the presence of sodium hydride–DMSO provided a new method for synthesizing β-hydroxyethyl ethers. A sugar hydroxyethylcarbonate was produced, which was suggested to collapse to the initial

[160] G. W. O'Donnell and G. N. Richards, *Austral. J. Chem.*, 1972, **25**, 907.
[161] G. W. O'Donnell and G. N. Richards, *Austral. J. Chem.*, 1972, **25**, 407.
[162] P. Kováč, *Carbohydrate Res.*, 1972, **22**, 464.
[163] S. Koto, Y. Takebe, and S. Zen, *Bull. Chem. Soc. Japan*, 1972, **45**, 291.
[164] L. F. Christensen and A. D. Broom, *J. Org. Chem.*, 1972, **37**, 3398.
[165] J. Stanek, A. Sele, R. Jaques, and A. Rossi, *Helv. Chim. Acta*, 1972, **55**, 434.

alkoxide, ethylene oxide, and carbon dioxide, with further reaction between the two former products resulting in the ether. However, it is much more likely that ejection of carbon dioxide occurs concurrently with ether



Scheme 14

formation. The process was utilized in the synthesis of 3- and 6-mono-ethers of D-glucose.[166] Alternatively, 2-*O*-(hydroxyethyl)-D-glucose was prepared either by reduction of the corresponding methoxycarbonylmethyl derivative with lithium aluminium hydride or by use of β-iodoethyl acetate and silver oxide. In both cases, *N*-(3,4,6-tri-*O*-acetyl-D-glucosyl)-piperidine was used as the D-glucosyl precursor.[167] Analogous *NN*-(diethylamino)ethyl ethers have been prepared from D-glucosides having the hydroxy-groups at C-2 and C-3 severally substituted and using *NN*-diethylaziridinium chloride as the alkylating agent. Substitution of the hydroxy-group at C-2 greatly enhanced the reactivity of the groups at C-3 and C-4 in dilute base, but had little effect in more concentrated base. Substitution at this position had little effect on the reactivity of the primary hydroxy-group. On the other hand, substitution of the C-3 hydroxy-group depressed the reactivity of the C-2 group throughout a range of base concentrations, but the reactivity of the hydroxy-group at C-6 was enhanced to a significant extent in dilute base and to a lesser extent in more concentrated base. The results were discussed and explanations to account for the foregoing patterns of reactivity were proposed.[168]

2,3-*O*-Epoxypropyl ethers and glycosides are discussed in Chapter 3. Various vinyl ethers of 1,6-anhydro-β-D-glucopyranose have been prepared.[169]

[166] H. C. Srivastava, K. V. Ramalingam, and A. S. Chaudhari, *Indian J. Chem.*, 1971, **9**, 1081.
[167] H. C. Srivastava, K. V. Ramalingam, and A. S. Chaudhari, *Indian J. Chem.*, 1971, **9**, 1083.
[168] E. J. Roberts, C. P. Wade, and S. P. Rowland, *Carbohydrate Res.*, 1972, **21**, 357.
[169] E. U. Ponomarenko, V. L. Lapenko, and G. G. Markova, *Trudy Veroneg. Univ.*, 1972, **95**, 72.

The potential of the but-2-enyl ether group for blocking purposes has been investigated. Treatment with potassium t-butoxide in DMSO regenerated the hydroxy-group under conditions that did not isomerize an allyl group to the prop-1-enyl group; the latter reaction could be carried out subsequently under more vigorous conditions. Uses of the new group were illustrated in the preparation of prop-1-enyl 2-benzamido-4,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside.[170]

Treatment of sucrose with two molar equivalents of trityl chloride has been shown to give the expected 6,6′-, 1′,6-, and 1′,6′-ditrityl ethers;[170a] the use of the first of these ethers in the preparation of a variety of sucrose derivatives has been referred to already.[113a]

Silyl Ethers.—A valuable survey of novel hydroxy-protecting groups is given in the introduction to a Note by Corey and Venkateswarlu [171] describing t-butyldimethylsilyl ethers. These silyl ethers are about 10^4 times more stable towards solvolysis than the trimethylsilyl group, enabling acetate groups to be removed in their presence. It is stable to hydrogenolysis and to many other combinations of reagents, but is hydrolysed either with tetra-n-butylammonium fluoride in THF at room temperature or with acid at a rate comparable with that of tetrahydropyranyl ethers. The derivatives were prepared using t-butyldimethylchlorosilane in DMF in the presence of imidazole.

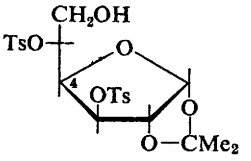
Intramolecular Ethers (Anhydro-sugars)

Epoxides.—Treatment of 1,2-*O*-isopropylidene-3,5-di-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose (38) with potassium acetate in DMF afforded the epoxide (39), 6-*O*-acetyl-1,2-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-β-L-idose (40) (produced by nucleophilic attack at C-6 of the epoxide), and an olefin (41) derived from the latter product by elimination of toluene-*p*-sulphonic acid (Scheme 15).[172]

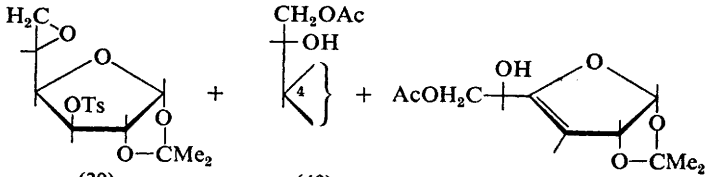
The 4-deoxy-DL-*lyxo*-epoxides (42) and (43) have been obtained from non-carbohydrate olefins in continuing studies on the synthesis of racemic sugar derivatives.[173]

Other Anhydrides.—Thermal degradation of 1,6-anhydro-β-D-glucopyranose labelled specifically at positions 1, 2, and 6 gave a variety of small fragments, which were studied in detail, and the nature of the complex reactions undergone in the presence of sodium hydroxide and zinc chloride was examined. Fourteen volatile products were characterized, but these corresponded to only a small fraction of starting material degraded, the residue being described euphemistically as 'tar' and 'char'.[174] In a more

[170] P. A Gent, R. Gigg, and R. Conant, *J.C.S. Perkin I*, 1972, 1535.
[170a] T. Otake, *Bull. Chem. Soc. Japan*, 1972, **45**, 2895.
[171] E. J. Corey and A. Venkateswarlu, *J. Amer. Chem. Soc.*, 1972, **94**, 6190.
[172] M. Miljković, D. Miljković, A. Jokić, V. Andrejević, and E. A. Davidson, *J. Org. Chem.*, 1972, **37**, 2536.
[173] A. Banaszek and A. Zamojski, *Roczniki Chem.*, 1971, **45**, 2089.
[174] F. Shafizadeh and Y. Z. Lai, *J. Org. Chem.*, 1972, **37**, 278.

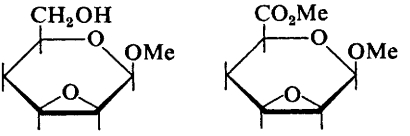


(38)

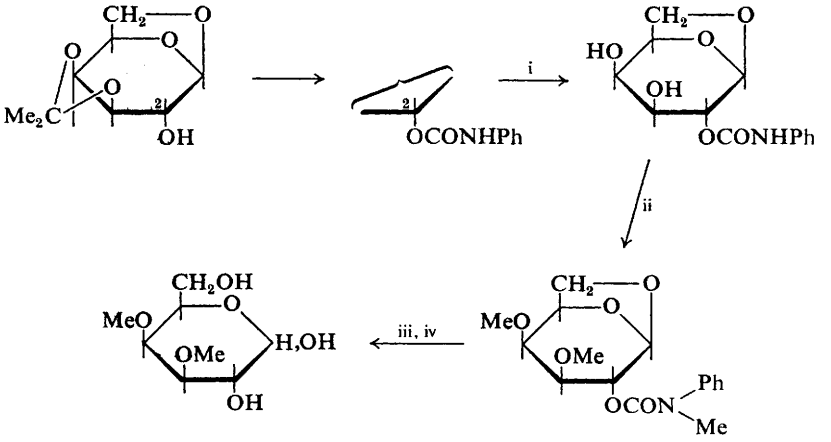


(39) (40) (41)

Scheme 15



(42) (43)



Reagents: i, MeOH–HCl; ii, MeI–Ag_2O–DMF; iii, $LiAlH_4$; iv, H_3O^+.

Scheme 16

specific reaction, an α-1,6-linked 2,3,4-trimethylated glucan was synthesized by treating 1,6-anhydro-2,3,4-tri-*O*-methyl-β-D-glucopyranose with chlorosulphonic acid in dichloromethane at −80 °C.[175]

Various unsaturated ethers of 1,6-anhydro-β-D-glucopyranose have been reported.[176]

D-Allose is known to equilibrate with its 1,6-anhydropyranose derivative in aqueous acid solution, but 1% of the 1,6-anhydrofuranose has now been detected by g.l.c. and it was isolated as its 2,3-*O*-isopropylidene derivative.[177] 1,6-Anhydro-3,4-*O*-isopropylidene-β-D-galactopyranose has been utilized in a synthesis of 3,4-di-*O*-methyl-D-galactose (Scheme 16).[178]

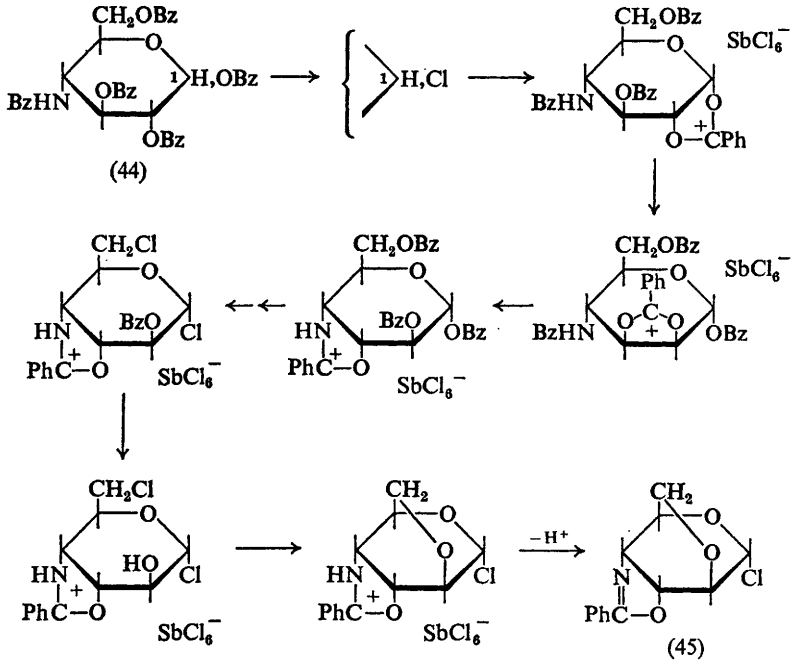
The crystal structure of 2,6-anhydro-β-D-fructofuranose has been reported,[179] and it was suggested in other work that various D-fructose anhydrides can be identified by their i.r. spectra in the fingerprint region.[180] In the octulose series, a crystalline compound presumed to be 2,7-anhydro-L-*glycero*-β-D-*manno*-octulopyranose has been described.[181]

The reaction of the benzamido-tetrabenzoate (44) with antimony pentachloride has been investigated with a view to determining the effect of introducing the amido-group at C-4. Instead of obtaining D-*ido*-products, as with the D-glucose analogues, the unusual 2,6-anhydro-D-altrosyl chloride (45) was produced. The complex path of the conversion is indicated in Scheme 17.[182]

Reference has been made in Chapter 3 to the use of 2,5-anhydroaldoses in a study of C-linked nucleosides and, for related purposes, Japanese workers have prepared the 2,5-anhydro-L-*ido*-compound (46)[183] (Scheme 18) and from it the L-*talo*-derivative (47).[183a]

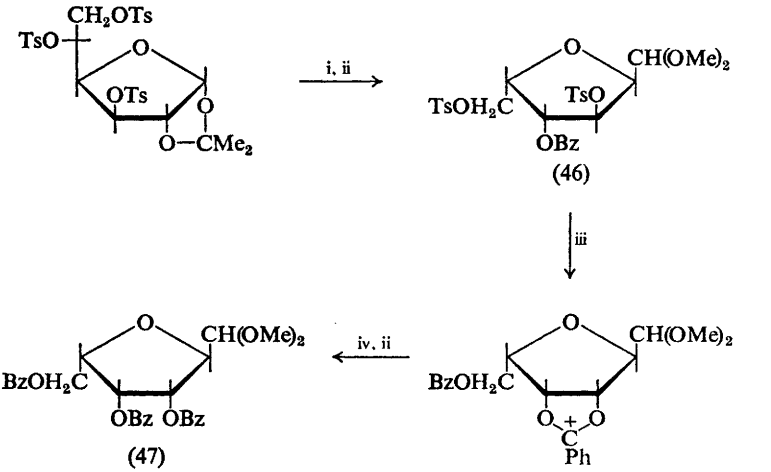
The crystal structure of methyl 3,6-anhydro-α-D-glucopyranoside has been reported,[184] and 3,6-anhydro-D-allose, which can exist only in the acyclic form, has been obtained as its 4,5-*O*-isopropylidene dimethyl acetal derivative (48). The latter derivative was obtained in minor amounts during acid-catalysed methanolysis of 1,2:5,6-di-*O*-isopropylidene-3-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose; 1,2-*O*-isopropylidene-5-*O*-methyl-3-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose similarly afforded 3,6-anhydro-5-*O*-methyl-D-allose dimethyl acetal (49) in good yield.[185]

[175] A. Klemer and C. Apostolides, *Carbohydrate Res.*, 1972, **22**, 432.
[176] B. I. Mikhant'ev, V. L. Lapenko, E. Yu. Ponomarenko, and N. L. Vasil'eva, *Zhur. obshchei Khim.*, 1972, **42**, 190.
[177] K. Heyns and P. Köll, *Chem. Ber.*, 1972, **105**, 2228.
[178] S. Hirase and K. Watanabe, *Bull. Chem. Soc. Japan*, 1972, **45**, 1569.
[179] W. Dreissig and P. Luger, *Carbohydrate Res.*, 1972, **23**, 447.
[180] W. W. Binkley, R. W. Binkley, and D. R. Diehl, *Internat. Sugar J.*, 1971, **73**, 259.
[181] N. K. Richtmyer, *Carbohydrate Res.*, 1972, **23**, 319.
[182] H. Paulsen and Ö. Kristinsson, *Chem. Ber.*, 1972, **105**, 3456.
[183] T. Ogawa, M. Matsui, H. Ohrui, H. Kuzuhara, and S. Emoto, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1449.
[183a] T. Ogawa, M. Matsui, H. Ohrui, H. Kuzuhara, and S. Emoto, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1655.
[184] J. W. Campbell and M. M. Harding, *J.C.S. Perkin II*, 1972, 1721.
[185] M. H. Randall and S. J. Angyal, *J.C.S. Perkin I*, 1972, 347.



(44) (45)

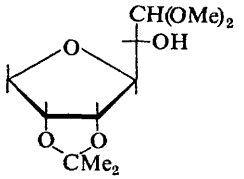
Scheme 17



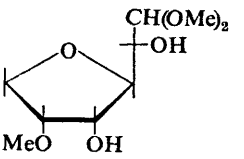
(46) (47)

Reagents: i, MeOH–HCl; ii, BzCl–py; iii, NaOBz–HMPT; iv, H_2O

Scheme 18

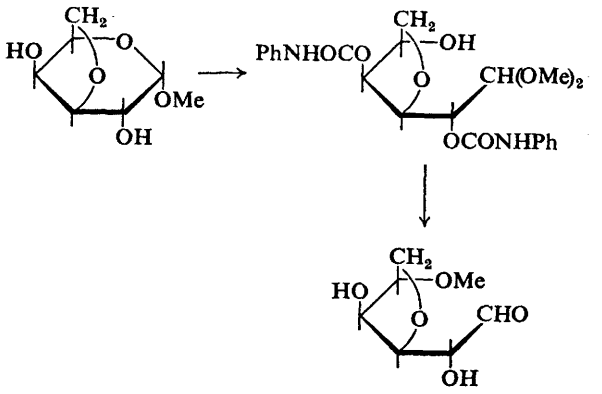


(48)

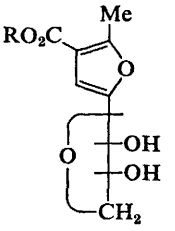


(49)

3,6-Anhydro-5-*O*-methyl-D-galactose has been prepared from methyl 3,6-anhydro-α-D-galactopyranoside as shown in Scheme 19.[186]



Scheme 19

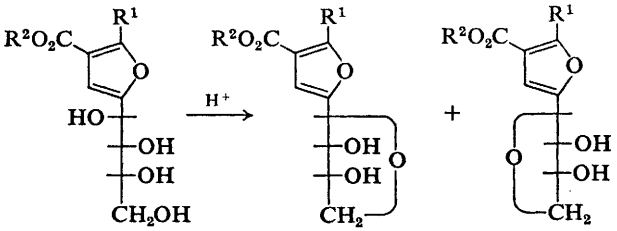


(50)

The reactions of acetoacetic esters and 3,6-anhydro-D-glucose afforded compounds in the series (50),[187] and related compounds have been prepared as illustrated in Scheme 20.[188]

4,7-Anhydro- and 4,8-anhydro-3-deoxy-octulosonic acids have been isolated following acid hydrolysis of a bacterial polysaccharide[189] (see Chapter 17).

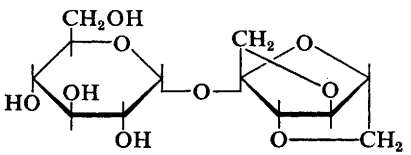
[186] S. Hirase and K. Watanabe, *Bull. Chem. Soc. Japan*, 1972, **45**, 1844.
[187] A. Gómez-Sánchez and A. Rodríguez-Roldán, *Anales de Quím.*, 1972, **68**, 609.
[188] A. Gómez-Sánchez and A. Rodríguez-Roldán, *Carbohydrate Res.*, 1972, **22**, 53.
[189] W. A. Volk, N. L. Salomonsky, and D. Hunt, *J. Biol. Chem.*, 1972, **247**, 3881.



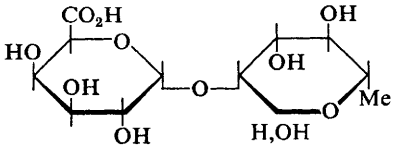
$R^1 = Me$
$R^2 = Me, Et, H, etc.$

Scheme 20

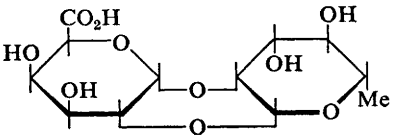
Several reports have dealt with sucrose anhydrides. The product of alkaline treatment of 6,1′,6′-tri-*O*-toluene-*p*-sulphonylsucrose penta-acetate was shown by *X*-ray analysis to be 3,6-anhydro-α-D-glucopyranosyl 1′,4′:3′,6′-dianhydro-β-D-fructofuranoside.[190] A similar product was obtained following treatment of the corresponding pentabenzoate with sodium methoxide.[191] 1′,4′:3′,6′-Dianhydrosucrose (51) has been prepared from 1′,6′-ditritylsucrose hexa-acetate by detritylation, mesylation, and treatment with base.[192]



(51)



(52)



(53)

Treatment of the aldobiouronic acid (52) with acid gave the interunit anhydride (53), which was also found in hydrolysates of spruce and in sulphite-spent liquors. On mild acid treatment, (53) partly reverted to the disaccharide (52) as well as to L-rhamnose and D-galacturonic acid.[193]

The crystal structures of several anhydro-sugar derivatives are noted in Chapter 24.

[190] N. W. Isaacs and C. H. L. Kennard, *J.C.S. Perkin II*, 1972, 582.
[191] R. Khan, *Carbohydrate Res.*, 1972, **22**, 441.
[192] J. G. Buchanan and D. A. Cummerson, *Carbohydrate Res.*, 1972, **21**, 293.
[193] K. Larsson and O. Samuelson, *Acta Chem. Scand.*, 1972, **26**, 837.

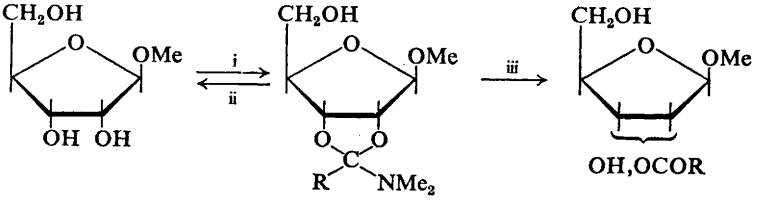
5
Acetals

Reactions and Properties

The general acid-catalysed hydrolysis of acetals, ketals, and orthoesters has been reviewed with reference to several carbohydrate and carbohydrate-like systems.[194]

In a reaction related to the photochemically induced cleavage of 2-nitrobenzyl glycosides,[123, 124] 2-nitrobenzylidene derivatives of carbohydrates have been irradiated to give 2-nitrosobenzoates, thereby providing a means of obtaining partially protected compounds. Whereas in some cases the acetal cleavage was specific, in others both possible monoesters were obtained.[195] Other workers have studied the photolysis of 2,4-dinitrobenzylidene acetals of hexitols to give 2-nitroso-4-nitroesters. In the case of acetals spanning primary and secondary diol groupings, the esters formed had the substituent located at the primary positions.[196]

A full paper on α-(dimethylamino)benzylidene and α-(dimethylamino)-ethylidene acetals has appeared in which the general properties of the derivatives were considered. The acetals were produced in high yield (Scheme 21) and on acid hydrolysis gave monoesters — primary mono-



R = Ph or Me

Reagents: i, $RC(OMe)_2NMe_2$; ii, MeOH–H_2O; iii, pH 4—5

Scheme 21

esters were formed if primary centres were involved. Various reactions were carried out in the presence of these protecting groups, which were generally assessed for their usefulness in selective esterification reactions,

[194] T. H. Fife, *Accounts Chem. Res.*, 1972, **5**, 264.
[195] P. M. Collins and N. N. Oparaeche, *J.C.S. Chem. Comm.*, 1972, 532.
[196] I. Tanasescu and A. Otea, *Studia Univ. Babes-Boljai, Ser. Chem.*, 1972, **17**, 113.

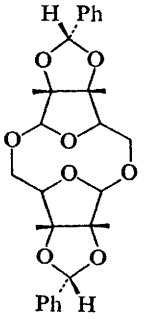
particularly with regard to their potential value in oligonucleotide synthesis.[197]

Treatment of 2,2-dialkyldioxolans with *N*-bromosuccinimide afforded only 2-alkyl-2-(1-bromoalkyl)dioxolans, but unsubstituted or 2-monosubstituted derivatives afforded mainly brominated esters, presumably by a concerted mechanism involving configurational inversion.[198] N.m.r. evidence has been presented for the existence of glycolaldehyde in a monomeric and two dimeric forms in solution.[199]

Acetals Derived from Carbohydrate Hydroxy-groups

A slightly modified synthesis of simple *O*-benzylidene acetals from diols involved the use of benzal chloride in pyridine. Dioxan or dioxolan derivatives can be produced under these conditions and, surprisingly in view of the solvent used, the thermodynamically stable isomers were obtained.[200]

From Diol Groups on Cyclic Carbohydrates.—Benzylidenation of D-ribose under different conditions yielded a variety of products, most of which were formed as mixtures of diastereoisomers. The conformations assumed by the furanose ring in the 2,3-*O*-benzylidene-β-D-riboses were discussed in relation to the coupling constants obtained by comparing the experimental and computed spectra. One of the products obtained was a dibenzylidene derivative (54) of D-ribose dianhydride.[201]

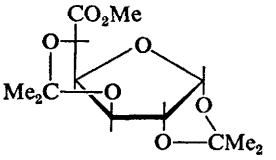


(54)

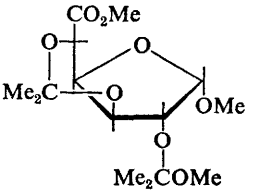
A series of diastereoisomeric pairs of D-glucose acetals, *viz.* 3,4,6-tri-*O*-acetyl-1,2-*O*-ethylidene- and -benzylidene-α-D-glucopyranose and 3-*O*-acetyl-1,2:4,6-di-*O*-ethylidene-α-D-glucopyranose, has been prepared by sodium borohydride reduction of intermediate dioxolenium ions; the stereochemistry of the products was examined in detail by n.m.r. spectroscopy.[202]

[197] S. Hanessian and E. Moralioglu, *Canad. J. Chem.*, 1972, **50**, 233.
[198] J. Gelas and S. Michaud, *Bull. Soc. chim. France*, 1972, 2441.
[199] C. I. Stassinopoulou and C. Zioudrou, *Tetrahedron*, 1972, **28**, 1257.
[200] P. J. Garegg, L. Maron, and C.-G. Swahn, *Acta Chem. Scand.*, 1972, **26**, 518.
[201] T. B. Grindley and W. A. Szarek, *Carbohydrate Res.*, 1972, **25**, 187.
[202] W. E. Dick, jun., D. Weisleder, and J. E. Hodge, *Carbohydrate Res.*, 1972, **23**, 229.

Convenient syntheses of methyl 4,6-*O*-benzylidene-α- and -β-D-glucopyranoside have been described involving the use of benzaldehyde dimethyl acetal in DMF.[203] In the D-glucose series, treatment of α-D-glucofuranurono-6,3-lactone with 2,2-dimethoxypropane and acetone in the presence of acid afforded the ester derivative (55), whereas under prolonged reaction or in the absence of acetone the major product was (56).[204]

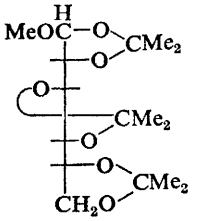


(55)



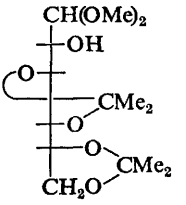
(56)

Among compounds isolated from the acid-catalysed reaction of D-glucose with acetone–methanol were the 2,3:4,5-di-*O*-isopropylidene derivatives of methyl α- and β-D-glucoseptanoside, the 1,2:3,5- and 1,2:5,6-diacetals of α-D-glucofuranose, and the acyclic derivatives (57)—(59).[205] The crystal structure of 3-*O*-acetyl-1,2:4,5-di-*O*-isopropylidene-

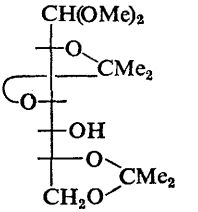


and C-1 isomer

(57)



(58)



(59)

α-D-glucoseptanose has shown that the sugar ring adopts a distorted chair shape.[206]

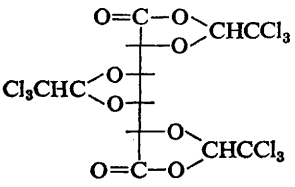
4,6-*O*-(1-Carboxyethylidene)-D-mannose has been found as a structural unit of a capsulated bacterial polysaccharide.[207]

[203] M. E. Evans, *Carbohydrate Res.*, 1972, **21**, 473.
[204] C. H. Lee, *Carbohydrate Res.*, 1972, **22**, 230.
[205] J. D. Stevens, *Carbohydrate Res.*, 1972, **21**, 490.
[206] E. T. Pallister, N. C. Stephenson, and J. D. Stevens, *J.C.S. Chem. Comm.*, 1972, 98.
[207] G. G. S. Dutton and M. T. Yang, *Canad. J. Chem.*, 1972, **50**, 2382.

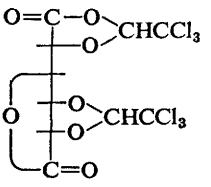
'Dibenzylidenefructose' has been identified as 2,3:4,5-di-*O*-benzylidene-β-D-fructopyranose by the application of i.r., mass, and n.m.r. spectral methods. A lanthanide shift reagent was used in the n.m.r. studies.[208]

Methyl 4,6-*O*-benzylidene-2-deoxy-2-halogeno-α-D-altropyranosides have been shown to undergo facile acid-catalysed migration of the acetal rings to give 3,4-*O*(*R*)- and -(*S*)-benzylidene derivatives in contradiction to the generalization that such acetals prefer the six-membered ring-form.[209]

From Diol Groups on Acyclic Carbohydrates.—The complex, acid-catalysed reaction between D-glucitol and acetone has been studied under a variety of conditions and 1,2-, 3,4-, 1,2:3,4-, 3,4:5,6-, 1,2:5,6-, and 1,2:3,4:5,6-derivatives have been shown to be formed. The C-1 primary hydroxy-group showed greater reactivity than that at C-6.[210] 1,3:2,4:5,6-Tri-*O*-benzylidenegalactitol was formed when either the 2,3:4,5-dibenzylidene derivative or the corresponding isopropylidene analogue was treated with benzaldehyde and zinc chloride. Barker and Bourne's rules (*J. Chem. Soc.*, 1952, 905) have been postulated to be valid only if the acetalization of alditols is performed under conditions of kinetic control.[211]



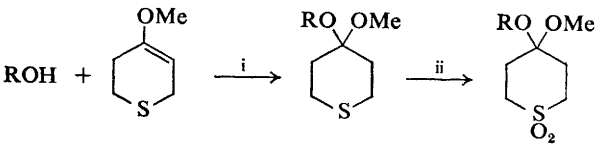
(60)



(61)

Galactaric acid on condensation with chloral in the presence of sulphuric acid gave the diester derivative (60), and potassium hydrogen D-glucarate gave the lactone diacetal (61).[212]

From Single Alcoholic Groups.—Two new sulphur-containing acetal groups have been developed for the protection of hydroxy-groups (Scheme 22).



Reagents: i, H^+; ii, 3-chloroperbenzoic acid

Scheme 22

[208] T. A. Koerner, E. S. Younathan, and J. D. Wander, *Carbohydrate Res.*, 1972, **21**, 455.
[209] T. D. Inch, *Carbohydrate Res.*, 1972, **21**, 37.
[210] T. G. Bonner, E. J. Bourne, R. F. J. Cole, and D. Lewis, *Carbohydrate Res.*, 1972, **21**, 29.
[211] R. Bonn and I. Dijong, *Chem. Ber.*, 1972, **105**, 3833.
[212] R. Brettle, *Carbohydrate Res.*, 1972, **22**, 450.

Whereas the sulphide derivatives were somewhat more labile toward acid than oxygen-containing analogues, the sulphones were more stable. These derivatives are achiral and do not suffer from the disadvantages consequent upon the use of tetrahydropyranyl ethers.[213]

[213] J. H. van Boom, P. van Deursen, J. Meeuwse, and C. B. Reese, *J.C.S. Chem. Comm.*, 1972, 766.

6
Esters

There have been fewer references than in previous years in all sections of this chapter.

Carboxylic Esters

Improved preparations of 1,2,3,6- and 1,2,4,6-tetra-acetyl-*O*-β-D-glucopyranose [214] have been reported and also of β-D-galactofuranose penta-acetate.[215] 6-*O*-Acetyl-D-glucose has been prepared by esterification of the free sugar with dilute acetic acid at 100 °C and removal of the unreacted sugar by fermentation with yeast.[216]

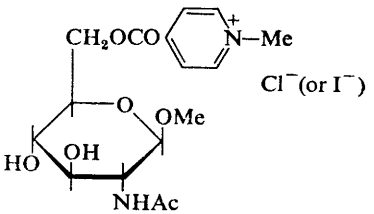
Sucrose octa-acetate can be deacetylated selectively on alumina to give the 2,3,4,6,1′,3′,4′-hepta-acetate.[217] Acetyl migrations from O-4 to O-6 and from O-3 to O-4 occurred during methylation of ethyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside with methyl iodide and silver oxide in DMF.[218] Other acetyl migrations have been reviewed [218] and details of the rearrangements of acetylated cyclitols [219] and cyclohexane-1,2-diols [220] are given in Chapter 19.

An improved synthesis of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2-benzoate has been described using *N*-benzoylimidazole.[220a] The alkaline hydrolysis of glycol benzoates in aqueous DMSO, aqueous ethanol, or aqueous methanol has been studied. It was shown that the rate of hydrolysis is markedly dependent on the solvent used and that this dependency could be rationalized in terms of solvation of the transition state.[221]

The benzoylpropionyl ester group has been shown to be a good protecting group, which is removable at pH 7 in the presence of benzoate groups, whereas removal in the presence of acetate groups caused side-reactions to occur.[222]

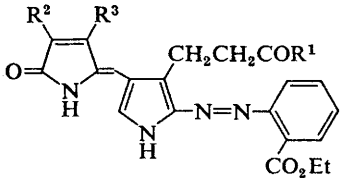
[214] B. H. Koeppen, *Carbohydrate Res.*, 1972, **24**, 154.
[215] G. J. F. Chittenden, *Carbohydrate Res.*, 1972, **25**, 35.
[216] V. Bilik, *Chem. Zvesti*, 1972, **26**, 82.
[217] J. M. Ballard, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1972, **24**, 152.
[218] R. M. Rowell, *Carbohydrate Res.*, 1972, **23**, 417.
[219] H. Paulsen and H. Höhne, *Chem. Ber.*, 1972, **105**, 3445.
[220] I. Lundt and C. Pedersen, *Acta Chem. Scand.*, 1972, **26**, 1938.
[220a] N. L. Holder and B. Fraser-Reid, *Synthesis*, 1972, 83.
[221] M. Balakrishnan, G. V. Rao, and N. Venkatasubramanian, *Tetrahedron Letters*, 1972, 4617.
[222] N. Belorizky, G. Excoffier, D. Gagnaire, J. P. Utille, M. Vignon, and P. Vottero, *Bull. Soc. chim. France*, 1972, 4749.

Nicotinic esters of D-glucose, D-galactose, D-mannose, L-arabinose, and D-xylose have been described.[223] The ester (62) has been synthesized and it

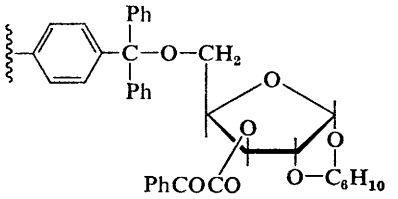


(62)

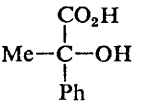
binds to lysozyme in a similar way to methyl 2-acetamido-2-deoxy-β-D-glucopyranoside.[224] Pigments prepared by diazotization of dog bile with ethyl anthranilate have been characterized (mostly by mass spectrometry) as (63)—(66).[225]



	R^1	R^2	R^3
(63)	β-D-xylopyranosyloxy	Me	$CH=CH_2$
(64)	β-D-xylopyranosyloxy	$CH=CH_2$	Me
(65)	β-D-glucopyranosyloxy	Me	$CH=CH_2$
(66)	β-D-glucopyranosyloxy	$CH=CH_2$	Me



(67)



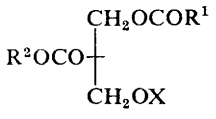
(68)

The polymer-bound sugar (67) has been used successfully in a synthesis of (*R*)-atrolactic acid (68), which was obtained in 77% chemical yield and 65% optical yield.[226]

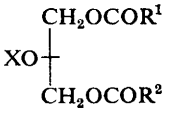
Diacylglycerol sulphonates (69) and (70) each gave mixtures of the products (71) and (72) when treated with carboxylate anions ($R^3CO_2^-$),

[223] E. A. Forlano, J. O. Deferrari, and R. A. Cadenas, *Carbohydrate Res.*, 1972, **21**, 484.
[224] J. Verhoeven and R. Schwyzer, *Helv. Chim. Acta*, 1972, **55**, 2572.
[225] F. Compernolle, G. P. van Hees, J. Fevery, and K. P. M. Heirwegh, *Biochem. J.*, 1971, **125**, 811.
[226] M. Kawana and S. Emoto, *Tetrahedron Letters*, 1972, 4855.

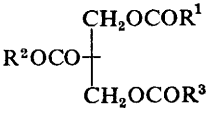
presumably by way of such intermediates as (73).[227] 3-*O*-β-D-Galacto-pyranosyl-1,2-di-*O*-palmitoyl-*sn*-glycerol has been described.[228] Glycosyl



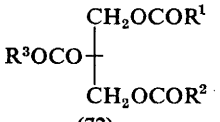
(69)



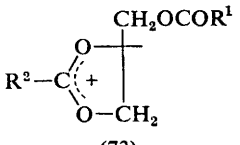
(70)



(71)



(72)

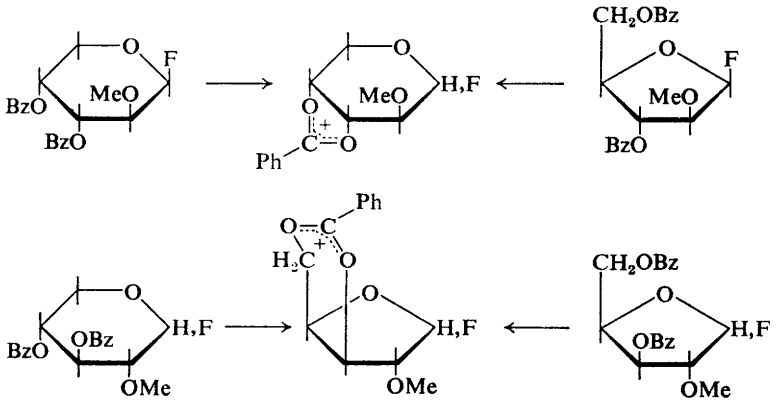


(73)

$X = MeSO_2$ or p-$MeC_6H_4SO_2$
$R^1 = C_{17}H_{35}$; $R^2 = C_{17}H_{35}$ or $C_{15}H_{31}$

esters of amino-acids have been prepared by way of the pentachlorophenyl esters.[229]

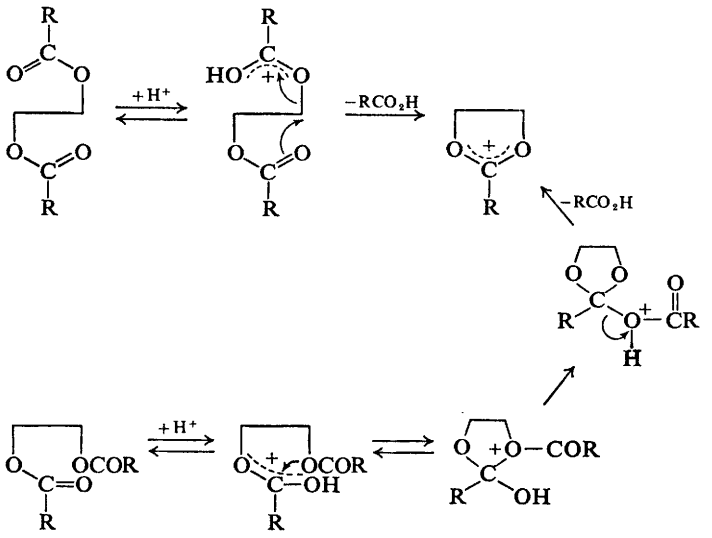
A detailed study has been made of the reactions of esters of 2-*O*-methyl-D-xylose and -D-arabinose with anhydrous hydrogen fluoride. The glycosyl fluorides were formed first and the cyclic acyloxonium ions were then produced more slowly. *cis*-Bicyclic ions were produced with, if necessary, a change in size of the sugar ring (Scheme 23).[230]



Scheme 23

[227] R. Aneja and A. P. Davies, *Tetrahedron Letters*, 1972, 4497.
[228] A. I. Bashkatova, G. V. Smirnova, V. N. Volynskaya, V. I. Shvets, and R. P. Evstigneeva, *Doklady Akad. Nauk S.S.S.R.*, 1971, **201**, 1231.
[229] D. Keglević, A. Kornhauser, and Š. Valenteković, *Carbohydrate Res.*, 1972, **22**, 245.
[230] S. Jacobsen, S. R. Jensen, and C. Pedersen, *Acta Chem. Scand.*, 1972, **26**, 1561.

Trifluoromethanesulphonic acid has been found to promote both 'back-side' and 'front-side' formation of cyclic acyloxonium ions as shown in Scheme 24.[231]



Scheme 24

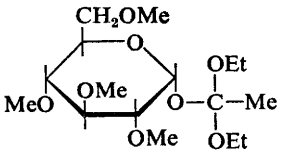
1-*O*-Acyl derivatives of sugars have been obtained by the reaction of acetohalogeno-D-glucose and -mannose with t-butyl esters of carboxylic acids (acetic, benzoic, *etc.*) using silver carbonate as catalyst.[232]

Carboxylic Orthoesters

Sugar orthoesters have been reviewed from the viewpoint of their use in synthesis.[233]

Acid-catalysed hydrolysis of 3,4,6-tri-*O*-methyl-1,2-*O*-(1-alkyloxyethylidene)-α-D-glucopyranose yielded the 2- and 1-acetates, the proportion of which varied with the solvent and concentration of acid. Low concentrations of acid gave mostly the 2-ester, whereas higher concentrations gave chiefly the 1-ester. A mechanism based on the intermediacy of a 1,2-acetoxonium ion was proposed.[234] The same workers have also studied the ethanolysis of the orthoester (74) catalysed by 2,6-dichlorobenzoic acid and showed that ethyl 2,3,4,6-tetra-*O*-methyl-D-glucopyranosides (β : α

[231] H. Paulsen and H. Meyborg, *Tetrahedron Letters*, 1972, 3973.
[232] N. K. Kochetkov, E. M. Klimov, S. A. Pogosjan, and V. A. Derevitskaya, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1972, 1657.
[233] N. K. Kochetkov and A. F. Bochkov, *Recent Develop. Chem. Natural Carbon Compounds*, 1971, **4**, 75.
[234] L. R. Schroeder, D. P. Hultman, and D. C. Johnson, *J.C.S. Perkin II*, 1972, 1063.



(74)

ratio 29.5 : 1) were minor products (36.6%) accompanying the principal product 2,3,4,6-tetra-*O*-methyl-D-glucose (63.3%). Addition of lithium toluene-*p*-sulphonate caused the D-glucosides to predominate, but the anomeric $\beta : \alpha$ ratio was then reduced to 2.1 : 1. These findings were rationalized mechanistically.[235]

Phosphates

Glycosyl phosphates[236] and the synthesis of sucrose phosphates[237] have been reviewed; details of the synthesis of sucrose 6′-phosphate have been given.[238] One of the main, neutral products from the γ-radiolysis of D-glucose 6-phosphate was 1,6-di-*aldehydo*-D-glucose.[239]

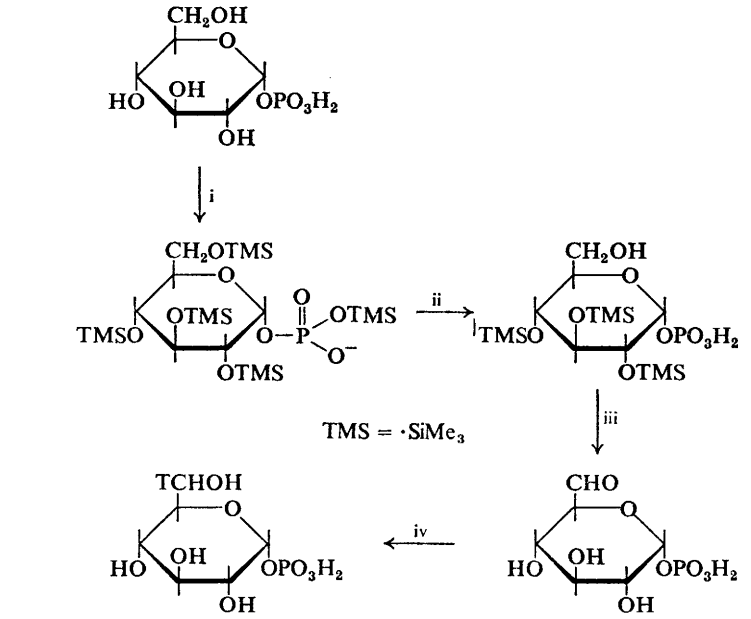
α-D-[6-^{3}H]Glucopyranosyl phosphate has been synthesized as shown in Scheme 25,[240] and β-D-galactofuranosyl phosphate has been prepared by a number of routes.[215]

The use of *o*-phenylenephosphorochloridate in the synthesis of glycosyl phosphates has been studied and the reagent was used in the preparation of β-D-mannopyranosyl 1-phosphate.[241] Pyrophosphodiesters of monosaccharides and isoprenoid alcohols such as (75), which are intermediates in the biosynthesis of bacterial cell-wall components, have been synthesized.[242]

Phosphorylation of protected sugars has been carried out with HMPT,[243] and with dimethylcarbamoyl- and dimethylamido-1,3-butylene phosphite.[244]

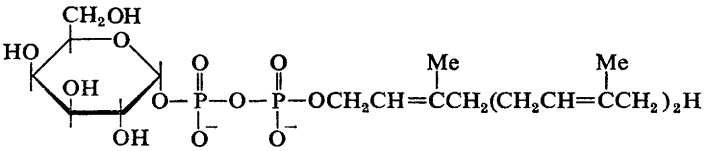
The 1,6-diphosphates of methyl α- and β-D-fructofuranoside and 2,5-anhydro-D-glucitol and -mannitol were shown to be inhibitors of D-fructose 1,6-phosphatase.[245]

[235] D. P. Hultman, L. R. Schroeder, and F. C. Haigh, *J.C.S. Perkin II*, 1972, 1525.
[236] Yu. Yu. Kosov and V. N. Shibaev, *Uspekhi Biol. Khim.*, 1971, **12**, 182.
[237] J. G. Buchanan, in 'Sugar: Chemical, Biological, and Nutritional Aspects of Sucrose', ed. J. Yudkin, Butterworths, London, 1971.
[238] J. G. Buchanan, D. A. Cummerson, and D. M. Turner, *Carbohydrate Res.*, 1972, **21**, 283.
[239] N. K. Kochetkov, L. I. Kudrjashov, M. A. Chlenov, and L. P. Grineva, *Doklady Akad. Nauk S.S.S.R.*, 1972, **202**, 847.
[240] V. N. Shibaev, Yu. Yu. Kusov, and N. K. Kochetkov, *Doklady Adak. Nauk S.S.S.R.*, 1972, **203**, 612.
[241] H. S. Prihar and E. J. Behrman, *Carbohydrate Res.*, 1972, **23**, 456.
[242] C. D. Warren and R. W. Jeanloz, *Biochemistry*, 1972, **11**, 2565.
[243] N. K. Kochetkov, E. E. Nifantev, I. P. Gudkova, N. L. Ivanova, K. V. Vlasova, and V. A. Leskin, *Zhur. obshchei Khim.*, 1972, **42**, 450.
[244] E. E. Nifantev, I. P. Gudkova, and Chan Din Dus, *Zhur. obshchei Khim.*, 1972, **42**, 506.
[245] S. J. Benkovic, J. J. Keinschuster, M. M. de Maine, and I. J. Siewers, *Biochemistry*, 1971, **10**, 4881.



Reagents: i, Me_3SiCl; ii, K_2CO_3–MeOH; iii, DMSO–H_2O; iv, NaB^3H_4

Scheme 25



(75)

The synthesis of 1-*O*-(1,2-di-*O*-stearoyl-glyceryl-3-phosphoryl)-*myo*-inositol [246] has been described.

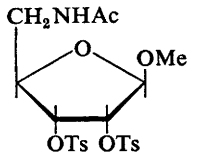
An extended Hückel calculation of the electronic structure of the ground and first excited states of the secondary anion of D-glucose 1-phosphate was able to correlate the weakest calculated bonds with the sites of enzymic attack.[247] D-Fructose 6-phosphate and 1,6-diphosphate have been studied by both ^{13}C [248] and ^{31}P [249] n.m.r. spectroscopy.

[246] A. I. Lutik, V. N. Krylova, B. A. Klyashchitskii, S. P. Kozlova, V. I. Shvets, R. P. Evstigneeva, and B. S. Zhdandovitch, *Zhur. obshchei Khim.*, 1971, **41**, 2747.
[247] R. Gaspar, *European Biophys. Congr. Proc.*, 1971, **4**, 215.
[248] S. J. Benkovic, J. L. Engle, and A. S. Mildvan, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 852.
[249] G. R. Gray, *Biochemistry*, 1971, **10**, 4705.

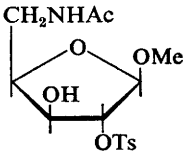
Sulphonates

A systematic study of the selective tosylation of methyl 4,6-*O*-benzylidene-D-glycosides (both anomers) in the *allo*, *altro*, *gluco*, and *manno* series has been made. Preferential esterification of equatorial hydroxy-groups in *cis*-diol systems was observed and, as with other systems, hydrogen-bonding effects directed the reaction.[250] Selective tosylation of methyl 2-benzamido-2-deoxy-α-D-glucopyranoside gave a mixture of the 6-, 3,6-, and 4,6-sulphonates (25, 20, and 12%, respectively).[251] The 6-toluene-*p*-sulphonate has been prepared independently and was shown to undergo conventional displacement and elimination reactions.[252] 1,2:3,5-Di-*O*-benzylidene-6-(naphthylamino-5-vinylsulphonyl)-α-D-glucofuranose has been prepared from the sulphone and the sugar.[252a]

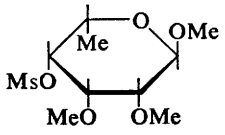
Displacements with a variety of nucleophiles on the D-ribofuranose derivative (76) gave only the *xylo*-product (77) by way of acetamido-group participation.[253]



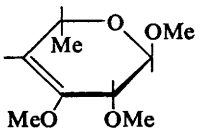
(76)



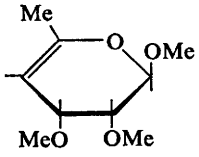
(77)



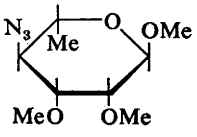
(78)



(79)



(80)



(81)

Treatment of methyl 6-deoxy-2,3-di-*O*-methyl-4-*O*-methylsulphonyl-α-L-talopyranoside (78) with azide ion in DMF gave the hex-3-enoside (79) as the major product together with the hex-4-enoside (80) and the azide (81); similar results were obtained with the benzyl glycoside analogue of (78). The reasons for the preponderance of elimination over substitution reactions were discussed.[254]

4-Sulphonic esters of sucrose have been used as intermediates in the synthesis of α-D-galactopyranosyl β-D-fructofuranoside.[255]

[250] S. E. Creasey and R. D. Guthrie, *Carbohydrate Res.*, 1972, **22**, 487.
[251] R. Khan and L. Hough, *Carbohydrate Res.*, 1972, **24**, 141.
[252] R. Khan and L. Hough, *Carbohydrate Res.*, 1972, **24**, 147.
[252a] I. A. Germanova and M. A. Chekalin, *Zhur. Khim. obshchest.*, 1972, **17**, 358.
[253] J. Hildesheim, J. Cleophax, and S. D. Gero, *Bull. Soc. chim. France*, 1972, 3450.
[254] A. K. Al-Radhi, J. S. Brimacombe, and L. C. N. Tucker, *Carbohydrate Res.*, 1972, **22**, 103.
[255] R. Khan, *Carbohydrate Res.*, 1972, **25**, 232.

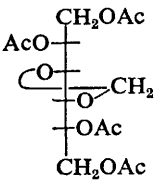
Other Esters

The 2,4-phenylboronate of 1,6-anhydro-β-D-glucopyranose has been described.[256] The transesterification of borates {$(RO)_3B$} by monosaccharides in water has been studied polarimetrically. The reaction path involved a transition state in which one molecule of $(RO)_3B$ co-ordinated with the pyranose ring-oxygen atom whilst a second molecule attacked the glycosyl hydroxy-group.[257]

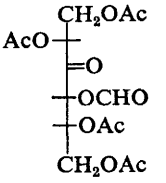
Mass spectral fragmentations of cyclic phenylboronates with five-, six-, and seven-membered rings have been studied and the results were applied to a study of phenylboronates of alditols.[258]

A number of 5,6-carbonates of D-glucofuranoses have been prepared by simply heating the 5,6-diol with ethylene carbonate in a soda-glass tube at 140 °C.[259]

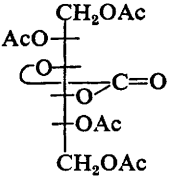
The oxidation of cyclic methylene acetals of acetylated sugars with chromium trioxide–acetic acid was profoundly affected by the presence of acetic anhydride. For example, (82) gave, instead of (83), the cyclic carbonate (84). A variety of methylene acetals were studied and it was shown



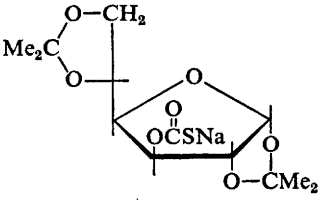
(82)



(83)



(84)



(85)

that six- and seven-membered cyclic acetals gave poor yields of cyclic carbonates.[260]

The preparation and properties of monothiocarbonate derivatives, for example (85), have been examined.[261] Dithiobis(thioformates)

[256] S. P. Valueva, E. P. Cherneva, V. A. Kargin, and N. M. Merlis, *Vysokomol. Soedineniya* (*B*), 1971, **13**, 468.
[257] V. V. Gertsev and L. A. Frolova, *Doklady Akad. Nauk S.S.S.R.*, 1971, **200**, 1107.
[258] I. R. McKinley and H. Weigel, *J.C.S. Chem. Comm.*, 1972, 1051.
[259] P. A. Gent, R. Gigg, and R. Conant, *J.C.S. Perkin I*, 1972, 248.
[260] S. J. Angyal and M. E. Evans, *Austral. J. Chem.*, 1972, **25**, 1513.
[261] B. S. Shasha, W. M. Doane, and C. R. Russell, *Carbohydrate Res.*, 1972, **24**, 202.

[ROC(S)SSC(S)OR, where R is carbohydrate] decomposed to the hydroxy-compound when they reacted with two equivalents of 4-chlorobenzenethiol in pyridine. Use of less than two equivalents of the reagent gave the intermediate mixed disulphide.[262]

Tri- and tetra-(*N*-phenylcarbamate) and -(*N*-methyl-*N*-phenylcarbamate) derivatives of methyl α- and benzyl β-D-glucopyranoside have been prepared. The latter compounds were resistant to hydrogenolysis. D-Glucopyranose 2,3,4-tri-(*N*-phenylcarbamate) and -(*N*-methyl-*N*-phenylcarbamate) have been prepared from 1,6-anhydro-β-glucopyranose and, on treatment with phosphoric anhydride, gave in the first case a cross-linked polymer and in the second one a monomeric diphosphate.[263]

Sugar amidosulphates have been obtained by reaction of pyridinium salts of sugar sulphates with ethoxyacetylene followed by amine treatment.[264]

The conformations of a number of nitrate esters have been determined by *X*-ray and c.d. methods.[265]

[262] B. S. Shasha, E. I. Stout, W. M. Doane, and C. R. Russell, *Carbohydrate Res.*, 1972, **24**, 115.
[263] R. Eby and C. Schuerch, *Carbohydrate Res.*, 1972, **25**, 133.
[264] N. K. Kochetkov, A. I. Usov, and V. V. Deriabin, *Zhur. obshchei Khim.*, 1972, **42**, 2763.
[265] R. E. Barton and L. D. Hayward, *Canad. J. Chem.*, 1972, **50**, 1719.

7
Halogenated Sugars

Glycosyl Halides

The formation of glycosyl chlorides by treatment of aldose 1,2-orthoester derivatives with dichloromethyl methyl ether has been reported. Using this procedure, 3,4,6-tri-*O*-acetyl-β-D-mannopyranose 1,2-(methyl orthoacetate) and 3,4,6-tri-*O*-acetyl-α-D-glucopyranose 1,2-(ethyl orthoacetate) were converted into 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl chloride and the corresponding β-D-glucopyranosyl chloride, respectively.[266]

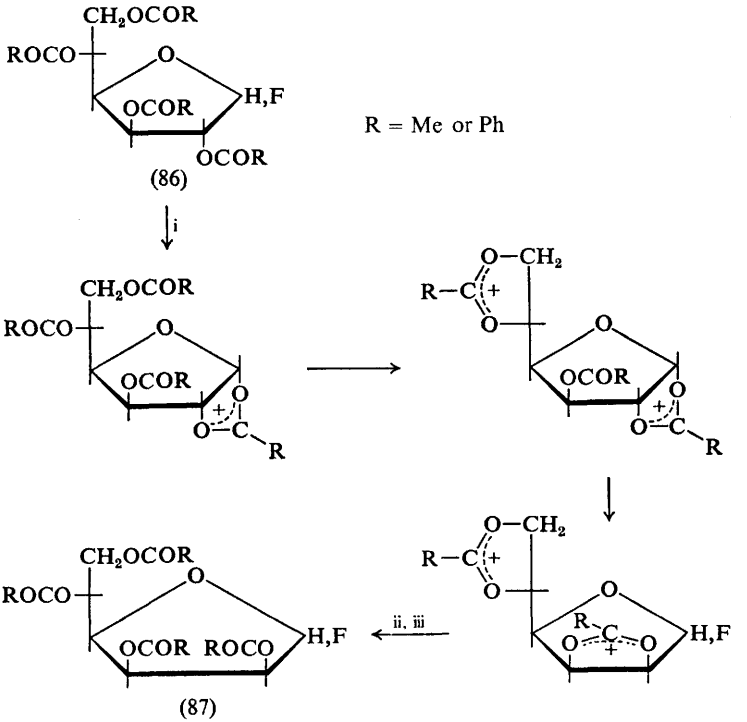
Reference is made to the alcoholysis of some glycosyl halides in Chapter 3. Phillips and his co-workers have continued their longstanding interest in this area by investigating the hydrolyses and halogen exchanges of aceto-bromo-sugars in acetone. The results indicated that bimolecular halogen exchange appears to be the rate-controlling step preceding rapid hydrolysis.[267] A related study of the alcoholysis of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide and the 5-thio-analogue has shown that both compounds react by an S_N1 process. Various alcohols were used and the effect of adding bromide ion was found to vary with the alcohol.[268] The reactions of acetobromo-derivatives of 2-deoxy-2-(2,4-dinitroanilino)-D-glucopyranose and a number of related monomethyl ethers with ethanol either in nitromethane in the presence of silver carbonate or in chloroform–pyridine have been studied. Under the former conditions, all compounds gave mainly β-glycosides, whereas in chloroform–pyridine solutions α-products predominated, except in the case of the 6-*O*-methylglycosyl bromide. This indicated the possibility of the α-glycosides arising by a process that involves participation by the acetoxy-group at C-6. It was suggested that pyridine could stabilize the C-1—C-6 acetoxonium ion formed after assisted displacement of bromide anion.[269] Hydrolytic studies on various β-D-glucosyl chlorides and corresponding α-1,2-orthoesters have shown that the products are very dependent on the acidity of the solution. As the acidity increased, less of the products of direct hydrolysis (*i.e.* the free sugar) was formed and higher proportions of isomeric 2-hydroxyglycosyl acetates were

[266] I. Farkas, Z. Dinya, I. F. Szabó, and R. Bognár, *Carbohydrate Res.*, 1972, **21**, 331.
[267] M. J. Duffy, M. Jeffries, G. Pass, and G. O. Phillips, *J.C.S. Perkin II*, 1972, 821.
[268] C. Yamagami, K. Shinkoda, T. Funabiki, and A. Sera, *Nippon Kagaku Kaishi*, 1972, 1266.
[269] P. F. Lloyd, B. Evans, and R. J. Fielder, *Carbohydrate Res.*, 1972, **22**, 111.

obtained. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl chloride, for example, gave 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose in 75% yield under appropriate conditions, and these procedures can be utilized for the general synthesis of 2-hydroxy- and 2-*O*-substituted-D-glucose derivatives.[270]

Continuing their studies on D-arabinofuranosyl derivatives with a benzyl group at C-2, Glaudemans and Fletcher have shown that the reaction of compounds of either anomeric configuration with hydrogen halides gives initially 1,2-*trans* products (*i.e.* α-glycosyl halides), which then partially anomerize.[271]

The 2,3,5,6-tetra-*O*-acyl-D-glucofuranosyl fluorides (86) were converted into D-mannofuranose derivatives (87) following treatment with anhydrous hydrogen fluoride as shown in Scheme 26.[272] The conformation of 2,3,4-tri-*O*-acetyl-β-D-arabinopyranosyl bromide in the crystalline form is essentially the same as that observed in solution.[272a]



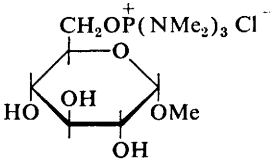
Reagents: i, HF; ii, H_2O; iii, $(RCO)_2O$–py

Scheme 26

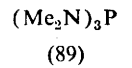
[270] W. E. Dick, jun., *Carbohydrate Res.*, 1972, **21**, 255.
[271] C. P. J. Glaudemans and H. G. Fletcher, *J. Org. Chem.*, 1971, **36**, 3598.
[272] K. Bock and C. Pedersen, *Acta Chem. Scand.*, 1972, **26**, 2360.
[272a] P. W. R. Corfield, J. D. Mokren, P. L. Durette, and D. Horton, *Carbohydrate Res.*, 1972, **23**, 158.

Other Halogenated Derivatives

Hanessian and his colleagues have described in full their general method for replacing primary hydroxy-groups in sugars by halogeno-groups; treatment in DMF with two equivalents each of triphenylphosphine and the appropriate *N*-halogenosuccinimide effected the transformation. Groups commonly used in the protection of hydroxy- and amino-groups were unaffected under the mild conditions of the reaction, and selective halogenation of hydroxymethyl-groups in polyhydroxy-compounds can be achieved.[273] A related reaction involved the use of alkoxyphosphonium salts [*e.g.* (88)], which were prepared using carbon tetrachloride and tris-(dimethylamino)phosphine (89) in DMF. The alkoxyphosphonium salts

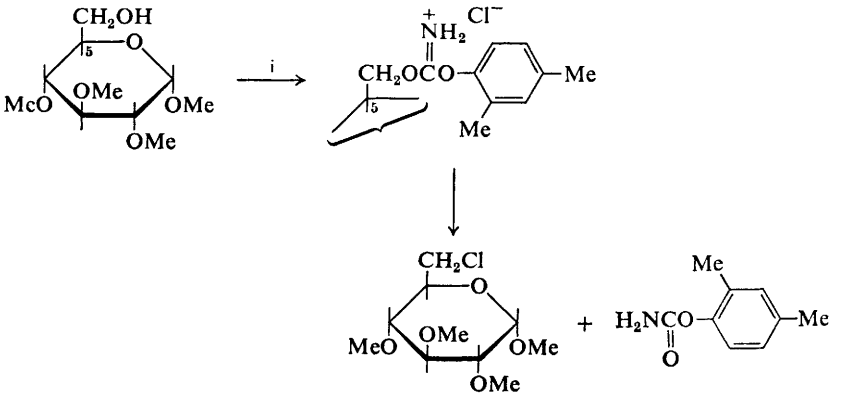


(88)



(89)

underwent nucleophilic displacements to yield primary halides, *etc.*[274] A similar approach to the synthesis of chloro-sugars utilized the reaction between sugar alcohols and aryl cyanates in the presence of hydrogen chloride to give thermally unstable intermediates, which decomposed to give aryl carbamates and the chlorodeoxy-compounds (Scheme 27). Secondary as well as primary hydroxy-groups reacted under these conditions; methyl 3-chloro-3-deoxy-2,4,6-tri-*O*-methyl-β-D-allopyranoside, for



Reagents: i, 2,4-di-MeC_6H_3OCN–HCl

Scheme 27

[273] S. Hanessian, M. M. Ponpipom, and P. Lavallee, *Carbohydrate Res.*, 1972, **24**, 45.
[274] B. Castro, Y. Chapleur, B. Gross, and C. Selve, *Tetrahedron Letters*, 1972, 5001.

example, has been synthesized in 17% yield by this method from the corresponding D-*gluco*-alcohol.[275]

Treatment of 1,2:5,6-di-*O*-isopropylidene-β-D-talofuranose with triphenylphosphine in carbon tetrachloride gave the 3-chloro-3-deoxy-D-*ido*-compound, the structure of which was determined crystallographically.[276]

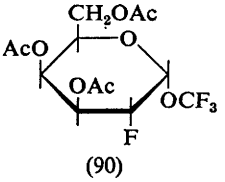
Deoxyfluoro-sugars have continued to command appreciable attention and their synthesis and reactivity have been reviewed.[277] The Jarovenko reagent (Et_2NCF_2CHFCl), by which alcohols can be converted directly into fluoro-compounds, has been reviewed with examples taken from carbohydrate chemistry.[278] A further reaction has been reported which is of potential value for the same purpose; phenyltetrafluorophosphorane ($PhPF_4$) used with silyl ethers resulted in the formation of fluorinated derivatives (Scheme 28).[279]

$$ROH \xrightarrow{i} ROSiMe_3 \xrightarrow{ii} [ROPF_3Ph] \longrightarrow RF + PhP(O)F_2$$

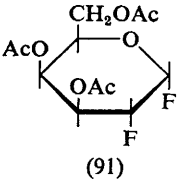
Reagents: i, Me_3SiCl–py; ii, $PhPF_4$

Scheme 28

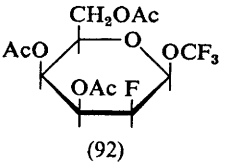
3,4,6-Tri-*O*-acetyl-D-galactal on treatment with trifluoro(fluoroxy)-methane at −80 °C afforded the products (90)—(93); 2-deoxy-2-fluoro-D-galactose was obtained by acidic hydrolysis of (90) and (91) (*cf.* Vol. 3, p. 59).[280]



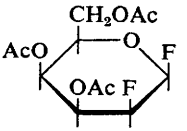
(90)



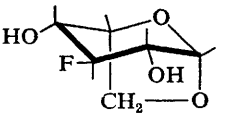
(91)



(92)



(93)



(94)

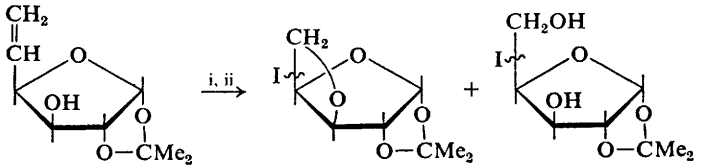
3-Deoxy-3-fluoro-D-glucose inhibited the utilization of lactose and other sources of carbon by *E. coli* for reasons which were discussed[281] and, for biological purposes, the 1- and 6-phosphates of this fluorinated sugar have

[275] A. Klemer and G. Mersmann, *Carbohydrate Res.*, 1972, **22**, 425.
[276] F. W. B. Einstein and K. N. Slessor, *Canad. J. Chem.*, 1972, **50**, 93.
[277] P. W. Kent, *Carbon–Fluorine Compounds: Chem., Biochem., Biol. Activ. Symp.*, 1971, 169.
[278] F. Liska, *Chem. listy*, 1972, **66**, 189.
[279] D. U. Robert and J. G. Riess, *Tetrahedron Letters*, 1972, 847.
[280] J. Adamson and D. M. Marcus, *Carbohydrate Res.*, 1972, **22**, 257.
[281] R. J. Miles and S. J. Pirt, *Biochem. J.*, 1972, **127**, 60P.

been prepared by standard reactions.[282] Electrolytic oxidation afforded the corresponding aldonic acids (see Chapter 17).

Derivatives of 3-deoxy-3-fluoro-L-idose have been prepared from 3-deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose by partial hydrolysis and inversion of the configuration at C-5 utilizing a nucleophilic displacement reaction.[283] In subsequent work, the free sugar was obtained by mild hydrolysis of 3-deoxy-3-fluoro-1,2-*O*-isopropylidene-β-L-idofuranose, but under more vigorous conditions the crystalline 1,6-anhydride (94) was formed. ^{19}F N.m.r. spectroscopy of 3-deoxy-3-fluoro-L-idose indicated that the equilibrium distribution of tautomers is similar to that of L-idose itself. In the course of this work, the conformation of each pyranose form was deduced from F–H coupling constants and the chemical shift of the fluorine atom.[43] 2,3,6-Tri-*O*-acetyl-4-deoxy-4-fluoro-α- and -β-D-glucopyranosyl fluorides have been prepared and the mechanisms of observed five-bond fluorine–fluorine couplings were discussed.[283a] ^{19}F N.m.r. spectroscopy of a number of fluoro-sugars is discussed in more detail in Chapter 23.

Reduction of 6-deoxy-6-fluoro-α-D-galactose to the corresponding alditol has been effected by either sodium borohydride or a polyol dehydrogenase; the biological reduction occurred approximately three times faster than the corresponding reduction of D-galactose. In the same work, 6-deoxy-6-fluoro-α-D-galactopyranosyl dipotassium phosphate was prepared by both chemical and enzymic procedures.[284] Other workers have entered the area of fluorinated alditols by synthesizing 1,6-dideoxy-1,6-difluoro-galactitol and -D-mannitol (also 1,6-dibromo-1,6-dideoxy-L-mannitol) as potential antitumour agents.[285] Continuing their work on the modification of αα-trehalose, Hough and his co-workers have now described 6,6′-dideoxy-6,6′-difluoro-αα-trehalose.[286]



Reagents: i, CF_3CO_2Ag–I_2–MeCN; ii, MeOH

Scheme 29

[282] J. A. Wright, N. F. Taylor, R. V. Brunt, and R. W. Brownsey, *J.C.S. Chem. Comm.*, 1972, 691.

[283] J. S. Brimacombe, A. M. Mofti, and J. H. Westwood, *Carbohydrate Res.*, 1972, **21**, 297.

[283a] A. B. Foster, J. H. Westwood, B. Donaldson, and L. D. Hall, *Carbohydrate Res.*, 1972, **25**, 228.

[284] P. W. Kent and J. R. Wright, *Carbohydrate Res.*, 1972, **22**, 193.

[285] E. M. Acton, M. Keyanpour-Rad, J. E. Christensen, H. H. Tong, R. P. Kwok, and L. Goodman, *Carbohydrate Res.*, 1972, **22**, 477.

[286] L. Hough, A. K. Palmer, and A. C. Richardson, *J.C.S. Perkin I*, 1972, 2513.

A new method of introducing iodine into sugars involved the addition of iodine trifluoroacetate, a reagent used with unsaturated steroids to give iodo-trifluoroacetoxy adducts. An application of this method to sugars is illustrated in Scheme 29; when the 3-hydroxy-group of the initial olefin was protected, formation of the anhydro-sugar was eliminated.[287]

Mass spectrometry of 6-deoxy-6-halogeno-sugars is discussed in Chapter 24. The preparation of 5-bromo-5-deoxypentoses has been noted [18] already and the selective, reductive dechlorination of secondary chloro-groups on sugars is mentioned in Chapter 13.

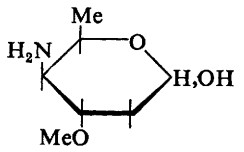
[287] R. G. S. Ritchie and W. A. Szarek, *Canad. J. Chem.*, 1972, **50**, 507.

8
Amino-sugars*

Natural Products†

The disaccharide 2-acetamido-2-deoxy-4-*O*-(β-*N*-acetylmuramyl)-D-glucose and an *O*-acetyl derivative have been isolated from cell walls of *Staphylococcus aureus* Copenhagen and were characterized by chemical and n.m.r. studies.[288]

Investigations of the interactions of hen egg-white lysozyme with three isomeric trisaccharides, *viz.* 4-nitrophenyl tri-*N*-acetylchitotrioside and 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)- and -(1 → 3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosides suggested that the lysozyme specificity, closely associated with the conformation of the active site of the enzyme, can vary extensively.[289] The 4-amino-sugar (95) occurs naturally in a steroidal glycoside and has been named D-holacosamine.[139a]



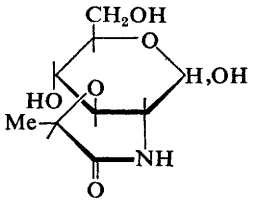
(95)

Synthesis

2-Amino-3-*O*-(L-1-carboxyethyl)-2-deoxy-D-glucose ('isomuramic acid') and its *N*-acetyl derivative have been prepared from benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α- and -β-D-glucopyranoside by condensation with D-2-chloropropionic acid followed by standard reaction sequences.[290] Syntheses of the D-*manno*-isomer [291] and the internal amide [292] (96) of muramic acid have been reported.

[288] D. J. Tipper, M. Tomoeda, and J. L. Strominger, *Biochemistry*, 1971, **10**, 4683.
[289] A. Ya. Khorlin, E. A. Shashkova, and S. E. Zurabyan, *Carbohydrate Res.*, 1972, **21**, 269.
[290] J. M. Petit, P. Sinäy, E. Walker, D. A. Jeanloz, and R. W. Jeanloz, *Carbohydrate Res.*, 1972, **24**, 415.
[291] P. Sinäy, M. D. A. Halford, M. S. Choudhary, P. H. Gross, and R. W. Jeanloz, *J. Biol. Chem.*, 1972, **247**, 391.
[292] P. Sinäy, J.-M. Petit, C. Merser, and R. W. Jeanloz, *Carbohydrate Res.*, 1972, **21**, 339.

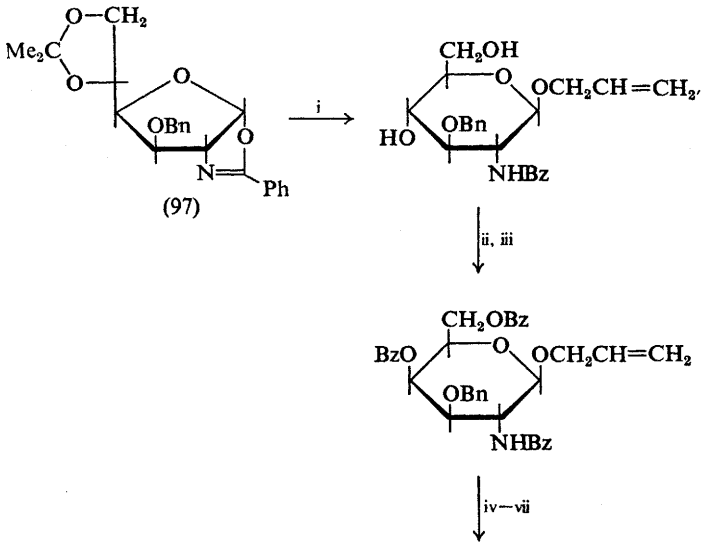
* See also Part I, Chapter 12.
† See also Part I, Chapter 20.



(96)

The easily accessible D-glucosamine derivative (97) has been used in a convenient synthesis of 2-amino-2-deoxy-D-galactose (Scheme 30).[293]

3-Deoxy-3-methylamino-D-xylose (gentosamine) has been prepared from 3-amino-3-deoxy-D-glucose derivatives by standard reactions, which included



(97)

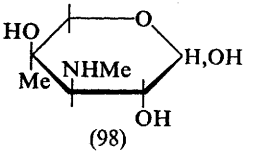
Reagents: i, CH_2=$CHCH_2OH$–TsOH; ii, MsCl–py; iii, NaBz–DMF; iv, NaOH; v, Ac_2O–py; vi, Pd–C; vii, 2N-HCl

Scheme 30

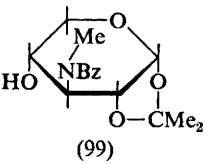
the periodate oxidation of a 5,6-diol in a furanose derivative. Garosamine (98) (3-deoxy-4-*C*-methyl-3-methylamino-L-arabinose) was also synthesized using the oxidation of (99) as the key step.[294]

An improved synthesis of acetylated 3-amino-3-deoxy-D-ribofuranose (Scheme 31) has been developed to facilitate the synthesis of puromycin;

[293] P. A. Gent, R. Gigg, and R. Conant, *J.C.S. Perkin I*, 1972, 277.
[294] W. Meyer zu Reckendorf and E. Bischof, *Chem. Ber.*, 1972, **105**, 2546.

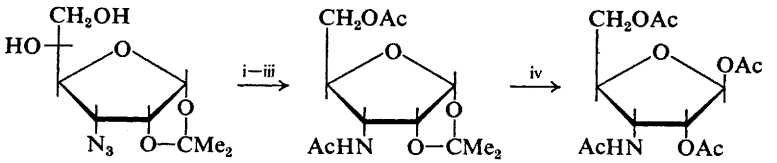


(98)



(99)

the overall yield from D-glucose has been increased from 5 to 29%.[295] 3-Amino-3-deoxy-D-ribose derivatives have also been prepared from 1,2:5,6-di-*O*-cyclohexylidene-D-glucofuranose using an oxidation, oximation, and reduction sequence to introduce the amino-group.[296] Oximes



Reagents: i, IO_4^-; ii, $NaBH_4$; iii, Ac_2O–py; iv, Ac_2O–AcOH–H_2SO_4

Scheme 31

have also been used in the synthesis of derivatives of 3-amino-3,5-dideoxy-1,2-*O*-isopropylidene-α-D-ribofuranose and -β-D-lyxofuranose,[297] 4-amino-4,6-dideoxy-D-allose,[298] and 4-amino-4-deoxy-D-*glycero*-D-*allo*-heptose.[28] Methyl 3-amino-3,4,6-trideoxy-α-D-*xylo*-hexopyranoside and its *N*-methyl derivative have been prepared by way of an oxiran intermediate.[298a] Methyl 2-benzamido-2,3,6-trideoxy-α-D-*ribo*-hexopyranoside has been described.[251]

A potentially useful synthesis of amino-sugars has been developed by treating hydroxy-groups with phthalimide, diethyl azodicarboxylate, and triphenylphosphine. The resulting phthalimido-sugars were readily converted into amino-sugars by treatment with n-butylamine in methanol. The reagent worked satisfactorily on primary hydroxy-groups and on a 2-hydroxy-group (with inversion) adjacent to a methylene group. However, no reaction occurred with either 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose or methyl 4,6-*O*-benzylidene-2-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside.[299] A phthalimido-derivative was also used in a synthesis of 4-amino-4-deoxy-D-erythronic acid from D-erythrono-lactone.[300]

Various amino-derivatives of sucrose and αα-trehalose have been reported. The former category included derivatives of diethylamine,

[295] A. K. M. Anisuzzaman and R. L. Whistler, *J. Org. Chem.*, 1972, **37**, 3187.
[296] K. Onodera, N. Kashimura, and N. Miyazaki, *Carbohydrate Res.*, 1972, **21**, 159.
[297] J. M. J. Tronchet and R. Graf, *Helv. Chim. Acta*, 1972, **55**, 2286.
[298] C. L. Stevens and K. K. Balasubramanian, *Carbohydrate Res.*, 1972, **21**, 166.
[298a] K. Čapek and J. Jarý, *Coll. Czech. Chem. Comm.*, 1972, **37**, 484.
[299] A. Zamojski, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1972, **23**, 460.
[300] T. Kamiya, Y. Saito, M. Hashimoto, and H. Seki, *Tetrahedron*, 1972, **28**, 899.

p-toluidine, and piperidine [301] as well as simple amino-compounds;[302] in all cases, the groups were located at primary positions. In the latter category, 2,2′-diacetamido-2,2′,3,3′-tetradeoxy-,[303] 6-amino-6-deoxy-,[302, 304] and 6,6′-diamino-6,6′-dideoxy-compounds [302] have been described. 3,3′-Diacetamido-3,3′-dideoxy-α-D-allopyranosyl α-D-allopyranoside has been prepared from αα-trehalose and 3-acetamido-3-deoxy-α-D-allopyranosyl β-D-fructofuranoside from sucrose.[305]

Jeanloz's group have continued their work on amino-sugar disaccharides and the modification thereof. Compounds described included 6-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactose,[306] 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-3-*O*-α-D-mannopyranosyl-β-D-glucopyranosylamine,[307] 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-6-*O*-α-D-mannopyranosyl-β-D-glucopyranosylamine,[308] and 2-acetamido-3,4,6-tri-*O*-acetyl-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1 and 4-oyl]-2-deoxy-β-D-glucopyranosylamine.[309] Other groups have described preparations of 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose [310] and 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose.[311]

The synthesis of 5′-amino-5′-deoxyguanosine derivatives containing a wide variety of *N*-alkyl groups has been reported.[312] An improved preparation of methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside has been described,[313] as has the synthesis of the 4-nitrophenyl β-glycosides of chitotriose and chitotetraose for use in lysozyme assay.[314] The synthesis of 3-amino-3-deoxy-D-glucuronic acid has been described from accessible 3-azido-intermediates.[315]

Two new types of branched amino-sugar have been reported, namely aminomethylcyclopropyl derivatives [315a] and a natural compound with a branch at the amino-bearing carbon atom [316, 317, 317a] (see Chapter 20). Nucleosides containing amino-functions are reported in Chapter 21.

[301] R. Neumann and J.-A. Ibarra, *J. prakt. Chem.*, 1972, **314**, 365.
[302] I. Jezo, *Chem. Zvesti*, 1971, **25**, 364.
[303] A. C. Richardson and E. Tarelli, *J.C.S. Perkin I*, 1972, 949.
[304] S. Hanessian and P. Lavallee, *J. Antibiotics*, 1972, **25**, 683.
[305] I. Jezo, *Chem. Zvesti*, 1971, **25**, 369.
[306] K. Miyai and R. W. Jeanloz, *Carbohydrate Res.*, 1972, **21**, 57.
[307] M. A. E. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1972, **21**, 347.
[308] M. A. E. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1972, **23**, 243.
[309] H. G. Garg and R. W. Jeanloz, *Carbohydrate Res.*, 1972, **23**, 437.
[310] K. L. Matta and O. P. Bahl, *Carbohydrate Res.*, 1972, **21**, 460.
[311] D. Bundle and N. Shaw, *Carbohydrate Res.*, 1972, **21**, 211.
[312] K. Schattka and B. Jastorff, *Chem. Ber.*, 1972, **105**, 3824.
[313] D. Dunstan and L. Hough, *Carbohydrate Res.*, 1972, **23**, 425.
[314] S. E. Zurabyan, V. V. Pimenova, E. A. Shashkova, and A. Ya. Khorlin, *Khim. prirod. Soedinenii*, 1971, **7**, 689.
[315] H. Paulsen, W. Koebernick, and E. Autschbach, *Chem. Ber.*, 1972, **105**, 1524.
[315a] W. Meyer zu Reckendorf and U. Kamprath-Scholtz, *Chem. Ber.*, 1972, **105**, 686.
[316] W. D. Weringa, D. H. Williams, J. Feeney, J. P. Brown, and R. W. King, *J.C.S. Perkin I*, 1972, 443.
[317] R. M. Smith, A. W. Johnson, and R. D. Guthrie, *J.C.S. Chem. Comm.*, 1972, 361.
[317a] A. W. Johnson, R. M. Smith, and R. D. Guthrie, *J.C.S. Perkin I*, 1972, 2153.

Physical Properties

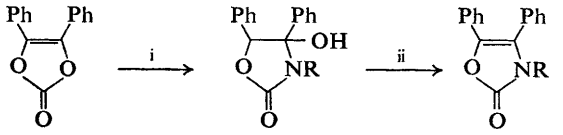
A detailed study has been made of the mutarotation of 2-amino-2-deoxy-D-glucose.[318]

The crystal structure of 2-amino-2-deoxy-β-D-galactopyranose hydrochloride has shown that the sugar ring assumes the *C*1(D) conformation.[319]

Reactions

Adducts (2 : 1) of 2-amino-2-deoxy-D-glucose with the bivalent cations Ca^{2+}, Ba^{2+}, or Sr^{2+} have been found to be hygroscopic and to be stable only under anhydrous conditions.[320] The epimerization of 2-acetamido-2-deoxy-D-glucose was improved by addition of Ni^{2+} ions, when the yield of the D-*manno*-compound was as high as 25%.[321] The latter compound was treated with benzyl alcohol in the presence of boron trifluoride and hydrogen chloride to give glycosides in the proportions α-pyranoside (66%), β-pyranoside (11%), α-furanoside (16%), and β-furanoside (7%). Acyloxonium-ion rearrangements of derivatives bearing an acylamido-group have been studied [182] (see Chapter 4).

4-Nitrophenyl formate has been recommended as a reagent for the preparation of *N*-formylated 2-amino-2-deoxyhexoses.[322] The 4,5-diphenyl-4-oxazolin-2-one group has been shown to protect both hydrogens of an amino-group and was readily removed by either catalytic hydrogenation or sodium in liquid ammonia (Scheme 32).[323] Methyl iodide in the presence



Reagents: i, RNH_2–DMF; ii, CF_3CO_2H

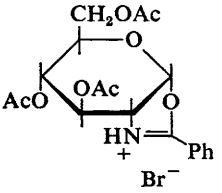
Scheme 32

of the dimethylsulphinyl carbanion has been used for the complete *O*- and *N*-methylation of 2-acetamido-2-deoxyglycosides.[158]

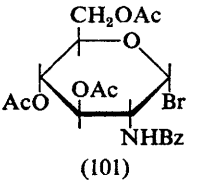
N.m.r. studies have shown that the hydrolysis of benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside by the 2-acetamido-2-deoxy-β-D-glucosidase from boar epididymis proceeds with retention of the anomeric configuration.[324] The equilibrium between (100) and (101) has been studied and was shown to be solvent-dependent.[325] As a model for polysaccharide–

[318] A. P. Fletcher and A. Neuberger, *J.C.S. Perkin II*, 1972, 12.
[319] M. Takai, S. Watanabe, T. Ashida, and M. Kakudo, *Acta Cryst.*, 1972, **B28**, 2370.
[320] S. P. Moulik and A. K. Mitra, *J. Indian Chem. Soc.*, 1971, **48**, 905.
[321] J. Yoshimura, H. Sakai, N. Oda, and H. Hashimoto, *Bull. Chem. Soc. Japan*, 1972, **45**, 2027.
[322] K. Yamamoto, *Bull. Chem. Soc. Japan*, 1972, **45**, 1253.
[323] J. C. Sheehan and F. S. Guziec, *J. Amer. Chem. Soc.*, 1972, **94**, 6561.
[324] A. Ya. Khorlin, S. E. Zurabyan, N. I. Dubrovina, V. F. Bystrov, G. V. Vikha, and E. D. Kaverzneva, *Carbohydrate Res.*, 1972, **21**, 316.
[325] H. Weidmann, D. Tartler, P. Stoeckl, and H. Hoenig, *Monatsh.*, 1972, **103**, 883.

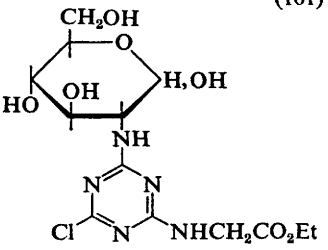
protein cross-linking, the reaction of cyanuric chloride with various amino-sugars and glycine ethyl ester has been studied. The reaction takes place preferentially at the amino-group and the hydroxy-groups need not be protected. Compounds of type (102) were prepared.[326]



(100)



(101)



(102)

In the presence of 2-amino-2-deoxy-D-glucose (or a number of other organic bases), violuric acid formed protein-transfer complexes which, in general, are pink; a 1 : 1 complex has been isolated and its physico-chemical properties were investigated.[327]

Full details of the deamination of methyl 4-amino-4-deoxy-2,3-*O*-isopropylidene-α-L-talopyranoside have been given (see Vol. 4, p. 64).[328] Deamination of the corresponding 5-aminofuranoside (103) gave (104) (2.9%), (106) (26.4%), (105) and (107) (together 12.3%), and (108) (43.5%). The D-*allo*-isomer (5-epimer) of (103) gave (104) (12.5%), (106) (25.8%), (105) and (107) (together 20.4%), (108) (7.7%), and (109) (17.5%) and its 1-*O*-acetate (14.1%).[329] Deamination of 2-amino-2-deoxy-D-mannose gave D-glucose in 72% yield.[20]

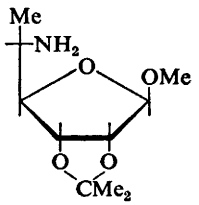
The reaction between 2-amino-2-deoxy-D-glucose and phenylboronic acid isobutyl ester in ethanol gave a boro-oxazolidine.[330]

The hydrolysis of 1-acetamido-1-deoxyketoses has been studied.[331]

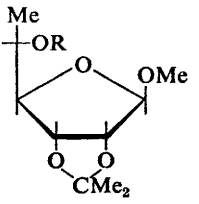
Diamino-sugars

The 2,6-diamino-2,6-dideoxy-D-glucose derivative (110) (prepared from neamine) has been mesylated and the product was then subjected to the

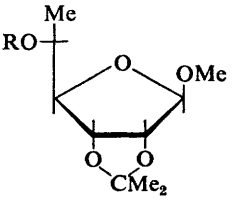
[326] A. S. Chaudhari and C. T. Bishop, *Canad. J. Chem.*, 1972, **50**, 1987.
[327] S. P. Moulik, C. R. Sahu, and A. K. Mitra, *Carbohydrate Res.*, 1972, **25**, 197.
[328] A. K. Al-Radhi, J. S. Brimacombe, and L. C. N. Tucker, *J.C.S. Perkin I*, 1972, 315.
[329] J. S. Brimacombe and J. Minshall, *Carbohydrate Res.*, 1972, **25**, 267.
[330] A. M. Yurkevich and O. V. Shevtsova, *Zhur. obshchei Khim.*, 1972, **42**, 1172.
[331] P. A. Finot and J. Mauron, *Helv. Chim. Acta*, 1972, **55**, 1153.



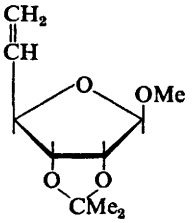
(103)



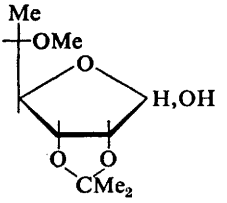
(104) R = Ac
(105) R = H



(106) R = Ac
(107) R = H



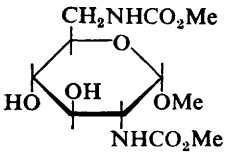
(108)



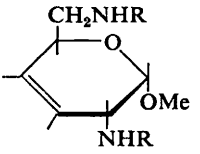
(109)

Tipson–Cohen reaction to give (111) and thereafter (112) and (113)[332] [note that this is the first synthesis of methyl purpurosaminide C (113), although no comment was made to this effect].

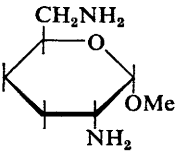
2,4-Diamino-2,4-dideoxy-D-galactose has been synthesized from (114) by standard procedures and the D-*gluco*-isomer was prepared similarly.[333]



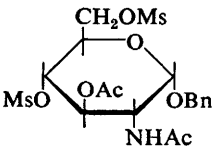
(110)



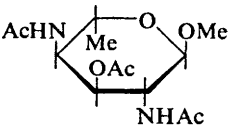
(111) R = CO_2Me
(112) R = H



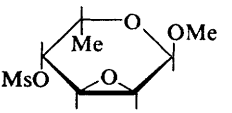
(113)



(114)



(115)



(116)

[332] S. Umezawa, T. Tsuchiya, and Y. Okazaki, *Bull. Chem. Soc. Japan*, 1971, **44**, 3494.
[333] W. Meyer zu Reckendorf and N. Wassiliadou-Micheli, *Chem. Ber.*, 1972, **105**, 2998.

Methyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-α-L-altropyranoside (115) and an isomer believed to be the related L-*ido*-compound were obtained by standard transformations on (116).[334] *N*-(2,4-Dinitrophenyl) derivatives of diamino-dideoxyhexoses have been prepared as intermediates for use in glycoside synthesis.[335]

[334] U. Zehavi and N. Sharon, *J. Org. Chem.*, 1972, **37**, 2141.
[335] W. Meyer zu Reckendorf, R. Weber, N. Wassiliadou-Micheli, and L. Roff, *Tetrahedron Letters*, 1972, 3461.

9
Hydrazones, Osazones, and Related Compounds

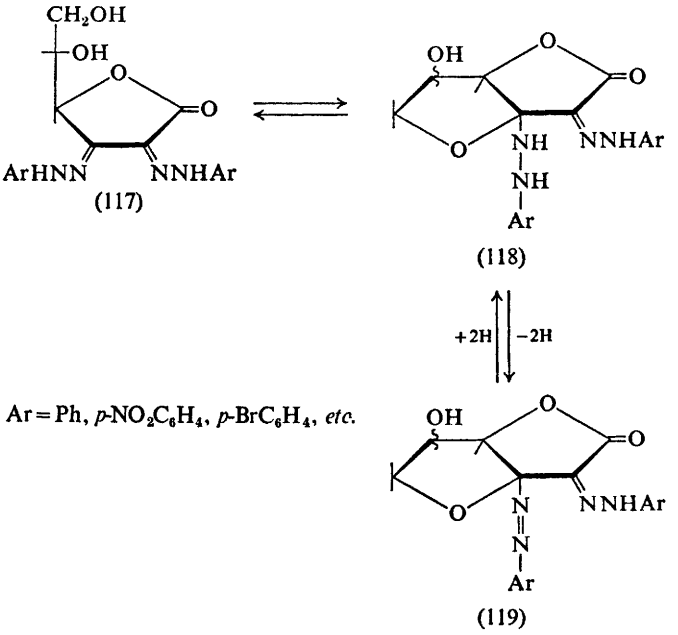
An attempt has been made to explain the stereospecificity of reactions leading to the formation of anhydro-osazones.[336] The suggested mechanism was extended to triazoles and other hetero-aromatic ring systems undergoing anhydro-ring formation, and the formation of dehydro-osazones appeared to follow a closely related pathway.

Mixtures of related 2-oxo-1,3-bis(phenylhydrazone) and tris(phenylhydrazone) derivatives have been prepared in low-to-moderate yield by treatment of enolic cyclohexane-1,3-diones in aqueous acetic acid with an excess of phenylhydrazine at room temperature.[337] E.s.r. spectroscopy indicated that free-radical anionic intermediates are partly involved. Formation of radical-anions was also observed when, on treatment with phenylhydrazine, enolic cyclohexane-1,2-diones (or α-hydroxy- or α-acetoxy-cyclohexanones) afforded mixtures of the corresponding mono- and bis-(phenylhydrazones).

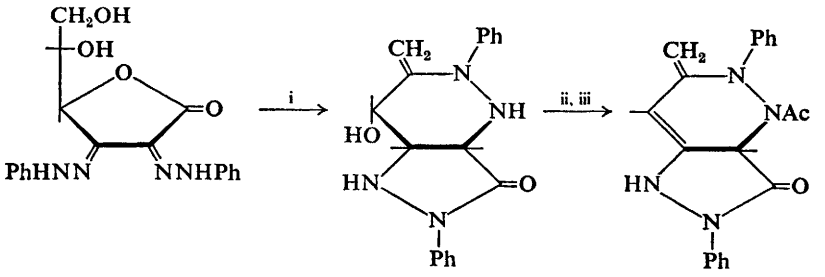
A number of derivatives of dehydro-L-ascorbic acid bis(hydrazones) have been described.[338] For example, oxidation of the lactone (117) with either cupric chloride or iodine led to formation of the bicyclic compound (119), presumably by way of (118) (Scheme 33); this reaction is characteristic of dehydro-L-ascorbic acid 2,3-bis(hydrazones). When similarly oxidized, aldosulose 1,2-bis(hydrazones) (*i.e.* sugar osazones) gave the 2-aryl-1,2,3-triazoles (sugar osotriazoles). The difference in behaviour can be attributed to chelation of the second hydrazone residue in sugar osazones preventing the rearrangement to the cyclic hydrazino-hydrazone forms. Other reported[339] transformations of dehydro-L-ascorbic acid phenylosazone are illustrated in Scheme 34.

Oxidation of acetylated saccharide aroylhydrazones with iodine–mercuric oxide afforded 5-aryl-2(polyacetoxyalkyl)-1,3,4-oxadiazoles; for example, the D-galactose hydrazone (120) was converted into (121). On deacetylation, (121) rearranged *via* (122) into the tautomeric, cyclic iminolactone (123), and this process was reversed on reacetylation of (123). Attempted

[336] H. El Khadem, *Carbohydrate Res.*, 1972, **23**, 311.
[337] A. J. Fatiadi, *Carbohydrate Res.*, 1972, **25**, 173.
[338] H. El Khadem, M. H. Meshreki, S. H. El Ashry, and M. El Sekeili, *Carbohydrate Res.*, 1972, **21**, 430.
[339] H. El Khadem, Z. M. El-Shafei, and M. El Sekeili, *J. Org. Chem.*, 1972, **37**, 3523.



Scheme 33

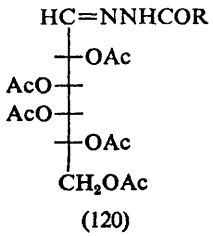


Reagents: i, $LiAlH_4$; ii, Ac_2O–py; iii, NH_3

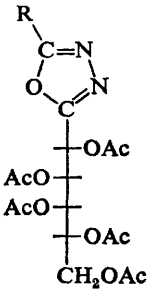
Scheme 34

dehydration of sugar acetyl- and benzoyl-hydrazones by treatment with boiling acetic anhydride resulted in the formation of peracetylated *NN*-diacetylhydrazones and *N*-acetyl-*N*-benzoylhydrazones, respectively.[340] The synthesis of 'reversed' 1,3,4-oxadiazolyl *C*-nucleosides [*e.g.* (125)] was accomplished similarly by oxidation of such *aldehydo*-dialdose benzoyl-hydrazones as (124) with a mixture of iodine and manganese and mercuric oxides.[341]

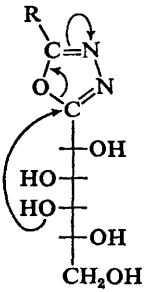
[340] H. El Khadem, M. Shaban, and M. Nassr, *Carbohydrate Res.*, 1972, **23**, 103.
[341] J. M. J. Tronchet and R. E. Moskalyk, *Helv. Chim. Acta*, 1972, **55**, 2816.



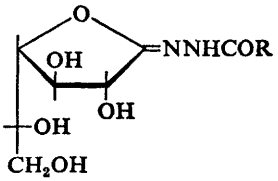
(120)



(121)

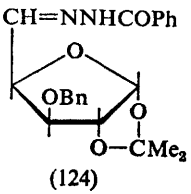


(122)

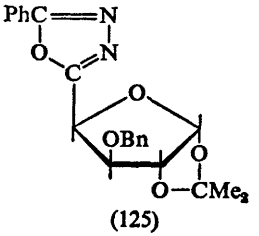


(123)

R = Ph, *p*-MeC_6H_4, *p*-$MeOC_6H_4$, *etc.*



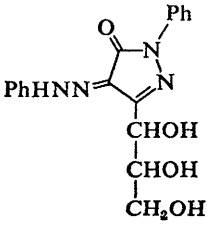
(124)



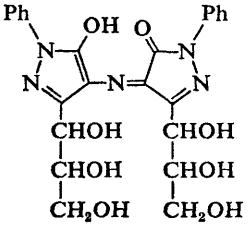
(125)

Careful reduction of the L-*threo*- and D-*erythro*-isomers of 1-phenyl-3-(1,2,3-trihydroxypropyl)-4,5-pyrazoledione-4-phenylhydrazone (126) with zinc and acetic acid afforded the corresponding substituted rubiazonic acid (127).[339]

The phenylhydrazone (128) on treatment with mild base was converted into 4,6-*O*-benzylidene-3-deoxy-D-*erythro*-hexosulose 2-phenylhydrazone

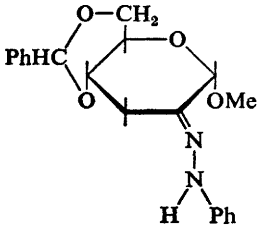


(126)

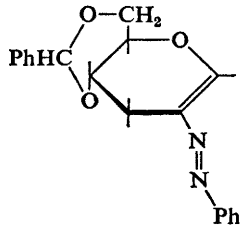


(127)

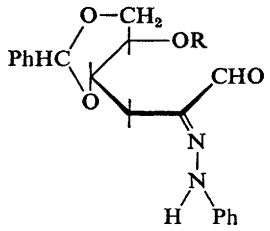
(130), presumably by hydrolysis of the intermediate (129).[342] Compound (130) exists in the *aldehydo*-form and afforded oxime and phenylosazone derivatives as well as the acetate (131).



(128)

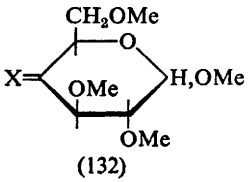


(129)

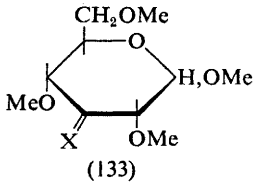


(130) R = H
(131) R = Ac

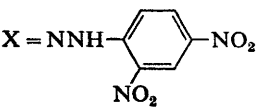
Oxidation of methanolysates of permethylated polysaccharides with DMSO-based oxidants produced anomeric mixtures of dicarbonyl sugars, which were converted into crystalline 2,4-dinitrophenylhydrazones.[343] Thus, (132) was obtained from cellulose, (133) from laminarin, and (134)

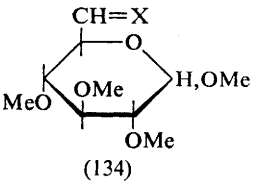


(132)



(133)





(134)

from dextran. The various anomers could be separated by t.l.c. and gave distinctive mass spectra. The use of hydrazones as easily crystallizable derivatives for the isolation of 3-deoxy-aldos-2-uloses from aldoses and ketoses has been described (see Chapter 16).

Treatment of phenylosazones with alcoholic solutions of mineral acids caused epimerization at C-3 and the formation of 3-*O*-alkyl- and 3,6-anhydro-derivatives. 2,3-Unsaturated derivatives were indicated to be key intermediates in these transformations.[344]

[342] P. M. Collins, S. Kumar, and W. G. Overend, *Carbohydrate Res.*, 1972, **22**, 187.
[343] N. Kashimura, Kj. Yoshida, and K. Onodera, *Carbohydrate Res.*, 1972, **25**, 264.
[344] A. Kraus and H. Simon, *Chem. Ber.*, 1972, **105**, 954.

Various benzylidene and methylene derivatives of D-glucose phenylosotriazole have been obtained (some under non-isomerizing conditions) and various aspects of the stereochemistry of the reactions have been discussed.[345]

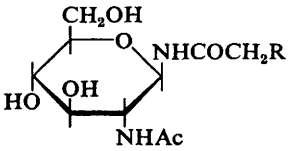
The conformations adopted by various acetylated phenylosotriazoles are referred to in Chapter 23.

[345] R. M. Carman, B. C. McKenzie, I. M. Shaw, and G. W. Zerner, *Austral. J. Chem.*, 1972, **25**, 1823.

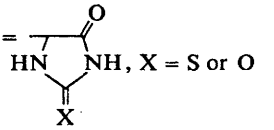
10
Miscellaneous Nitrogen-containing Compounds

Glycosylamines and Related Compounds

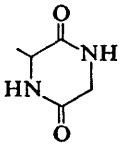
The validity of Hudson's Rules of Isorotation for *N*-arylglycosylamines has been examined. For free *gluco*-compounds the 2*B* values compared well with those of the analogous *O*-glycosides, but for tetra-*O*-acetyl and -methyl derivatives the difference between *O*- and *N*-glycosides increased with increasing polarizability of the aglycones.[346] The same workers have also investigated the anomeric forms of methylated *N*-arylhexosylamines in more detail.[346a] The synthesis of various *N*-glycosylasparagines has been described from mono- and oligo-saccharide derivatives.[347, 348] Several novel analogues of 2-acetamido-1-*N*-(β-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine have been synthesized as potential regulators of the biosynthesis, secretion, and function of immunoglobulins. The compounds prepared included (135)—(139).[349]



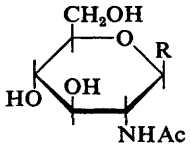
(135) R = $-CH(NHTs)CONMe_2$



(136) R = , X = S or O



(137) R =



(138) R = $OCH_2CH_2CH(NHAc)CONH_2$

(139) R = $NHSO_2Me$

[346] A. Lipták and R. Bognár, *Acta Chim. Acad. Sci. Hung.*, 1972, **72**, 309.
[346a] A. Lipták and R. Bognár, *Acta Chim. Acad. Sci. Hung.*, 1972, **73**, 335.
[347] D. Dunstan and L. Hough, *Carbohydrate Res.*, 1972, **23**, 17.
[348] D. Dunstan and L. Hough, *Carbohydrate Res.*, 1972, **25**, 246.
[349] T. Y. Shen, J. P. Li, C. P. Dorn, D. Ebel, R. Bugianesi, and R. Fecher, *Carbohydrate Res.*, 1972, **23**, 87.

It has been claimed that *N*-(4-nitrophenyl)-β-D-fructofuranosylamine has been prepared by use of β-fructofuranosidase, but no proof of structure was given.[350] Some new peracylated glycosylamines have been described [351] and so too have a number of *N*-arylethyl ($ArCH_2CH_2NH$-) glycosylamines [352] and fully acetylated glycopyranosylamines containing the indole nucleus.[353] *N*-Glycosides of tryptamine were obtained from the reaction between the amine and either D-glucose or 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide.[354]

The Amadori rearrangement of *N*-phenyl-D-glucosylamine has been shown to be first order both in the presence or the absence of acid.[355] The Maillard reaction between L-rhamnose and ethylamine afforded pyrrolic products.[356]

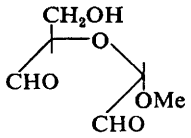
D-Glucose has been shown to react with peptides in pyridine–acetic acid by loss of water to give a product having either an imino or ring-closed glycosylamine structure.[357] Acetobromoaldopyranoses of D-glucose, D-galactose, and L-arabinose on treatment with the mercuric chloride salt of 2,3-dimethyl-1*H*-pyrrolo[3,2-*c*]pyridine afforded the corresponding β-glycosides.[358]

Nitro-compounds

Baer's group have continued to be active in this area. Alkaline equilibrations of methyl 3,6-dideoxy-3-nitro-hexopyranosides with α-L-*gluco*, α-L-*galacto*, α-L-*manno*, and α-L-*talo* configurations have been studied by ^1H n.m.r. spectroscopy. In the presence of equimolar proportions of base, when nitronate anion species are involved, the order of stabilities was *talo* > *manno* > *galacto* ~ *gluco*. When traces only of alkali were present and the nitro-glycosides were involved in the equilibrium, the order was *gluco* > *galacto* ~ *manno* > *talo*. These findings were rationalized with particular reference to the $A^{(1,3)}$ interactions.[359]

As has been extensively reported previously, condensation of the dialdehyde (140) and nitromethane under basic conditions gives a mixture of methyl 3-deoxy-3-nitro-α-D-glycosides, which are best separated as the 4,6-*O*-benzylidene acetals. Analysis of this mixture by a ^1H n.m.r. study of the benzylidene methine protons showed the composition to be *manno*

[350] A. Baseer and S. Shall, *Internat. J. Biochem.*, 1971, **2**, 503.
[351] S. Delphy and A. S. Cerezo, *Anales Asoc. quim. argentina*, 1971, **59**, 409.
[352] N. S. Volodarskaya, V. I. Mamonov, V. I. Svergun, and V. G. Yashunskii, *Zhur. obshchei Khim.*, 1971, **41**, 2328.
[353] A. A. Magnin, A. M. Stephen, and R. J. H. Davies, *Tetrahedron*, 1972, **28**, 3069.
[354] M. M. Vigdorchik, R. P. Oladko, N. P. Kostuchenko, Yu. N. Scheinker, and N. N. Suvorv, *Zhur. org. Khim.*, 1972, **8**, 880.
[355] S. Kolka and J. Sokolowski, *Roczniki Chem.*, 1972, **46**, 147.
[356] H. Kato, H. Shisematsu, T. Kurata, and M. Fujimaki, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1639.
[357] H. B. F. Dixon, *Biochem. J.*, 1972, **129**, 203.
[358] O. V. Stetsenko and I. N. Kupchevska, *Ukraino khim. Zhur.*, 1972, **38**, 503.
[359] J. Kovár, K. Čapek, and H. H. Baer, *Canad. J. Chem.*, 1971, **49**, 3960.

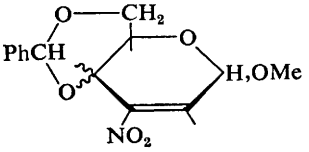


(140)

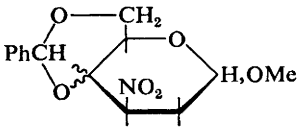
(42%), *talo* (8%), *gluco* (41%), and *galacto* (9%). Treatment of the original product mixture with aqueous alkali, prior to acetal formation, caused epimerization, and the corresponding ratios were then 15.5 : 52.5 : 20 : 13.[360] Studies were also made on the related β-glycosides.

Methyl 2,3-di-*O*-benzyl-α- and -β-D-*xylo*-pento-1,5-dialdehydofurano-sides have been condensed with nitromethane to yield mainly the 6-deoxy-6-nitro-D-glucofuranosides.[361]

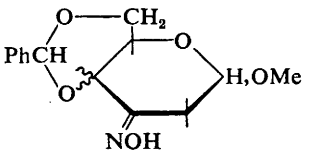
The four stereoisomeric nitro-olefins generally represented by structure (141) were smoothly reduced with sodium borohydride in ethanol to yield the corresponding saturated compounds (142) having the nitro-group



(141)



(142)



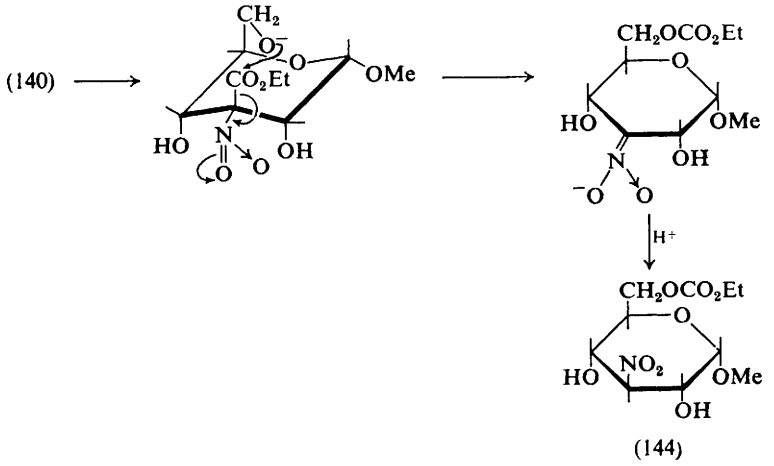
(143)

equatorially oriented. Reduction with zinc and acetic acid gave the oximes (143), which were transformed into 3-ulose derivatives by transoximation with levulinic acid.[362]

An interesting $C \rightarrow O$ ethoxycarbonyl migration occurred when (140) and ethyl nitroacetate reacted together under basic conditions. A number of isomers were obtained, the main one of which was (144), but the D-*manno* and other (probably D-*galacto* and D-*talo*) isomers were also formed.[363] A possible pathway is shown in Scheme 35.

Several 1,2-dideoxy-1-nitroalditols have been prepared and their o.r.d. and c.d. curves measured.[364]

[360] H. H. Baer and W. Rank, *Canad. J. Chem.*, 1972, **50**, 1216.
[361] V. D. Gusev, T. K. Mitrofanova, O. N. Tolkachev, and R. P. Evstigneeva, *Khim. prirod. Soedinenii*, 1972, 8.
[362] H. H. Baer and W. Rank, *Canad. J. Chem.*, 1972, **50**, 1292.
[363] F. W. Lichtenthaler and G. Bambach, *J. Org. Chem.*, 1972, **37**, 1621.
[364] C. Satoh and A. Kiyomoto, *Carbohydrate Res.*, 1972, **23**, 450.

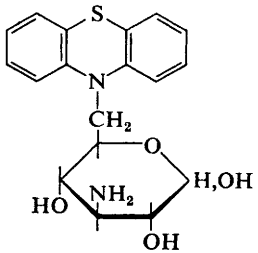


(144)

Scheme 35

Heterocyclic Derivatives

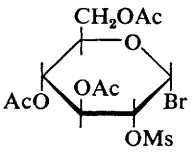
The phenothiazine derivative (145) has been prepared by way of opening of the oxiran ring in 5,6-anhydro-3-azido-3-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose.[365]



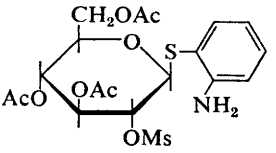
(145)

Several papers have described the preparation and properties of benzothiazine derivatives. 2-Aminophenyl 3,4,6-tri-*O*-acetyl-2-*O*-methylsulphonyl-1-thio-β-D-glucopyranoside (147), which was prepared from the corresponding bromide (146) by treatment with 2-aminobenzenethiol, was converted into D-mannopyrano[*cis*-1,2*b*]dihydrobenzothiazine (148) on heating under reflux in aqueous ethanol containing sodium acetate.[366] Attempts to prepare D-*talo*-dihydrobenzothiazine and D-*manno*- and D-*talo*-dihydrobenzo-oxazine from the appropriate thioglycoside or glycosides were unsuccessful. Compound (148) was converted by way of

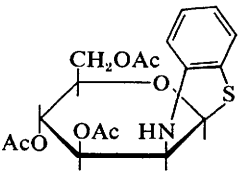
[365] W. Meyer zu Reckendorf, *Arch. Pharm.*, 1972, **305**, 29.
[366] M. Sekiya and S. Ishiguro, *Carbohydrate Res.*, 1972, **22**, 325.



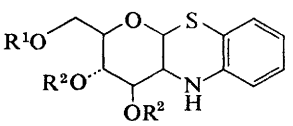
(146)



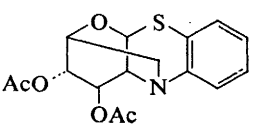
(147)



(148)



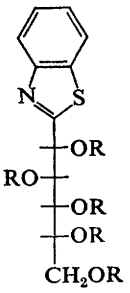
(149) $R^1 = R^2 = H$
(150) $R^1 = Ts$, $R^2 = Ac$



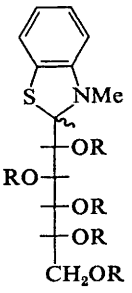
(151)

(149) and (150) into (151), and other related compounds were also prepared.[367] C.d. spectral characteristics of such derivatives as (152) and (153) have been reported.[368]

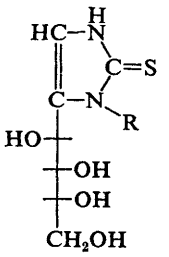
3-Aryl(alkyl)-4-(D-*arabino*-tetrahydroxybutyl)imidazoline-2-thiones [*e.g.* (154)—(156)] have been synthesized by treatment of 1-amino-1-deoxy-D-fructose with the appropriate thiocyanates.[369]



(152) R = H or Ac



(153)

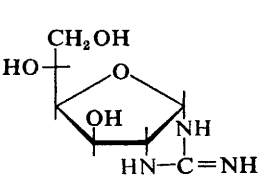


(154) R = Ph
(155) R = *p*-BrC_6H_4
(156) R = Me

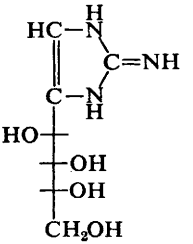
'2-Deoxy-2-guanidino-D-glucose' was shown to exist mainly as (157) and was converted into (158) in alkali.[370] D-Ribofuranosyl azides having a non-participating cyclic carbonate group on C-2 and C-3 have been used in the synthesis of 5-amino-1-(α-D-ribofuranosyl)imidazole-4-carboxamide

[367] M. Sekiya and S. Ishiguro, *Carbohydrate Res.*, 1972, **22**, 337.
[368] G. Snatzke, F. Werner-Zamojska, L. Szkágyi, R. Bognár, and I. Farkás, *Tetrahedron*, 1972, **28**, 4197.
[369] F. García González, J. Fernández-Bolaños, and J. F. Mota, *Carbohydrate Res.*, 1972, **22**, 436.
[370] J. Yoshimura, T. Sekiya, and T. Iida, *Bull. Chem. Soc. Japan*, 1972, **45**, 1227.

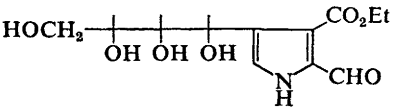
and its 4-methoxycarbonyl analogue.[371] The reaction between 1-amino-1-deoxy-D-fructose and ethyl 4,4-diethoxy-3-oxobutanoate has been shown to produce (159).[372]



(157)

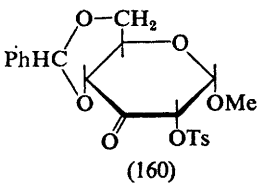


(158)

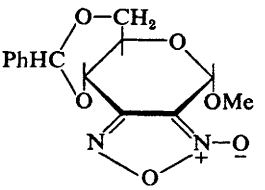


(159)

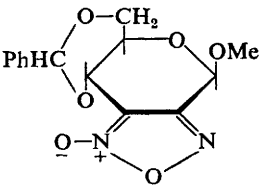
Treatment of the 2-tosyloxy-3-uloside (160) with hydroxylamine in aqueous pyridine followed by sodium nitrite in the same solvent gave the furoxan derivative (161). The related β-3-tosyloxy-2-uloside analogue of (160) gave the furoxan derivative (162).[373]



(160)

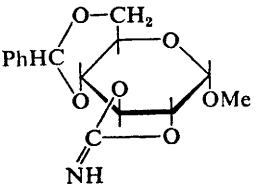


(161)



(162)

The reaction of cyanogen bromide with methyl 4,6-*O*-benzylidene-α-D-glucopyranoside gave mainly (163) as well as small proportions of the 2- and 3-carbamates.[374]

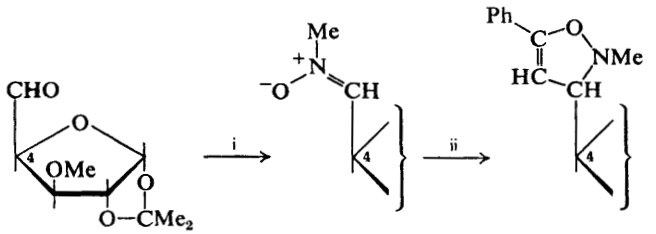


(163)

[371] C. G. Beddows and D. V. Wilson, *J.C.S. Perkin I*, 1972, 1773.
[372] F. García González, J. Fernández-Bolaños, and F. Alcudia, *Anales real Soc. espaň. Fis. Quim.*, 1972, **68**, 571.
[373] C. S. Wu, W. A. Szarek, and J. K. N. Jones, *J.C.S. Chem. Comm.*, 1972, 1117.
[374] L. Ahrgren, L. Kågedal, and S. Åkerström, *Acta Chem. Scand.*, 1972, **26**, 285.

The structure of the product arising from solvolysis of 3-acetamido-3-deoxy-1,2-*O*-isopropylidene-5,6-di-*O*-methylsulphonyl-α-D-glucofuranose has been confirmed by *X*-ray methods as 3,6-*N*-acetylepimino-3,6-dideoxy-1,2-*O*-isopropylidene-β-L-idofuranose.[375]

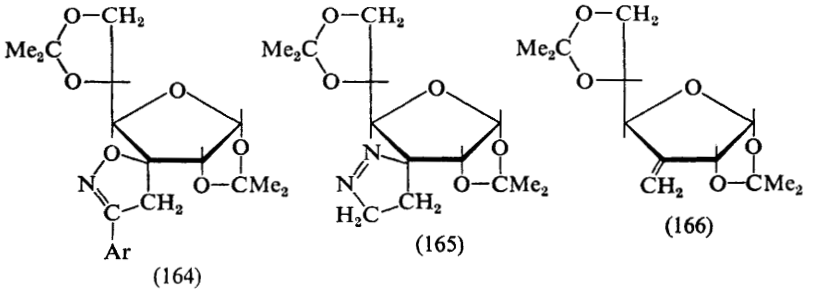
Carbohydrate nitrones are of great synthetic interest and syntheses of this class of compound have been described: see, for example, Scheme 36.[376]



Reagents: i, MeNHOH–HCl; ii, PhC≡CH

Scheme 36

Tronchet's group have continued their work on the synthesis of novel heterocyclic derivatives: for example, (164) and (165) resulted from the addition of aryl cyanates and diazomethane, respectively, to (166).[376a]



(164) (165) (166)

In another paper, they described the synthesis of the 'reverse *C*-nucleoside' (125) from the corresponding 5-aldehyde by means of a Wittig reaction.[341]

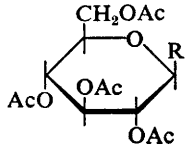
Miscellaneous Compounds

The reaction of azido-sugars with hydrazine has been studied. The products were considered to arise from two pathways; firstly by straightforward reduction of the azido-group and secondly through a di-imide intermediate that subsequently gave unsaturated or deoxy-compounds.[377]

[375] J. S. Brimacombe, J. Iball, and J. N. Low, *J.C.S. Perkin II*, 1972, 937.
[376] J. M. J. Tronchet and E. Mihaly, *Helv. Chim. Acta*, 1972, **55**, 1266.
[376a] J. M. J. Tronchet, J. M. Bourgeois, and Y. Suard, *Helv. Chim. Acta*, 1972, **55**, 2813.
[377] R. D. Guthrie and R. D. Wells, *Carbohydrate Res.*, 1972, **24**, 11.

β-D-Galactopyranosyl azide has been used as a substrate for the β-galactosidase of *E. coli*.[378] Glycosyl azides and αβ-unsaturated nitro-olefins have been converted into glycosyl triazoles and glycosyl nitro-olefins.[379]

aldehydo-D-Glucose semicarbazone, on treatment with an excess of acetic anhydride in pyridine, gave the cyclized derivative (167). With nitrous acid, (167) afforded the azide (168) in a new route to the latter compound.[380]

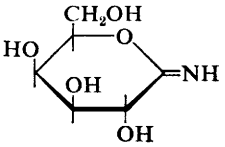


(167) R = ·NNHCONH$_2$
|
Ac

(168) R = N_3

Methyl 6-deoxy-6-hydrazino-α-D-glucopyranoside reacted with hydrogen or benzoyl peroxides to form a mixture of the 6-deoxy- and 6-hydroxy-glycosides and an unidentified compound. In non-aqueous solvents, the 6-deoxy-compound preponderated. Oxidation of the hydrazino-compound with either iodine or iodate ion yielded the 6-deoxy-6-iodo-glycoside as well as the other products. Oxidation in the presence of other nucleophiles (Cl^-, Br^-, N_3^-, and NCS^-) gave the corresponding 6-deoxy-derivatives.[381]

U.v.-photolysis of D-galactose oxime gave the iminolactone (169) (81%) and D-lyxose (14%); D-xylose oxime on similar irradiation gave low yields

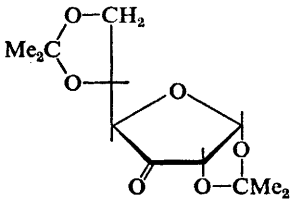


(169)

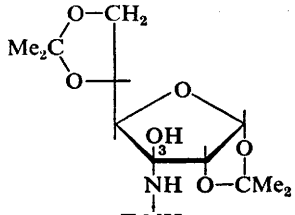
of D-xylonoiminolactone (14.8%) and D-threose (3.1%). However, the iminolactones only were obtained from irradiation of the oximes of D-ribose and D-arabinose. A mechanism was proposed for the photolytic reactions.[382]

Treatment of the ulose (170) with toluene-*p*-sulphonylhydrazine gave the carbinolamine derivative (171), which on dehydration gave the hydrazones (172) and (173).[383]

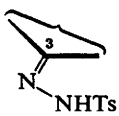
[378] M. L. Sinnott, *Biochem. J.*, 1971, **125**, 717.
[379] M. T. García-López, G. García-Muñoz, and R. Madroñero, *Anales real Soc. españ. Fis. Quim.*, 1972, **68**, 629.
[380] O. L. Galmarini and I. O. Mastronardi, *Carbohydrate Res.*, 1972, **21**, 476.
[381] S. Inouye, *Meiji Seika Kenkyu Nempo*, 1970, 75.
[382] R. W. Binkley and W. W. Binkley, *Carbohydrate Res.*, 1972, **23**, 283.
[383] W. B. Gleason and R. Barker, *Carbohydrate Res.*, 1972, **21**, 447.



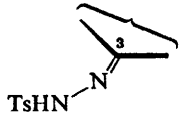
(170)



(171)

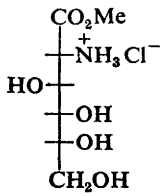


(172)

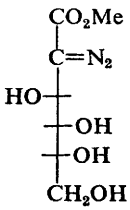


(173)

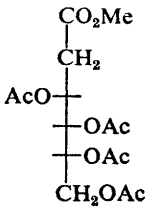
Diazo-sugars have been prepared by treating amino-sugars with nitrous acid; for example, (174) gave (175). The peracetate of (175), when photolysed in propan-2-ol, gave mainly (176), whereas in methanol a *cis–trans* mixture of (177) was formed. With phenylacetylene, the peracetate of (175) was converted into (178).[384]



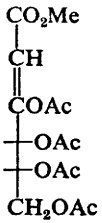
(174)



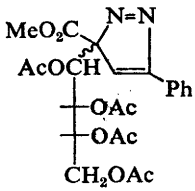
(175)



(176)



(177)

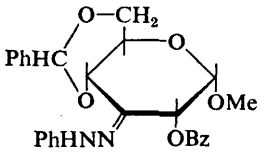


(178)

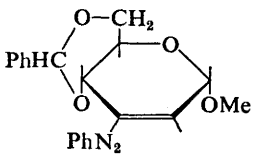
Full details of the syntheses of 3-cyano-3-deoxyglycose derivatives and the formation of related 2,3-unsaturated derivatives have been given[385] (*cf.* Vol. 2, p. 117 and Vol. 3, p. 106).

[384] D. Horton and K. D. Philips, *Carbohydrate Res.*, 1972, **22**, 151.
[385] B. E. Davison and R. D. Guthrie, *J.C.S. Perkin I*, 1972, 658.

Some 2- and 3-arylazo-derivatives of methyl 4,6-*O*-benzylidene-2,3-dideoxy-α- and -β-D-hex-2-enopyranosides were obtained by base-catalysed elimination of benzoic acid from the corresponding 2(3)-phenylhydrazone 3(2)-benzoates. In this way, (179) was converted into (180). The azoalkenes

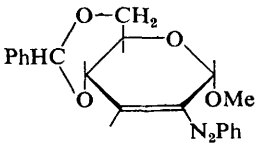


(179)

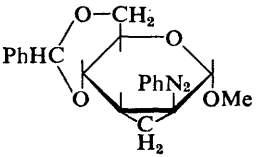


(180)

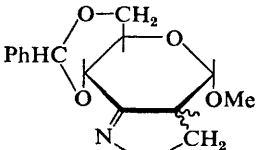
underwent 1,4-additions in a highly selective fashion with a variety of nucleophiles (methoxide and azide ions, metal hydrides, acetic acid, and amines) and the stereochemistry of the adducts was discussed.[386] Addition of dimethylsulphoxonium methylide to (181) afforded the cyclopropane derivative (182), whereas (180) yielded a mixture of isomeric pyrazolines (183) when similarly treated.[387]



(181)

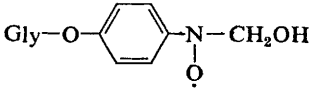


(182)

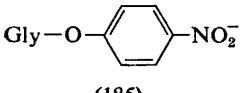


(183)

Photolysis of 3- and 4-nitrophenyl β-D-glycopyranosides in methanol yielded the corresponding nitroxide radical (184) in neutral conditions and the nitro-anion (185) in the presence of alkali.[388] Stable radicals were not



(184)



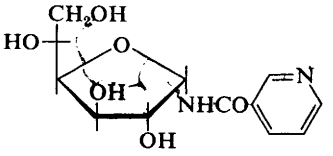
(185)

produced from 2-nitrophenyl glycosides owing to facile solvolysis of the glycosidic bond.

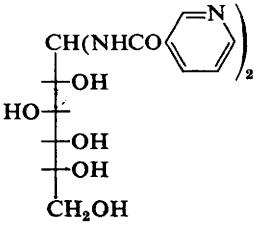
Treatment of penta-*O*-nicotinoyl-α-D-glucopyranose with aqueous ammonia gave *N*-nicotinoyl-D-glucofuranosylamine (186), 1-deoxy-1,1-bis(nicotinamido)-D-glucitol (187), and 2-(D-*arabino*-tetrahydroxybutyl)-

[386] P. M. Collins, D. Gardiner, S. Kumar, and W. G. Overend, *J.C.S. Perkin I*, 1972, 2596.
[387] P. M. Collins, D. Gardiner, S. Kumar, and W. G. Overend, *J.C.S. Perkin I*, 1972, 2611.
[388] P. J. Baugh, K. Kershaw, and G. O. Phillips, *Carbohydrate Res.*, 1972, **22**, 233.

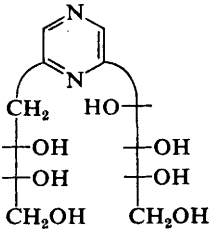
6-(D-*erythro*-2,3,4-trihydroxybutyl)pyrazine (188). The mechanism of formation of the latter compound was discussed.[389]



(186)



(187)

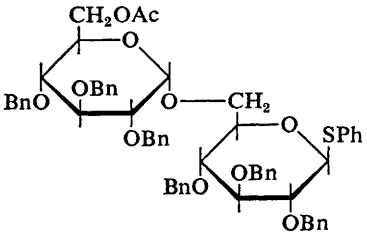


(188)

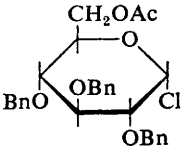
[389] E. A. Forlano, J. O. Deferrari, and R. A. Cadenas, *Carbohydrate Res.*, 1972, **23**, 111.

11
Thio-sugars

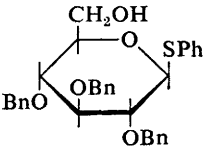
A series of acetylated n-alkyl 1-thio-β-D-xylopyranosides has been prepared by the reaction of 2,3,4-tri-*O*-acetyl-1-thio-β-D-xylopyranose with various n-alkyl iodides.[390] Thioglycosides bearing *O*-benzyl protecting groups have been prepared as intermediates for the systematic, sequential synthesis of oligosaccharides. The yield of the blocked isomaltoside (189), prepared by coupling (190) to (191), proved to be better than when (190) was coupled



(189)



(190)

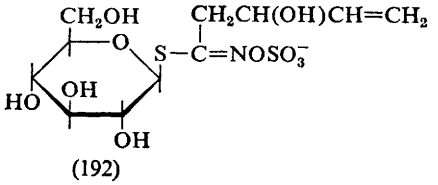


(191)

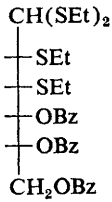
with methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside. However, under the conditions of *O*-debenzylation by sodium in liquid ammonia, the phenylthio-group was cleaved to a considerable extent with reduction at the anomeric carbon atom. 3-Phenylpropyl thioglucosides were stable under these conditions and this is thus a more satisfactory protecting group, which can be removed as required by treatment with aqueous mercuric chloride.[98] A synthesis of (*R*,*S*)-2-hydroxy-3-butenyl-D-glucosinolate (192) has been described.[390a]

The product from the reaction of 3,5,6-tri-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-glucofuranose with ethanethiol and hydrochloric acid, first described in 1932, has now been shown to be (193). It is presumed to arise

[390] M. Claeyssens and C. K. De Bruyne, *Carbohydrate Res.*, 1972, **22**, 460.
[390a] S. R. Jensen and A. Kjaer, *Acta Chem. Scand.*, 1971, **25**, 3891.



(192)

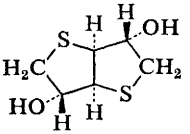


(193)

by a mechanism involving successive migrations of ethylthio-groups along the carbon chain.[391]

5-Methylthioribose has been isolated from *E. coli* B.[392] Methyl 2,3-anhydro-β-D-ribofuranoside gave mainly methyl 3-*S*-benzyl-3-thio-β-D-xylofuranoside on ring-opening with benzylthiolate ion. Under similar conditions, the corresponding α-anomer gave a mixture of the 3-*S*-benzyl-3-thio-D-*xylo*- and 2-*S*-benzyl-2-thio-D-*arabino*-isomers in the ratio 3 : 7.[393]

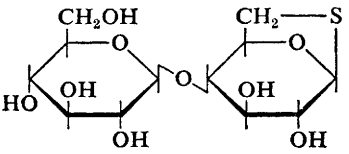
1,4:3,6-Bis(thioanhydro)-D-iditol (194) and its 2,5-diacetate have been



(194)

prepared from 3,4-di-*O*-methylsulphonyl-D-mannitol.[394] The optical isomers of dithiothreitol (Cleland's reagent) and the oxidized forms were obtained from L(+)- and D(−)-tartaric acid by an improved procedure. Their toxicity and ability to protect against radiation were investigated in mice; the D-form showed radioprotective properties, whereas the L-form did not.[395]

'6-Thiocellobiosan' (195) and some related 1,6-dithiocellobiose derivatives have been described.[396]

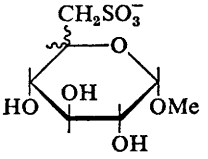


(195)

[391] G. S. Bethell and R. J. Ferrier, *J.C.S. Perkin I*, 1972, 1033.
[392] H. R. Schroeder, C. J. Barnes, R. C. Bohinski, R. O. Mumma, and M. F. Mallette, *Biochim. Biophys. Acta*, 1972, **273**, 254.
[393] T. Van Es, *Carbohydrate Res.*, 1972, **21**, 156.
[394] J. Kuszmann and P. Sohár, *Carbohydrate Res.*, 1972, **21**, 19.
[395] M. Carmack, C. J. Kelley, S. D. Harrison, and K. P. Dubois, *J. Medicin. Chem.*, 1972, **15**, 600.
[396] S. Tejima and Y. Okamori, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 2036.

Dethioacetalization can be accomplished very rapidly at room temperature using thallium(III) trifluoroacetate in THF.[397] Although carbohydrates were not specifically mentioned, the method should be applicable. Attempts to synthesize carbohydrate thioketones are described in Chapter 16.

Free-radical addition of hydrogen [^{35}S]sulphite in aqueous solution to several diastereoisomeric methyl 6-deoxy-hex-5-enopyranosides gave epimeric 6-(methyl 6-deoxy-glycopyranoside)-[^{35}S]sulphonic acids [*e.g.* (196)] as well as [^{35}S]sulphoacetate (197). The proportion of these products



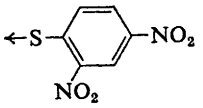
(196)

$^{-}O_3SCH_2CO_2H$

(197)

varied with the enoside used and theoretical aspects of the reaction were considered.[398] Related studies involving the free-radical addition of bisulphite have been reported [398a] and are fully discussed in Chapter 23.

The 2,4-dinitrobenzenesulphenyl group (198) has been used as a protecting group; it was as stable towards alkali as the acetate group and exhibited



(198)

comparable stability towards acid as the acetal group of 1,2-*O*-isopropylidenealdofuranoses. It can be removed in methanolic solution with aluminium in the presence of mercuric acetate or by hydrogenolysis over a Raney nickel catalyst.[399]

The crystal structure of ethyl 2-*S*-ethyl-1,2-dithio-α-D-mannofuranoside has been determined.[400]

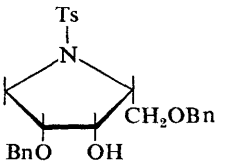
[397] T.-L. Ho and C. M. Wong, *Canad. J. Chem.*, 1972, **50**, 3740.
[398] J. Lehmann and W. Weckerle, *Carbohydrate Res.*, 1972, **22**, 317.
[398a] J. Lehmann and W. Weckerle, *Carbohydrate Res.*, 1972, **22**, 23.
[399] K. Takiura, S. Honda, and T. Endo, *Carbohydrate Res.*, 1972, **21**, 301.
[400] A. Ducruix and C. Pascard-Billy, *Acta Cryst.*, 1972, **B28**, 1195.

12

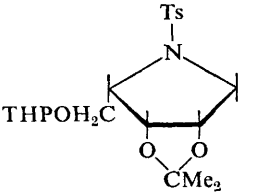
Derivatives with Nitrogen or Sulphur in the Sugar Ring

Nitrogen Derivatives

Studies on the formation of the N-analogues (199) and (200) of 2,5-anhydro-D- and -L-arabinitol derivatives, respectively, have been reported.[401]

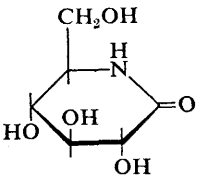


(199)



(200) THP = tetrahydropyranyl

Studies on the synthesis of nojirimycin (5-amino-5-deoxy-D-glucose) and its 6-deoxy-derivative have been described[402] and the derived lactam (201) is a potent inhibitor of β-glucuronidase.[403] 5-(Benzyloxycarbonylamino)-5,6-dideoxy-3-*O*-methylsulphonyl-L-idose was shown to exist in solution

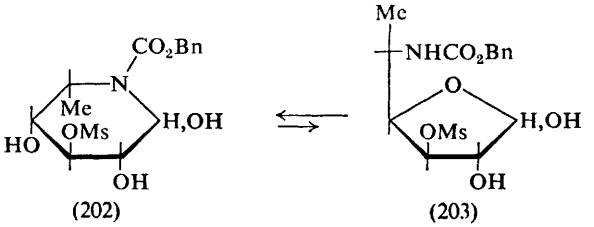


(201)

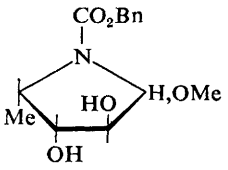
as a mixture of the six-membered (202) (80%) and furanose (203) (20%) ring forms. Conformational studies on (202) were reported.[404] Methyl glycosides of pyrrolidine-ring sugars having an unsubstituted nitrogen

[401] A. Gateau, A. M. Sepulchre, A. Gaudemer, and S. D. Gero, *Carbohydrate Res.*, 1972, **24**, 474.
[402] S. Inouye, *Meiji Seika Kenkyu Nempo*, 1970, 52.
[403] T. Niwa, T. Tsuruoaka, S. Inouye, Y. Naito, T. Koeda, and T. Niida, *J. Biochem. (Japan)*, 1972, **72**, 207.
[404] H. Paulsen and M. Friedmann, *Chem. Ber.*, 1972, **105**, 731.

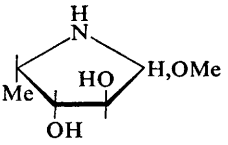
atom were found to lose methanol spontaneously and to dimerize;[405] for example, (204) was converted by hydrogenolysis over palladium into (206) by way of (205).



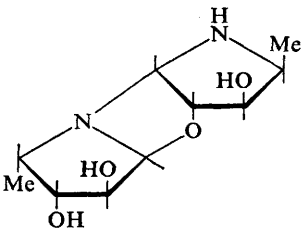
(202) (203)



(204)



(205)

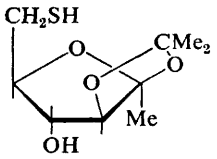


(206)

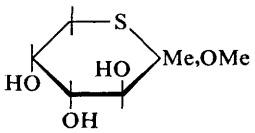
Sulphur Derivatives

Acetylation of 5-thio-D-glucose with cold acetic anhydride in pyridine afforded a mixture of 1,2,3,4,6-penta-*O*-acetyl-5-thio-α-D-glucopyranose (85%) and the β-anomer (15%), whereas only the α-anomer is obtained from D-glucose on similar treatment. 5-Thio-α-D-glucopyranose was shown to mutarotate more rapidly than α-D-glucopyranose in aqueous ethanol.[44]

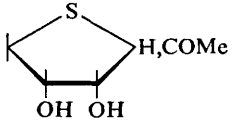
Compounds (208) and (209) were produced on partial methanolysis



(207)



(208)



(209)

of 1-deoxy-2,3-*O*-isopropylidene-6-thio-D-fructose (207) and, in acidic solution, (209) was converted into methyl 2-thienyl ketone.[406]

The alcoholysis of 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide has been compared with that of the corresponding D-xylothiapyranosyl bromide, and both were shown to be S_N1 processes.[268]

[405] H. Paulsen, H. Koebernick, and H. Schönherr, *Chem. Ber.*, 1972, **105**, 1515.
[406] T. van Es and M. S. Feather, *Carbohydrate Res.*, 1972, **22**, 470.

13
Deoxy-sugars

New methods of preparing iodo-sugars [287] and alkoxyphosphonium salts,[274] as well as other material mentioned in Chapter 7, can be thought of as introducing novel approaches to the synthesis of deoxy-sugars, as can the reduction of primary sulphonates (Chapter 22). Another general method is noted in the section below (see Scheme 37).

1-Deoxyhexuloses have been synthesized basically by an iodide exchange applied to nitrobenzene-*p*-sulphonates followed by a reduction step. In the course of this work, a new synthesis of 1-deoxy-L-psicose was developed. Somewhat surprisingly, 1-deoxy-D-fructose was found to be present in considerable proportions as the *keto*-form in aqueous solution.[26] In parallel work, Angyal and Pickles have shown that removal of an oxygen atom from either C-2 or C-3 of aldoses causes an increase in the furanose content of the equilibrium mixtures. Valuable comment was made on factors affecting the stability of furanoid ring-systems.[407]

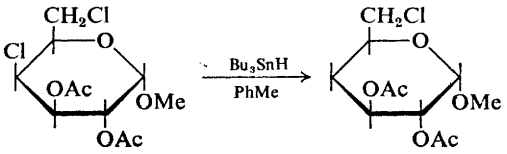
Two papers have described modified procedures for preparing 2-deoxy-D-*erythro*-pentose. The first used the well-known base treatment of 3-*O*-toluene-*p*-sulphonyl-D-glucose and reported a 42.5% overall yield,[408] whereas the second used the methoxymercuration of 3,4-di-*O*-acetyl-D-arabinal followed by reduction *etc.*, and claimed a 90% conversion from the glycal.[409] Photolysis of methyl 2-(acetoxymercuri)-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside in methanol gave methyl 3,4,6-tri-*O*-acetyl-2-deoxy-D-*arabino*-hexopyranoside thereby providing a non-reductive procedure for effecting this transformation.[410] A fluorometric method for determining 2-deoxy-D-*erythro*-pentose is mentioned in Chapter 26.

2-Deoxy-D-*arabino*-hexose was reported to increase hepatic tyrosine aminotransferase and tryptophan oxygenase activities in mice.[411] 3-Deoxy-D-*erythro*-hexos-2-ulose has been isolated from beef livers.[412]

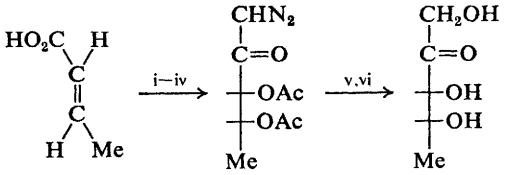
Chlorodeoxy-sugars can be reduced to the corresponding deoxy-sugars in high yield using tributyl tin hydride in toluene in the presence of

[407] S. J. Angyal and V. A. Pickles, *Austral. J. Chem.*, 1972, **25**, 1711.
[408] J. Tomasz, *Acta Chim. Acad. Sci. Hung.*, 1971, **70**, 263.
[409] S. Honda, K. Izumi, and K. Takiura, *Carbohydrate Res.*, 1972, **23**, 427.
[410] D. Horton, J. M. Tarelli, and J. D. Wander, *Carbohydrate Res.*, 1972, **23**, 440.
[411] R. W. Fuller, *Experientia*, 1972, **28**, 299.
[412] L. G. Eguyd, *Carbohydrate Res.*, 1972, **23**, 307.

αα′-azobis(isobutyronitrile). Interestingly, secondary halides were reduced selectively in the presence of primary halides (see Scheme 37).[413]



Scheme 37

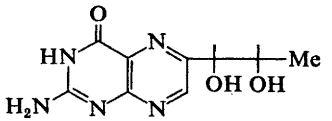


Reagents: i, H_2O_2–W_2O_3; ii, Ac_2O–py; iii, $SOCl_2$; iv, CH_2N_2; v, Ac_2O–$Cu(OAc)_2$; vi, MeONa

Scheme 38

5-Deoxy-DL-erythrulose has been synthesized as illustrated in Scheme 38 and was condensed thereafter with 2,4,5-triamino-6-hydroxypyrimidine to yield DL-biopterin (210).[414, 415]

A new synthesis of 6-deoxy-L-glucose was achieved in 60% overall yield by epimerization of L-rhamnose in the presence of molybdic acid.[416] Modification of αα-trehalose has afforded the 3,3′-dideoxy-compound.[303]

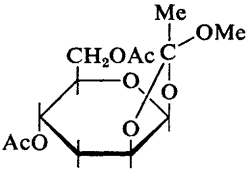


(210)

In the area of dideoxy-sugars, methyl 3-*O*-(3,6-dideoxy-α-D-*arabino*-hexopyranosyl)-α-D-mannopyranoside has been synthesized for the purpose of assessing the immunological importance of disaccharide units terminating in 3,6-dideoxy-α-D-*arabino*-hexose in *Salmonella* lipopolysaccharides. The key step involved use of the orthoester (211).[102, 417]

Methyl 2,3,6-trideoxy-α-L-*erythro*-hexopyranoside (the enantiomer of methyl amicetoside) and 2,3,6-trideoxy-α-L-*threo*-hexose (rhodinose) have

[413] H. Arita, N. Ueda, and Y. Matsushima, *Bull. Chem. Soc. Japan*, 1972, **45**, 567.
[414] M. Viscontini and W. F. Frei, *Helv. Chim. Acta*, 1972, **55**, 574.
[415] M. Viscontini, R. Provenzale, and W. F. Frei, *Helv. Chim. Acta*, 1972, **55**, 570.
[416] V. Bilik, W. Voelter, and E. Bayer, *Annalen*, 1972, **759**, 189.
[417] H. B. Borén, P. J. Garegg, and N.-H. Wallin, *Acta Chem. Scand.*, 1972, **26**, 1082.



(211)

been prepared from L-rhamnose; the synthetic sequence involved the use of methyl 2,3,6-trideoxy-hex-2-enoside derivatives, which were obtained from related vicinal diols using the Corey–Winter olefin synthesis.[418]

[418] A. H. Haines, *Carbohydrate Res.*, 1972, **21**, 99.

14
Unsaturated Derivatives

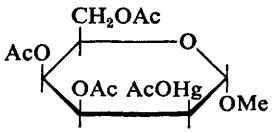
Several halogenated compounds obtained by additions to unsaturated carbohydrates are described in Chapter 7.

Glycals

The use of 3,4-di-*O*-acetyl-D-arabinal in a standard synthesis of 2-deoxy-D-*erythro*-pentose,[409] of 3,4,6-tri-*O*-acetyl-D-galactal in a synthesis of 2-deoxy-2-fluoro-D-galactose,[280] and of D-galactal in a modified, efficient synthesis of D-talose [19] has been referred to already.

D-Glucal was shown to be an inhibitor of *Aspergillus niger* glucose oxidase [419] and, in more extensive biological studies, D-glucal and D-galactal have been treated with β-D-glucosidase and β-D-galactosidase, respectively, in the presence of glycerol as an acceptor. The main products formed by addition processes were glyceryl 2-deoxy-β-D-hexopyranosides. In the case of the D-glucal–glycerol system, a minor product was identified as glyceryl 2,3-dideoxy-β-D-*erythro*-hex-2-enopyranoside, so that the enzyme-catalysed reactions resemble very closely those already known for enzyme-free systems.[420]

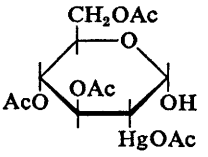
Methoxymercuration of glycals has received further attention. The stereochemistry of the adducts formed by methoxymercuration of the acetates of D-glucal, D-galactal, L-arabinal, and D-xylal has now been examined in detail. As was to be expected from previous work, *trans*-addition to the double bond occurred with the mercury atom entering at C-2, but the proportions of the two possible isomers are influenced by the configuration at C-4. With 3,4,6-tri-*O*-acetyl-D-galactal, for example, only diaxial addition occurred and the α-*talo*-glycoside (212) was formed specifically, whereas the β-*gluco*- and α-*manno*-glycosides were formed in a



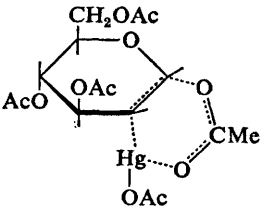
(212)

[419] M. J. Rogers and K. G. Brandt, *Biochemistry*, 1971, **10**, 4625.
[420] J. Lehmann and E. Schröter, *Carbohydrate Res.*, 1972, **23**, 359.

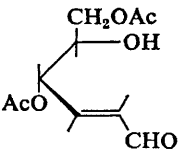
ratio of 45 : 55 with 3,4,6-tri-*O*-acetyl-D-glucal. The structures of the products were determined by n.m.r. methods, by stereoselective demercuration with thiourea to give 2,3-unsaturated glycosides (see later in this chapter), and by sodium borohydride reduction to give 2-deoxyglycosides.[421] The same workers also reported that 3,4,6-tri-*O*-acetyl-D-glucal on treatment with mercuric acetate in aqueous acetone gave the free-sugar derivative (213) in high yield (*cf.* methoxymercuration) and that, under anhydrous conditions, the corresponding acetate was formed; *i.e. cis*-addition had occurred, presumably by way of an intermediate such as (214). On reduction with sodium borohydride, the free sugar (213) gave



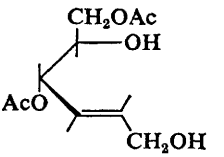
(213)



(214)



(215)



(216)

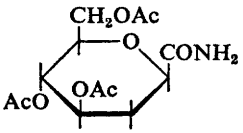
(215) and (216); other demercuration reactions of the adducts were also discussed.[422]

Photochemical addition of formamide to 3,4,6-tri-*O*-acetyl-D-glucal with acetone as an initiator gave the adducts (217)—(219) in the approximate ratio 2 : 2 : 3 and a trace of the D-*manno*-isomer of (219); the overall yield for the conversion was about 75%.[423] Other work has shown that acetone itself will add to this glucal, the oxetan (220) (33%) being isolated as the main product. Alcoholysis of (220) and deacetylation gave (221) and (222).[424]

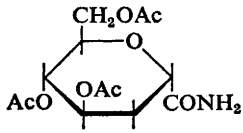
Interest has continued in the reactions between glycal derivatives and nucleoside bases. Addition of 6-chloropurine to the glycal (223) gave a product that was converted into the 2-deoxyhexosylpurine (224).[425]

Acid-catalysed reactions of benzotriazole and 5,6-dichloro- and 5,6-dimethyl-benzotriazoles with 3,4-di-*O*-acetyl-D-xylal in ethyl acetate gave

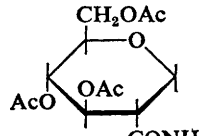
[421] K. Takiura and S. Honda, *Carbohydrate Res.*, 1972, **21**, 379.
[422] K. Takiura and S. Honda, *Carbohydrate Res.*, 1972, **23**, 369.
[423] A. Rosenthal and A. Zanlungo, *Canad. J. Chem.*, 1972, **50**, 1192.
[424] K.-S. Ong and R. L. Whistler, *J. Org. Chem.*, 1972, **37**, 572.
[425] G. J. Lourens and A. Jordaan, *J. Heterocyclic Chem.*, 1972, **9**, 975.



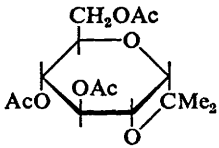
(217)



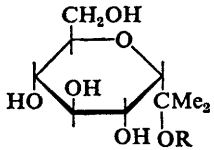
(218)



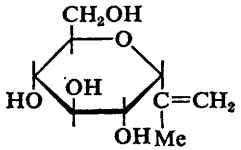
(219)



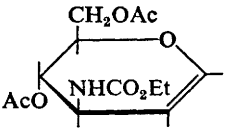
(220)



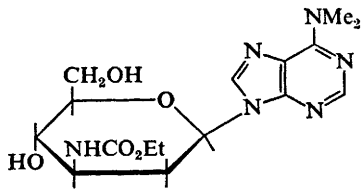
(221) R = Me or Et



(222)



(223)



(224)

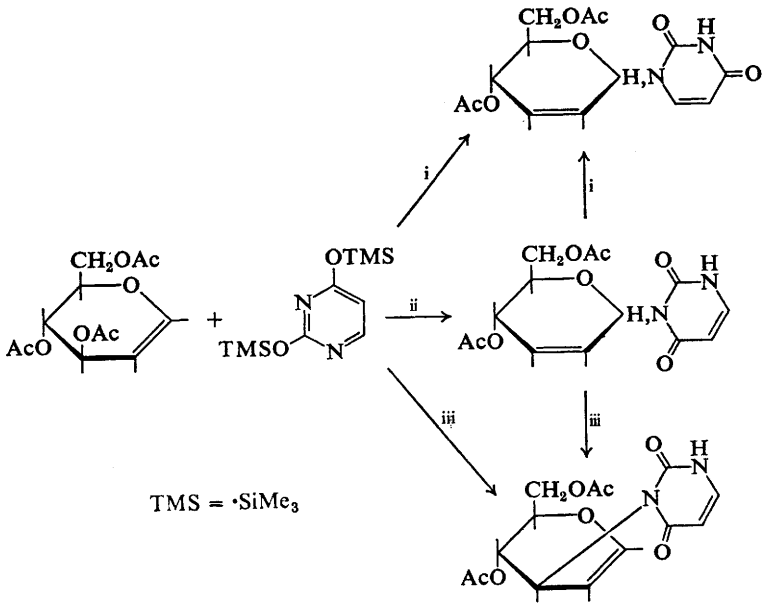
the expected 2,3-unsaturated glycosylbenzotriazoles and small amounts of 3-linked isomers (*cf.* Vol. 5, p. 90). Consistent with earlier findings, 3,4-di-*O*-acetyl-L-arabinal did not take part readily in displacement reactions of this type and also afforded 2′-deoxynucleoside analogues by addition.[426] The same group of workers have shown that acid-catalysed reactions of 3,4,6-tri-*O*-acetyl-D-glucal with benzotriazoles and purines gave mainly the 2,3-unsaturated α-glycosylamine derivatives, whereas 3,4,6-tri-*O*-acetyl-D-galactal yielded mainly 2-deoxy-adducts.[426a] Evidence is consistently accumulating that for allylic rearrangements of glycals to occur a *trans* configurational relationship is required at C-3 and C-4. A further development has revealed that allylic rearrangements can also be effected with pyrimidines (Scheme 39). The nature of the catalysts was especially important, since rearrangements were not observed with, for example, phosphorus pentachloride or protic acids.[427]

Fraser-Reid and Radatus have continued their work on branched-chain glycal derivatives and have reported on the synthesis of the homoallylic iodides (225) and (226). The former was produced in 85% yield by application of the Simmons–Smith reaction to a cyclopropyl compound (Scheme 40), but only trivial amounts of the C-3 epimer were obtained by

[426] M. Fuertes, G. García-Muñoz, R. Madroñero, and M. Stud, *Tetrahedron*, 1972, **28**, 623.

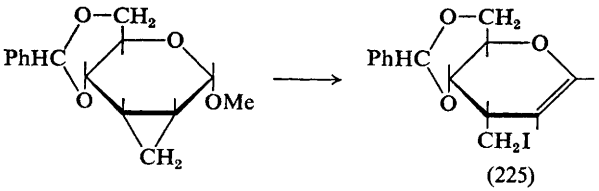
[426a] M. Fuertes, G. García-Muñoz, F. G. De Las Heras, R. Madroñero, and M. Stud, *Tetrahedron*, 1972, **28**, 4099.

[427] T. Kondo, H. Nakai, and T. Goto, *Agric. and Biol. Chem.* (*Japan*), 1971, **35**, 1990.



Reagents: i, $SbCl_5$ (20 °C); ii, BF_3 (−20 °C); iii, BF_3 (20 °C)

Scheme 39



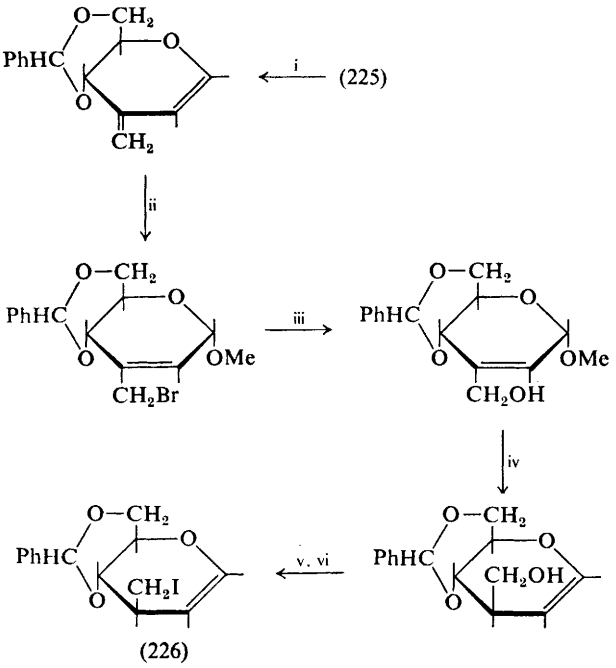
(225)

Scheme 40

this approach. The latter compound was synthesized instead by the steps illustrated in Scheme 41 and details of all the reactions involved were discussed at length.[428]

1,5-Diazabicyclo[5,4,0]undec-5-ene (DBU) (Vol. 5, p. 93) has been confirmed as an excellent base for the preparation of 2-hydroxyglycal derivatives from glycosyl halides. Derivatives from D-glucose, D-galactose, cellobiose, lactose, D-xylose, and D-ribose have been prepared by reactions that were more convenient and efficient than those based on the use of diethylamine.[429] *trans*-Elimination of hydrogen halide is involved in these cases, but Hughes has shown that 1,2-*trans*-substituted glycosyl halides can also be utilized when the base is used in the presence of lithium bromide

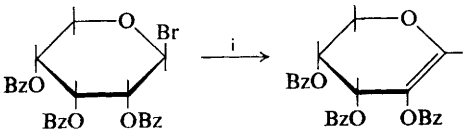
[428] B. Fraser-Reid and B. Radatus, *Canad. J. Chem.*, 1972, **50**, 2919.
[429] D. R. Rao and L. M. Lerner, *Carbohydrate Res.*, 1972, **22**, 345.



Reagents: i, NaOH; ii, MeOH–AgOAc–Br_2; iii, $AgNO_3$–H_2O; iv, $LiAlH_4$; v, TsCl–py; vi, NaI–Me_2CO

Scheme 41

in HMPT. The conversion illustrated in Scheme 42 was readily effected, presumably following bromide-catalysed anomerization of the starting material.[430]



Reagents: i, DBU–LiBr–HMPT

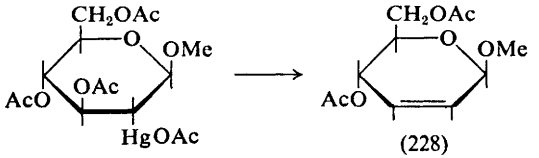
Scheme 42

Other Unsaturated Compounds

2,3-Unsaturated glycosides and glycosylamines have been mentioned earlier in this chapter and 2,3,6-trideoxy-sugars have been synthesized from the former group (see Chapter 13). A new method of preparing these compounds consisted of applying the alkoxymercuration reaction to glycals with subsequent elimination of mercuric acetate from the adducts.

[430] N. A. Hughes, *Carbohydrate Res.*, 1972, **25**, 242.

Iodide-induced elimination from compound (227), for example, gave the β-olefin (228) (Scheme 43), which is less readily available by other means than the α-anomer.[431]



(227) (228)

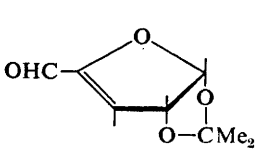
Scheme 43

2,3-Unsaturated glycosides having phenylazo-groups at either sp^2-hybridized carbon atom have been prepared from phenylhydrazone benzoates (see p. 81) by treatment with base, and 1,4-additions to these compounds have been considered earlier.[386, 387] Reactions of analogous nitro-olefins have also been dealt with.[362]

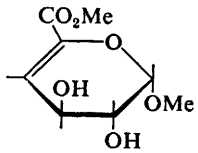
Interesting additions to hex-2-eno-4-ulosides and hex-3-eno-2-ulosides resulting in alkylation of the γ-position of the enone are considered in Chapter 15, and other enones and dienones are noted in Chapter 22.

Fraser-Reid and Holder have used the Tipson–Cohen procedure to obtain hex-3-enopyranoside derivatives from both *cis*- and *trans*-3,4-disulphonates.[432] A new 3,4-unsaturated furanoid compound is noted[172] in Chapter 4 and other elimination products (3,4- and 4,5-unsaturated hexosides) formed in addition to the desired saturated compounds have also been described earlier.[254] Studies with D-*xylo*-dialdose derivatives have led to the preparation of the αβ-unsaturated aldehyde (229).[433]

Specific syntheses of the 4,5-unsaturated hexuronic esters (230)[434] and (231)[435] have been accomplished. In the second paper, base-catalysed



(229)

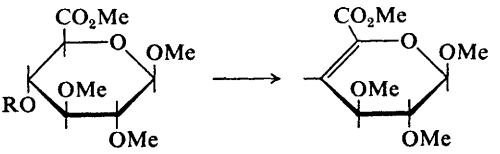


(230)

elimination of methylsulphonyloxy-groups was demonstrated to occur more readily than similar elimination of acetoxy-groups, which not unexpectedly were removed more readily than methoxy-groups (Scheme 44).

Treatment of methyl 2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-α-L-rhamnofuranoside with tetra-n-butylammonium fluoride in acetonitrile

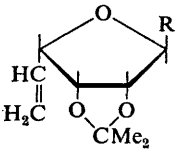
[431] A. DeBoer, G. J. Thanel, and G. A. Wilson, *Tetrahedron Letters*, 1972, 5137.
[432] B. Fraser-Reid and N. L. Holder, *J.C.S. Chem. Comm.*, 1972, 31.
[433] H. Wiedmann and H. Schwarz, *Monatsh.*, 1972, **103**, 218.
[434] J. W. Llewellyn and J. M. Williams, *Carbohydrate Res.*, 1972, **22**, 221.
[435] G. O. Aspinall and P. E. Barron, *Canad. J. Chem.*, 1972, **50**, 2203.



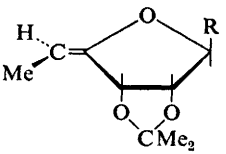
R = Ms, Ac, or Me (231)

Scheme 44

gave the isomeric olefins (232) and (233) in the ratio 1 : 2.2. It was suggested from a comparison of optical activities that the product obtained by treatment of the same rhamnofuranoside with methanolic potassium hydroxide,



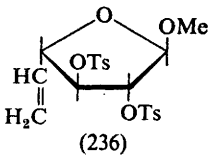
(232) R = OMe
(234) R = adenyl



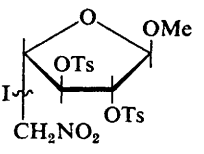
(233) R = OMe
(235) R = adenyl

which was claimed to be (233), is in fact a mixture of two isomers.[436] Both the terminal (234) and internal (235) olefins were also formed when (2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-α-L-rhamnofuranosyl)-adenosine was heated with sodium benzoate in DMF.[437] Other unsaturated sugar components of nucleosides are discussed in Chapter 21.

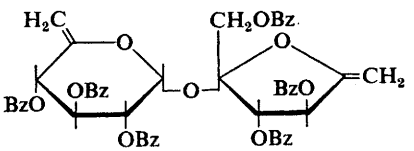
Reactions of the terminal olefin (236) with nitryl iodide, iodine nitrate, and iodine azide have been studied.[438] The β-iodo-*C*-nitro-adduct (237) was formed when (236) was treated with nitryl iodide in dry ether.



(236)



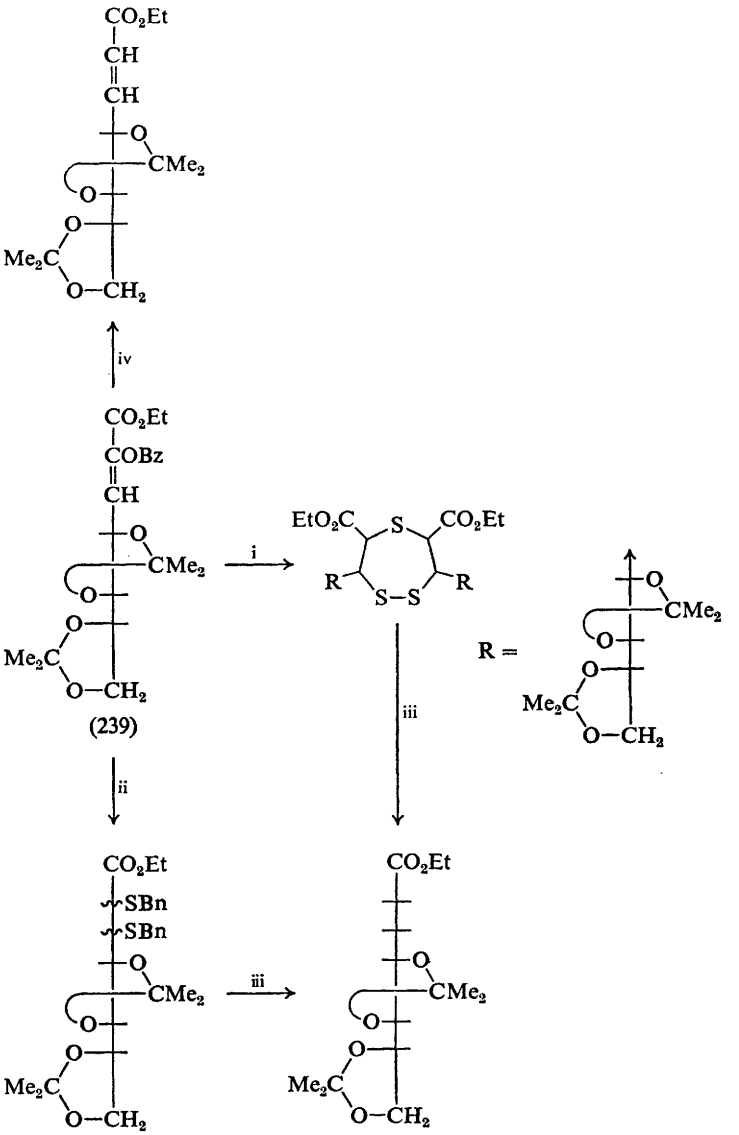
(237)



(238)

[436] G. Chaves O. and A. H. Haines, *Carbohydrate Res.*, 1972, **22**, 205.
[437] L. M. Lerner, *J. Org. Chem.*, 1972, **37**, 477.
[438] I. Szczerek, J. S. Jewell, R. G. S. Ritchie, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1972, **22**, 163.

The diolefin (238) has been obtained from 6,6′-di-*O*-toluene-*p*-sulphonyl-sucrose.[113b]

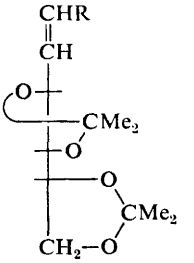


Reagents: i, H_2S–NEt_3; ii, BnSH–NEt_3; iii, Ni–H_2; iv, BnSH–py

Scheme 45

The reactions of the unsaturated aldonic acid (239) have been investigated (Scheme 45) as a new approach to the synthesis of thio-sugars.[439]

A series of *cis*- and *trans*-olefins (240) has been synthesized by the use of appropriate ylides in studies by Tronchet's group,[440] who have also reported 1,3-dipolar additions to 3-*C*-methylene-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranose (166).[376a] The conformations in solution of



(240) R = SMe, CN, or Ph

3,4,5,6,7-penta-*O*-acetoxy-*trans*-1-nitro-1-heptenes having the D-*galacto*, D-*gluco*, and D-*talo* configurations have been examined by ^{1}H n.m.r. spectroscopy and were in accord with results obtained for related acyclic derivatives.[441] Other acyclic, unsaturated derivatives, including poly-unsaturated compounds, are described in Chapter 22.

Carbohydrate sulphonic acids formed by additions to alkyl hex-5-enopyranosides are discussed[398, 398a] in Chapter 11, and the use of unsaturated sugars in syntheses of the antibiotic sugar purpurosamine C (Chapter 8) and negamycin (Chapter 20) are also referred to elsewhere.

[439] B. A. Dmitriev, N. E. Bairamova, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1972, 2554.
[440] J. M. J. Tronchet and B. Baehler, *Helv. Chim. Acta*, 1972, **55**, 2112.
[441] S. J. Angyal, R. Le Fur, and D. Gagnaire, *Carbohydrate Res.*, 1972, **23**, 135.

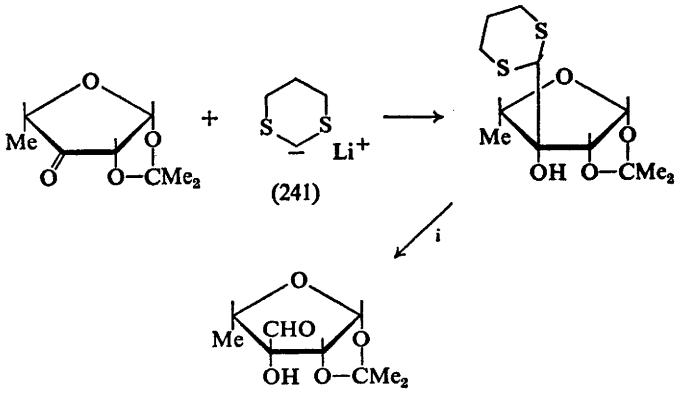
15
Branched-chain Sugars

The chemistry and biochemistry of branched-chain sugars have been reviewed [442] and the use of branched-chain sugars in the synthesis of chiral, non-carbohydrate compounds has been discussed.[443]

Compounds with an R^1-C-OR^2 Branch

2-*C*-Methyl-L-xylonic and -L-lyxonic acids (α- and β-L-galactosaccharinic acids) have been prepared (see Chapter 17).[444]

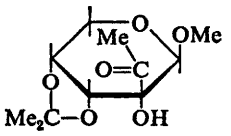
2-Lithio-1,3-dithian derivatives have been used in the preparation of branched-chain sugars from appropriately protected uloses. Dithian (241), for example, has been used in the synthesis of an L-streptose derivative as illustrated in Scheme 46.[445, 445a] Hamamelose has been prepared by a similar procedure. Compounds (242) and (243) were obtained using the dithian derivative (244), which was also used to convert (245) into (246).[446] ^{13}C N.m.r. spectroscopy has been used to determine the configuration at



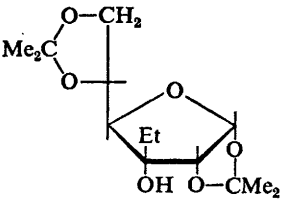
Reagents: i, $HgO-BF_3-THF$

Scheme 46

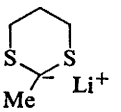
[442] H. Grisebach and R. Schmid, *Angew. Chem. Internat. Edn.*, 1972, **11**, 159.
[443] T. D. Inch, *Adv. Carbohydrate Chem.*, 1972, **27**, 191.
[444] A. Ishizu, K. Yoshida, and N. Yamazaki, *Carbohydrate Res.*, 1972, **23**, 23.
[445] H. Paulsen, V. Sinnwell, and P. Stadler, *Angew. Chem. Internat. Edn.*, 1972, **11**, 149.
[445a] H. Paulsen, V. Sinnwell, and P. Stadler, *Chem. Ber.*, 1972, **105**, 1978.
[446] A. M. Sepulchre, A. Gateau-Olesker, G. Lukacs, G. Vass, S. D. Gero, and W. Voelter, *Tetrahedron Letters*, 1972, 3945.



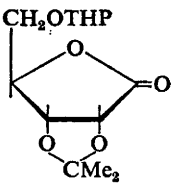
(242)



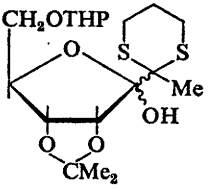
(243)



(244)



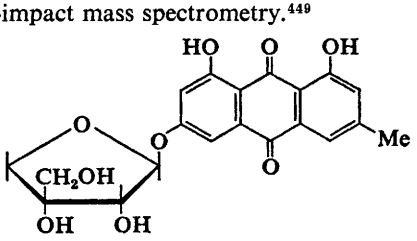
(245)



(246)

quaternary centres carrying 1,3-dithianyl branched chains in alicyclic and carbohydrate derivatives.[447]

Using cyanomethylenetriphenylphosphorane as a key reagent, uloses have been converted by way of *C*-formyl sugars into *C*-hydroxymethyl sugars.[448] The new apiose glycoside (247) has been studied by field desorption and electron-impact mass spectrometry.[449]

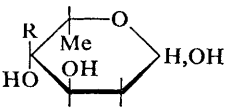


(247)

The biosynthesis of branched-chain sugars from the quinocycline complex has been investigated.[450] The sugar component from quinocycline A and isoquinocycline A has been identified as 2,6-dideoxy-4-*C*-(1′-hydroxyethyl)-L-*xylo*-hexopyranose (248) and that from quinocycline B and isoquinocycline B as 2,6-dideoxy-4-*C*-(1′-oxoethyl)-L-*xylo*-hexopyranose (249).[451]

The anomeric methyl 2-amino-2-deoxy-6-*O*-L-mycarosyl-β-D-glucosides have been prepared.[95]

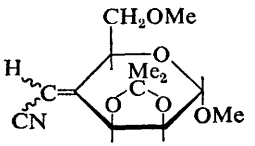
[447] G. Lukacs, A. M. Sepulchre, A. Gateau-Olesker, G. Vass, S. D. Gero, R. D. Guthrie, W. Voelter, and E. Breitmaier, *Tetrahedron Letters*, 1972, 5163.
[448] J. M. J. Tronchet, R. Graf, and R. Gurny, *Helv. Chim. Acta*, 1972, **55**, 613.
[449] H. Wagner and G. Demuth, *Tetrahedron Letters*, 1972, 5013.
[450] U. Matern and H. Grisebach, *European J. Biochem.*, 1972, **29**, 5.
[451] U. Matern, H. Grisebach, W. Karl, and H. Achenbach, *European J. Biochem.*, 1972, **29**, 1.



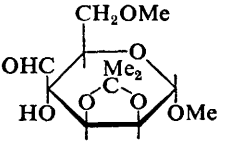
(248) R = MeCH(OH)
(249) R = MeCO

In addition to straight-chain alditols, branched-chain alditols such as 2-*C*-(hydroxymethyl)glycerol, 2-*C*-(hydroxymethyl)tetritol, and 2-*C*- and 3-*C*-(hydroxymethyl)pentitols have been identified following borohydride reduction of the reaction mixture formed by condensation of formaldehyde under catalysis by calcium hydroxide.[452] The products were analysed by combined g.l.c.–mass spectrometry of the trimethylsilyl ethers.

cis-Dihydroxylation with potassium permanganate of a mixture of *cis*- and *trans*-isomers of methyl 4-*C*-cyanomethylene-4-deoxy-2,3-*O*-isopropylidene-6-*O*-methyl-α-D-*lyxo*-hexopyranoside (250) was shown to be stereospecific and to lead to a branched-chain sugar (251) having a *gem*-

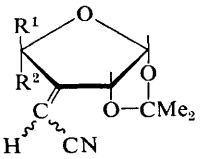


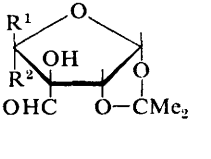
(250)



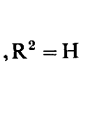
(251)

hydroxyformyl group to which the D-*manno*-configuration was assigned on the supposition of the reagent's easier access to the side of the double bond located *trans* with respect to the dioxolan ring.[453] Similarly, *cis*-dihydroxylation of (252) and (253) afforded (254) and (255), respectively.[453a]



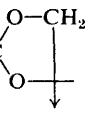


(252) $R^1 = Me_2C$, $R^2 = H$



(254) $R^1 = Me_2C$, $R^2 = H$

(253) $R^1 = H$, $R^2 = Me_2C$

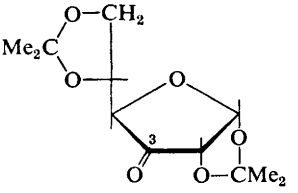


(255) $R^1 = H$, $R^2 = Me_2C$

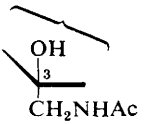
[452] R. D. Partridge, A. H. Weiss, and D. Todd, *Carbohydrate Res.*, 1972, **24**, 29.
[453] J. M. J. Tronchet and J. M. Chalet, *Carbohydrate Res.*, 1972, **24**, 283.
[453a] J. M. J. Tronchet and J. M. Bourgeois, *Helv. Chim. Acta*, 1972, **55**, 2820.

A study has been made of the stereochemistry of the products from reactions of (256) with a variety of reagents. Configurational assignments to such products as (257), (258), and (259) were made on the bases of chemical evidence and n.m.r. spectroscopy.[454]

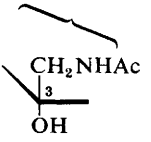
Osmium tetroxide oxidation of (260) afforded (261), which was reduced to a separable mixture of isomers (262).[455]



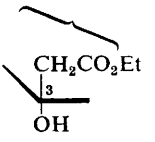
(256)



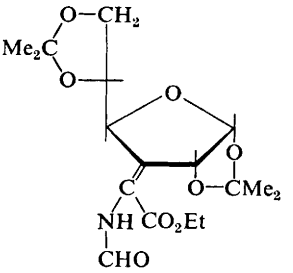
(257)



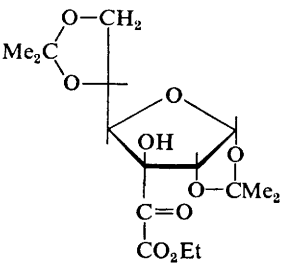
(258)



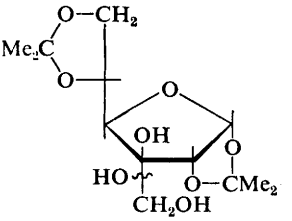
(259)



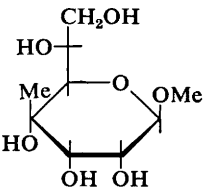
(260)



(261)



(262)



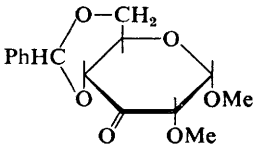
(263)

[454] J. Yoshimura, K. Kobayashi, K. Sato, and M. Funabashi, *Bull. Chem. Soc. Japan*, 1972, **45**, 1806.

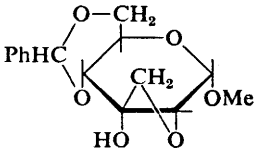
[455] A. J. Brink, J. Coetzer, A. Jordaan, and G. J. Lourens, *Tetrahedron Letters*, 1972, 5353.

4-*C*-Methylheptopyranosides (*e.g.* 263) have been prepared.[28]

The reaction of acetobromo-D-glucose with vinylmagnesium bromide in THF gave, after acetylation, compounds (34) and (35).[146] The photolysis of (264) yielded the oxetanol (265)[456] (see also Chapter 16).



(264)

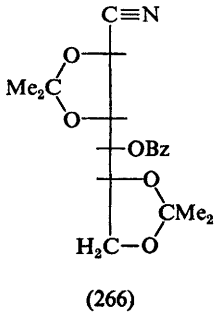


(265)

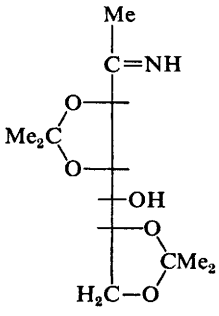
Compounds with an R—C—N Branch

Acidic hydrolysis of the antibiotic vancomycin (from *Streptomyces orientalis*) afforded the branched-chain amino-sugar vancosamine (3-amino-2,3,6-trideoxy-3-*C*-methyl-L-*lyxo*-hexopyranose).[457] The structure of vancosamine was confirmed by ^{13}C n.m.r. spectroscopy, providing an excellent example of the use of this technique for configurational assignment to a naturally occurring branched-chain sugar.[316–317a]

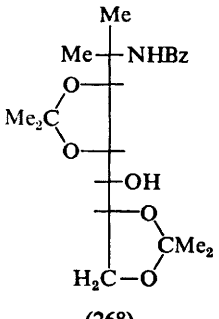
Treatment of (266) with an eight-molar excess of methylmagnesium iodide afforded (267) and (268).[458]



(266)



(267)

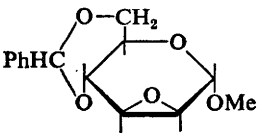


(268)

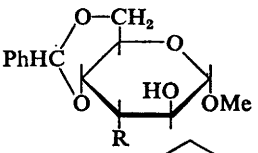
Compounds with an $R^1—C—R^2$ Branch

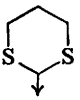
Stereospecific, diaxial ring-opening of epoxides with the nucleophilic reagent 2-lithio-1,3-dithian afforded branched-chain deoxy-sugar derivatives, which can be converted into *C*-formyl derivatives by hydrolysis and thence into *C*-hydroxymethyl and *C*-methyl derivatives by reduction, *etc.*[459, 459a]

[456] P. M. Collins, P. Gupta, and R. Iyer, *J.C.S. Perkin I*, 1972, 1671.
[457] G. Lukacs and R. M. Smith, *Bull. Soc. chim. France*, 1972, 3995.
[458] A. Kampf and E. Dimant, *Carbohydrate Res.*, 1972, **21**, 1.
[459] A. M. Sepulchre, G. Lukacs, G. Vass, and S. D. Gero, *Bull. Soc. chim. France*, 1972, 4000.
[459a] A. M. Sepulchre, G. Lukacs, G. Vass, and S. D. Gero, *Angew. Chem. Internat. Edn.*, 1972, **11**, 148.



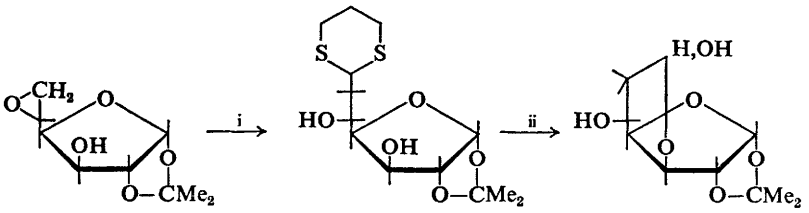
(269)



(270) R = 

(271) R = CHO
(272) R = CH_2OH
(273) R = Me

For example, (269) has been converted into (270), (271), (272), and (273), and the sequence illustrated in Scheme 47 was also reported.[459] Configurational assignments were made on the basis of ^1H and ^{13}C n.m.r. spectral data.

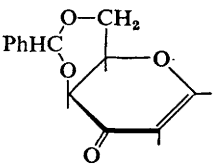


Reagents: i, $S(CH_2)_3S\bar{C}H\ Li^+$; ii, $HgO–BF_3–THF$

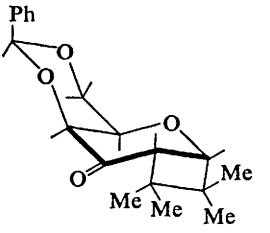
Scheme 47

Photochemical addition of 2,3-dimethylbut-2-ene to 4,6-*O*-benzylidene-1,2-dideoxy-D-*threo*-hex-1-en-3-ulopyranose (274) afforded the cyclobutane derivatives (275)—(277) and a mixture of isomeric dimers (278).[460]

When methyl 2,3-*O*-isopropylidene-6-*O*-methyl-α-D-*lyxo*-hexopyranosid-4-ulose was treated with various phosphonium ylides, the branched-chain sugars (279) and (280) were obtained in total yields approaching 80%. The reaction occurred with partial inversion of configuration at C-5 the

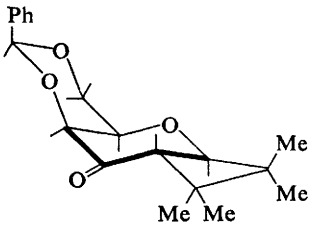


(274)

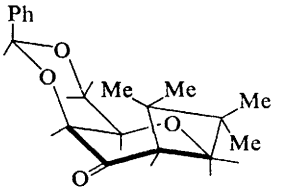


(275)

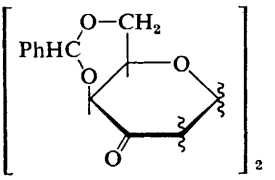
[460] P. M. Collins and B. Whitton, *Carbohydrate Res.*, 1972, **21**, 487.



(276)



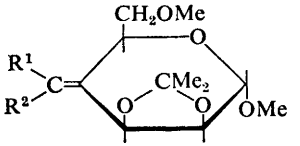
(277)



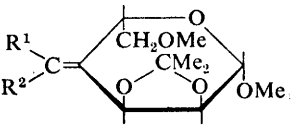
(278)

extent of which varied between 2 and 67%. The assignment of configurations at C-4 and C-5 was discussed as well as the interest presented by a study of this inversion to an understanding of the mechanism of the Wittig reaction.[461]

Treatment of the 4-*C*-methylthiomethylene derivatives of (279) with Raney nickel afforded mixtures of branched 4-deoxy-4-methyl-sugars with



(279)



(280)

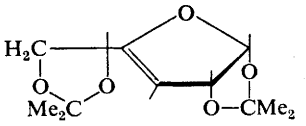
$R^1 = R^2 = H$
$R^1 = Ph$, $R^2 = H$
$R^1 = H$, $R^2 = Ph$
$R^1 = SMe$, $R^2 = H$
$R^1 = H$, $R^2 = SMe$
$R^1 = SO_2Me$, $R^2 = H$
$R^1 = H$, $R^2 = SO_2Me$
$R^1 = CN$, $R^2 = H$
$R^1 = H$, $R^2 = CN$

the D-*manno* and D-*talo* configurations.[453] Related reactions starting from pent-3-uloses have been reported.[462]

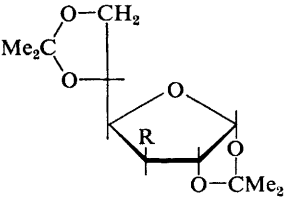
Addition of formamide to (281) in acetone under photochemical conditions afforded the branched-chain derivatives (282), (283), and (284);

[461] J. M. J. Tronchet and J. M. Chalet, *Carbohydrate Res.*, 1972, **24**, 263.
[462] J. M. J. Tronchet and G. Graf, *Helv. Chim. Acta*, 1972, **55**, 1141.

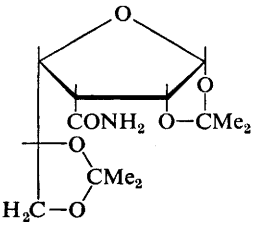
the latter compound was converted into an aminomethyl branched-chain D-gulose derivative by reduction.[463] Although this procedure constitutes a new route to branched-chain sugars, the yields of specific products were



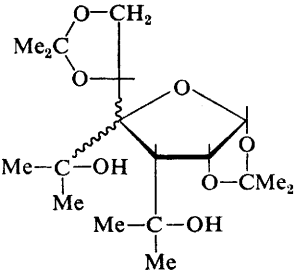
(281)



(282) R = $CONH_2$
(283) R = $C(OH)Me_2$



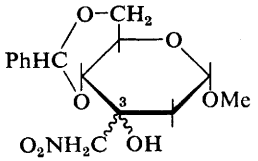
(284)



(285)

not encouraging. A minor product identified tentatively as (285) has been isolated following irradiation of (281) in acetone–propan-2-ol.

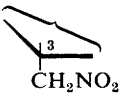
The previously reported epimeric nitro-compounds (286) (Vol. 4, p. 105) were acetylated and dehydroacetylated to yield the isomeric *cis*- and *trans*-olefins (287) from which (288) was prepared.[464] C.d. spectroscopy was investigated as a means of determining the configuration of the nitro-sugar.



(286)



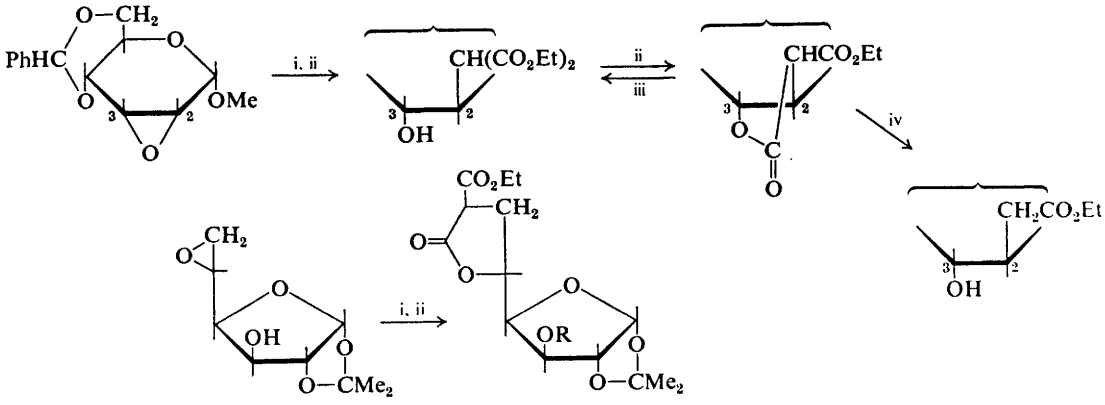
(287)



(288)

The carbanion of sodium diethylmalonate has been investigated as a reagent for the synthesis of branched-chain and extended-chain sugars.[465] The method, which is exemplified in Scheme 48, is of particular interest because of the functionalization of the branched-chain groups.

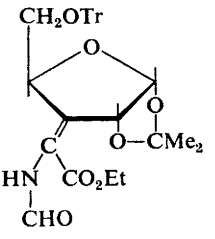
[463] A. Rosenthal and K. Shudo, *J. Org. Chem.*, 1972, **37**, 1608.
[464] A. Rosenthal and G. Schöllnhammer, *Canad. J. Chem.*, 1972, **50**, 1780.
[465] S. Hanessian and P. Dextraze, *Canad. J. Chem.*, 1972, **50**, 226.



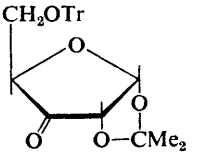
Reagents: i, $CH_2(CO_2Et)_2$; ii, NaH; iii, NaOEt–EtOH; iv, aq. NaOH.

Scheme 48

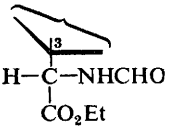
Branched-chain sugars such as (289) and (260) have been prepared by treating the corresponding uloses (290) and (256), respectively, with ethyl isocyanoacetate in the presence of base.[455] Suitable reduction of (289)



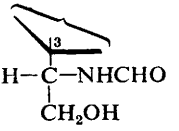
(289)



(290)



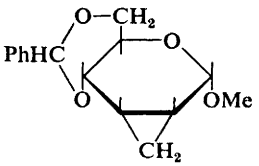
(291)



(292)

and (260) gave branched-chain sugars with such groups as (291) (with the *R* configuration) and (292). The crystal and molecular structure of 5-*O*-brosyl-3-deoxy-3-*C*-(*R*)-(ethoxycarbonylformamido)methyl-1,2-*O*-isopropylidene-α-D-ribofuranose has been determined.[466]

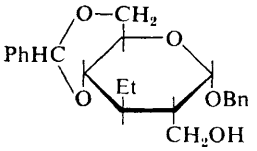
The n.m.r. spectra of the epimeric 3-deoxy-3-*C*-iodomethyl glycals (225) and (226) have been discussed in detail.[467] In (225), the chemical shifts of the methylene protons of the iodomethyl group are separated by 0.64 p.p.m., whereas in (226) the separation is only 0.1 p.p.m.; this difference was ascribed to the orientation of the protons relative to the carbon–carbon double bond. Compound (225) was prepared by Simmons–Smith reduction of (293), whereas (226) was prepared from (225) as described in Chapter 14.[428]

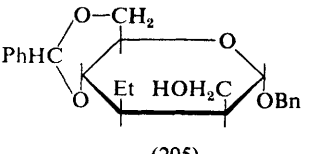


(293)

Sugars containing two branched points, for example benzyl 4,6-*O*-benzylidene-2,3-dideoxy-3-*C*-ethyl-2-*C*-hydroxymethyl-α-D-glucopyranoside (294) and -mannopyranoside (295), have been prepared. The former

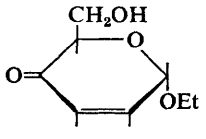
[466] J. Coetzer, A. Jordaan, G. J. Lourens, and M. J. Nolte, *Acta Cryst.*, 1972, **B28**, 3537.
[467] B. Fraser-Reid, B. J. Carthy, and B. Radatus, *Tetrahedron*, 1972, **28**, 2741.

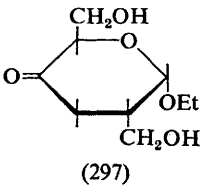

(294)

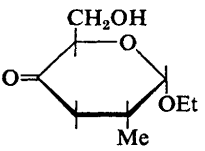

(295)

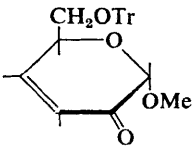
compound was degraded to give (+)-(*R*)-2,3-bisacetoxymethylpentyl acetate, which was also prepared from the alkaloid pilocarpine.[468]

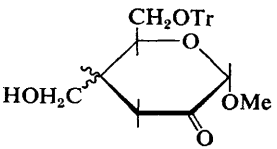
1,4-Addition reactions have been carried out photolytically in the conversion of unsaturated uloses into branched-chain sugars.[469] For


(296)


(297)


(298)

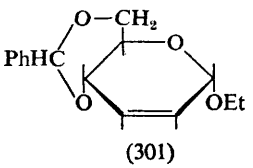

(299)

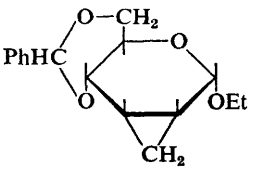

(300)

example, photolysis of (296) in methanol afforded (297), whereas with methylmagnesium iodide under ground-state conditions it gave (298). The conversion of (299) into (300) by photolysis in methanol in the presence of benzophenone was also described.

Cyclopropyl Derivatives

A detailed paper has reported on the Simmons–Smith reaction applied to the anomeric methyl 4,6-*O*-benzylidene-2,3-dideoxy-D-*erythro*-hex-2-eno-pyranosides, which were previously shown to give mainly cyclopropyl

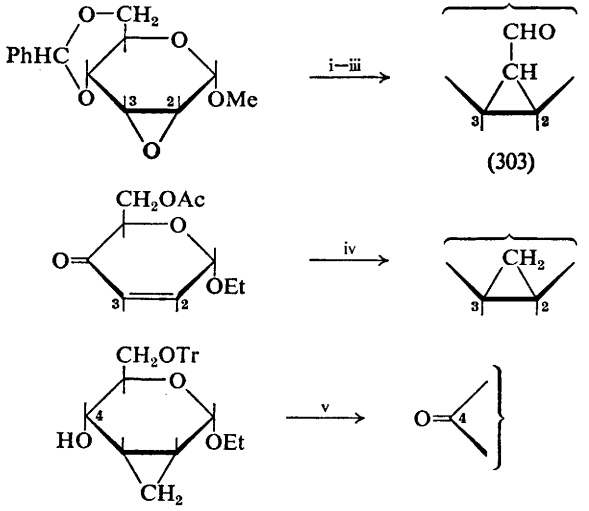

(301)


(302)

[468] T. D. Inch and G. J. Lewis, *Carbohydrate Res.*, 1972, **22**, 91.
[469] B. Fraser-Reid, N. L. Holder, and M. B. Yunker, *J.C.S. Chem. Comm.*, 1972, 1286.

products.[470] In addition to (293) (62%), small proportions of compounds (225) (7%), (301) (2%), and (302) (2%) were formed. It was shown that the cyclopropyl adducts are converted into the unsaturated products during the course of the reaction.

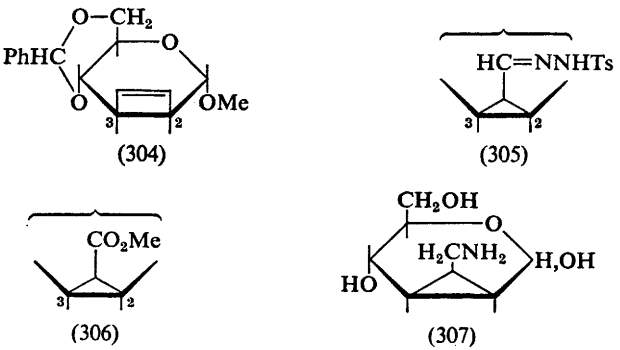
Three cyclopropyl glycosidulose derivatives have been prepared as illustrated in Scheme 49.[471] The cyclopropyl derivative (303) was converted



Reagents: i, $(EtO)_2POCH_2CO_2Et$; ii, $LiAlH_4$; iii, MnO_2; iv, CH_2I_2–Zn–Cu; v, RuO_4

Scheme 49

into the cyclobutene derivative (304) by treating the derived toluene-*p*-sulphonylhydrazone (305) with potassium t-butoxide.[472] The ester (306) has been converted into the related amine (307) by standard reactions.[315a]



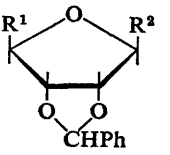
[470] B. Radatus and B. Fraser-Reid, *Canad. J. Chem.*, 1972, **50**, 2909.
[471] B. Fraser-Reid and B. J. Carthy, *Canad. J. Chem.*, 1972, **50**, 2928.
[472] W. Meyer zu Reckendorf and U. Kamprath-Scholtz, *Chem. Ber.*, 1972, **105**, 673.

16

Aldehydo-sugars, Alduloses, Dialduloses, and Diuloses

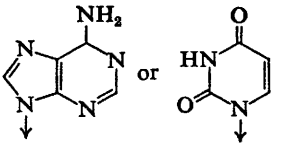
A number of compounds of this general class, which have been prepared by oxidation of suitably protected sugar derivatives, are described in Chapter 22.

5′-Aldehydo-derivatives of adenosine and uridine have been prepared by photolysis of suitably protected azido-derivatives; for example (308) was converted into (309).[473] The aldehyde derivatives afforded deuterium-

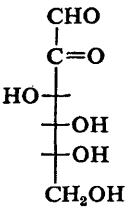


(308) $R^1 = CH_2N_3$
(309) $R^1 = CHO$

$R^2 =$



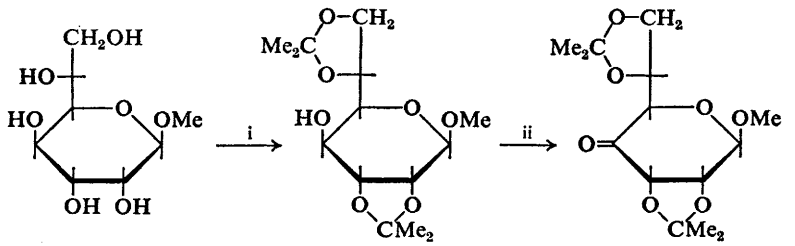
labelled primary alcohols following reduction with sodium borodeuteride, and the position of deuterium labelling was verified by high-resolution mass and n.m.r. spectrometry. Treatment of 1-amino-1-deoxy-D-fructose hydrochloride with 3,5-di-(t-butyl)-1,2-benzoquinone afforded D-*arabino*-hexos-2-ulose (310) in 25% yield.[474]



(310)

[473] D. C. Baker and D. Horton, *Carbohydrate Res.*, 1972, **21**, 393.
[474] H. J. Haas and P. Schlimmer, *Annalen*, 1972, **759**, 208.

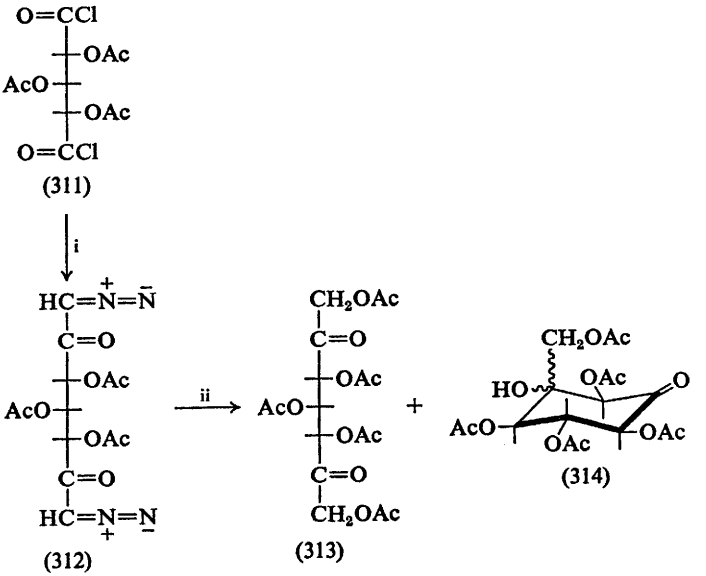
Methyl 2,3:6,7-di-*O*-isopropylidene-β-D-*allo*-heptopyranosid-4-ulose has been synthesized as illustrated (Scheme 50).[28] The reaction whereby aldose



Reagents: i, $Me_2C(OMe)_2$–TsOH; ii, DMSO–Ac_2O

Scheme 50

or ketose precursors are converted in acidified aqueous ethanol in the presence of *p*-toluidine into 3-deoxyaldos-2-uloses, which are then trapped as the crystalline bis(benzoylhydrazones), has been shown to be of wide generality (*cf.* Vol. 5, p. 111). Sugar precursors having four-, five-, six-, or seven-carbon chains and aroylhydrazines having 4-toluoyl, anisoyl, 4-chlorobenzoyl, 4-iodobenzoyl, or 4-nitrobenzoyl groups all gave crystalline products in a single operation.[475]



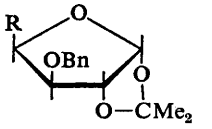
Reagents: i, CH_2N_2; ii, Cu^{2+}–AcOH (68 °C)

Scheme 51

[475] H. El Khadem, D. Horton, M. H. Meshreki, and M. A. Nashed, *Carbohydrate Res.*, 1972, **22**, 381.

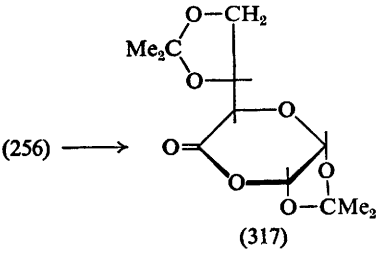
During attempted conversion of 2,3,4-tri-*O*-acetylxylaryl dichloride (311) into the *xylo*-2,6-heptodiulose derivative (313) by way of the bis-(diazomethyl) ketone (312), as illustrated in Scheme 51, ring-closure occurred to give the cyclitol derivative (314).[476]

Optimum conditions have been sought for converting (315) into (316) by use of the Wittig reagent methylenetriphenylphosphorane (*cf.* Vol. 4,

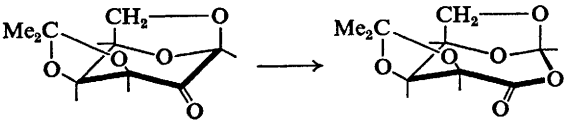


(315) R = CHO
(316) R = $CH{=}CH_2$

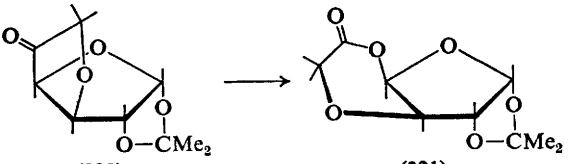
p. 103); for a successful reaction, great care must be taken in the preparation of the Wittig reagent.[477] The Wittig reaction of sugar aldehydes with bromoacetylmethylenetriphenylphosphorane ($BrCH_2COCH{=}PPh_3$) afforded $\alpha\beta$-unsaturated ketoses.[478]



(317)



(318) (319)

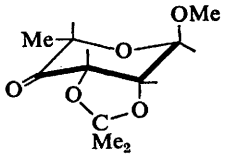


(320) (321)

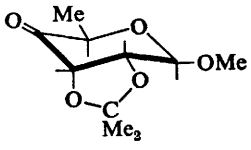
[476] D. E. Kiely and C. E. Cantrell, *Carbohydrate Res.*, 1972, **23**, 155.
[477] N. Baggett, J. M. Webber, and N. R. Whitehouse, *Carbohydrate Res.*, 1972, **22**, 227.
[478] Yu. Zhdanova and L. A. Uzlova, *Zhur. obshchei Khim.*, 1972, **42**, 759.

The conversions (256) → (317), (318) → (319), and (320) → (321) were carried out by Baeyer–Villiger oxidation with 3-chloroperbenzoic acid in solutions of ethanol-free chloroform at room temperature.[479]

Methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranosid-4-ulose (322) was partially epimerized at C-5 on heating in aqueous pyridine to the



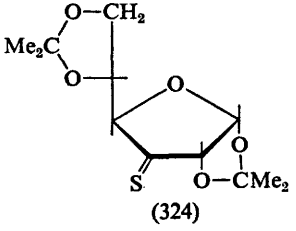
(322)



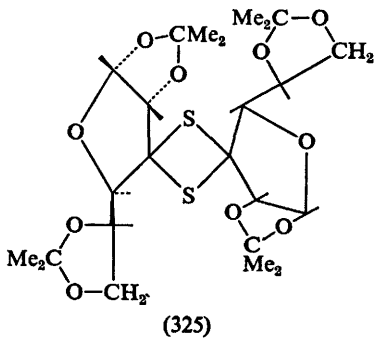
(323)

D-*ribo*-isomer (323). This procedure constitutes a useful way of changing the configuration of a sugar at C-5.[298]

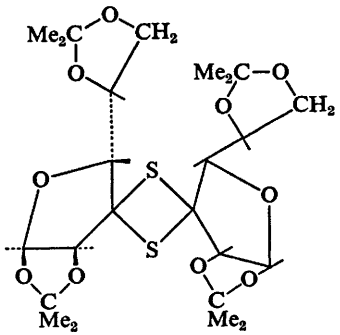
A mixture of (325) and (326), but not (324), was formed when 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose (256) was treated with phosphorus pentasulphide in boiling pyridine. The ketone (256) was



(324)



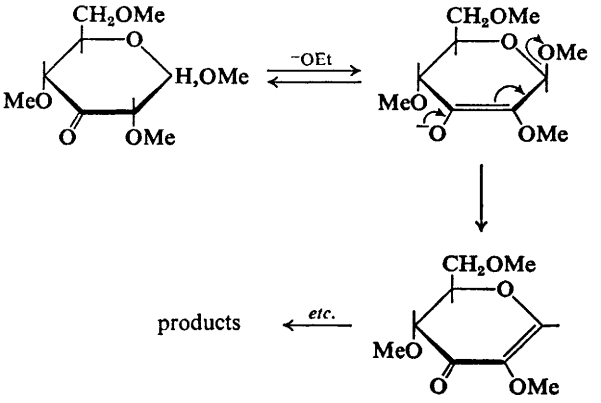
(325)



(326)

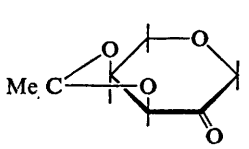
[479] P. Köll, R. Dürrfeld, U. Wolfmeier, and K. Heyns, *Tetrahedron Letters*, 1972, 5081.

converted into crude (324) on treatment with hydrogen sulphide, but rearrangement to (325) and (326) occurred during attempted purification.[480] Treatment of methyl 2,4,6-tri-*O*-methyl-α- and -β-D-*ribo*-hexopyranosid-3-uloses with ethanolic sodium ethoxide gave a complex mixture of products.[481] The degradation was presumed to involve loss of the aglycone (Scheme 52) to give a product that undergoes further reactions.

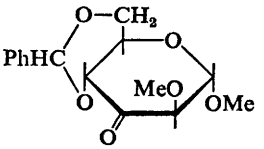


Scheme 52

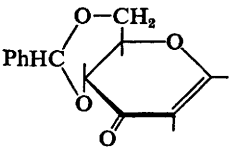
Further studies on the photochemistry of aldulose derivatives have been reported.[456] Methyl 3,4-*O*-isopropylidene-α- and -β-L-*erythro*-pentopyranosid-2-uloses on irradiation in t-butyl alcohol underwent Norrish Type II cleavage to give the 1,5-anhydro-compound (327). However,



(327)



(328)



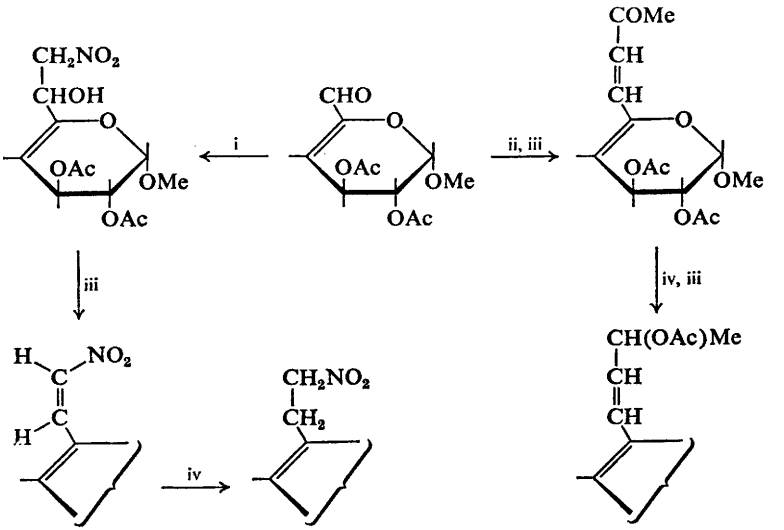
(329)

whereas (328) afforded (329) on photolysis, the C-2 epimer (264) gave the oxetanol (265); these differences were rationalized mechanistically.

D-*Glycero*-D-*galacto*-heptitol (perseitol) and L-*glycero*-D-*galacto*-heptitol have been prepared from acetylenic precursors formed by way of ethynylation of 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose.[482]

The synthetic potential of αβ-unsaturated aldehydo-sugars is illustrated by the base-catalysed reaction shown in Scheme 53.[483]

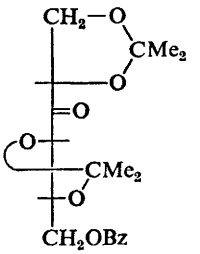
[480] J. N. Dominguez and L. N. Owen, *Carbohydrate Res.*, 1972, **22**, 225.
[481] L. Kenne, O. Larm, and S. Svensson, *Acta Chem. Scand.*, 1972, **26**, 2473.
[482] R. Hems, D. Horton, and M. Nakadate, *Carbohydrate Res.*, 1972, **25**, 205.
[483] D. Horton and A. Liav, *Carbohydrate Res.*, 1972, **24**, 105.



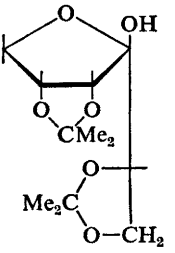
Reagents: i, $MeNO_2$–Et_3N; ii, Me_2CO–NaOMe; iii, Ac_2O–py; iv, $NaBH_4$

Scheme 53

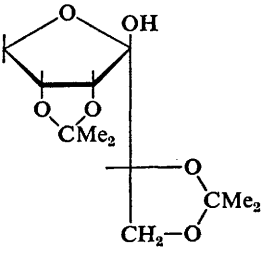
Debenzoylation of 6-*O*-benzoyl-1,2:4,5-di-*O*-isopropylidene-DL-*xylo*-hex-3-ulose (330) with sodium methoxide afforded the cyclic DL-*ribo*- (331) and DL-*arabino*- (332) derivatives, and not as reported previously the α- and β-anomers of 1,2:4,5-di-*O*-isopropylidene-DL-*xylo*-hex-3-ulofuranose.



(330)



(331)



(332)

Treatment of (331) or (332) with sodium methoxide in methanol led to their interconversion.[484]

Some branched-chain uloses are described in Chapter 15.

[484] S. J. Angyal and M. E. Evans, *Austral. J. Chem.*, 1972, **25**, 1347.

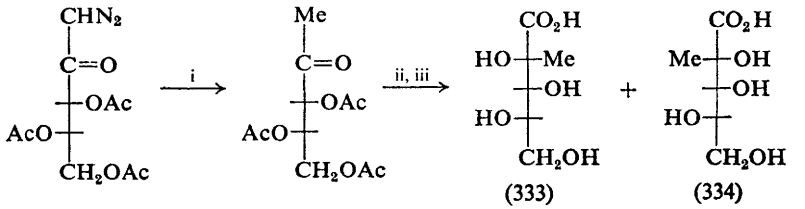
17
Sugar Acids and Lactones

Aldonic Acids

Several aldonic acid derivatives are referred to in Chapter 22. It has been confirmed that potassium D-gluconate has a planar, extended carbon chain and thus deviates from the normal situation for alditols in that the C-2—O and C-4—O bonds are eclipsed; reasons for this have been discussed.[485] Lactone formation has been shown to be responsible for the difference between the true and apparent ionization constants of D-gluconic acid.[486]

In the area of D-gluconic acid derivatives, a preparation of 2,3,4,6-tetra-*O*-methyl-D-glucono-1,5-lactone from methyl α-D-glucopyranoside has been described.[487] 6-*O*-(*NN*-Dimethylglycyl)-D-glucono-1,4-lactone has been related to pangamic acid (vitamin B_{15}),[488] and 3-deoxy-3-fluoro-D-gluconic acid (isolated as its calcium salt) has been obtained by electrolytic oxidation of 3-deoxy-3-fluoro-D-glucose.[489]

'α- and β-L-Galactosaccharinic acids' (333) and (334) have been synthesized as shown in Scheme 54, and they have also been obtained by way



Reagents: i, HI; ii, HCN; iii, H^+

Scheme 54

of an α-dicarbonyl intermediate when L-sorbose was treated with calcium hydroxide.[444]

Treatment of D-ribono-1,4-lactone with cyclohexanone in the presence of an acid catalyst gave the expected 2,3-acetal as well as a small proportion

[485] G. A. Jeffrey and E. J. Fasiska, *Carbohydrate Res.*, 1972, **21**, 187.
[486] L. H. Skibsted and G. Kilde, *Dansk Tidsskr. Farm.*, 1971, **45**, 320.
[487] P. Moeller, *Annalen*, 1972, **755**, 191.
[488] J. C. Micheau, A. Lattes, M. Podesta, G. Roux, and P. Gayrel, *Chim. Ther.*, 1972, **7**, 103.
[489] N. F. Taylor, B. Hunt, P. W. Kent, and R. A. Dwek, *Carbohydrate Res.*, 1972, **22**, 467

of the 3,4- acetal of the 1,5-lactone.[490] Azidopentitols were prepared from these lactones as described in Chapter 10.

2-Deoxy-2-nitroheptonate derivatives have been obtained by the addition of ethyl nitroacetate to *aldehydo*-pentose derivatives.[491]

Aldaric Acids

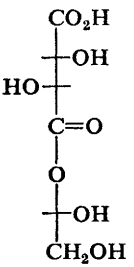
Acetals obtained from galactaric acid and potassium hydrogen D-glucarate [212] are mentioned in Chapter 5.

Ulosonic Acids

The synthesis of D-*arabino*-hexulosonic acid 6-phosphate from D-gluconic acid 6-phosphate has been described, and the chemical and physical properties of this ulosonic acid have been reported.[492] A 3-deoxy-5-*O*-methyloctulosonic acid of unknown configuration at C-4 and C-5 has been prepared according to Scheme 55 and comments on the quantitative determination of compounds of this class have been given.[493]

Hydrolysis of a bacterial lipopolysaccharide from *Xanthomonas sinensis* gave 4,7- and 4,8-anhydro-3-deoxyoctulosonic acids, which were proposed to arise by the reactions shown in Scheme 56.[189]

The unstable, hemiacetal structure (335) was suggested as an intermediate in the transformation of '5-ketogluconic acid' into L-tartaric acid.[494]



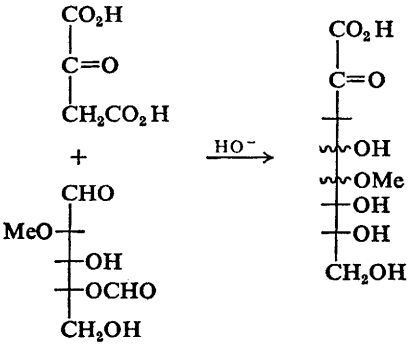
(335)

Uronic Acids

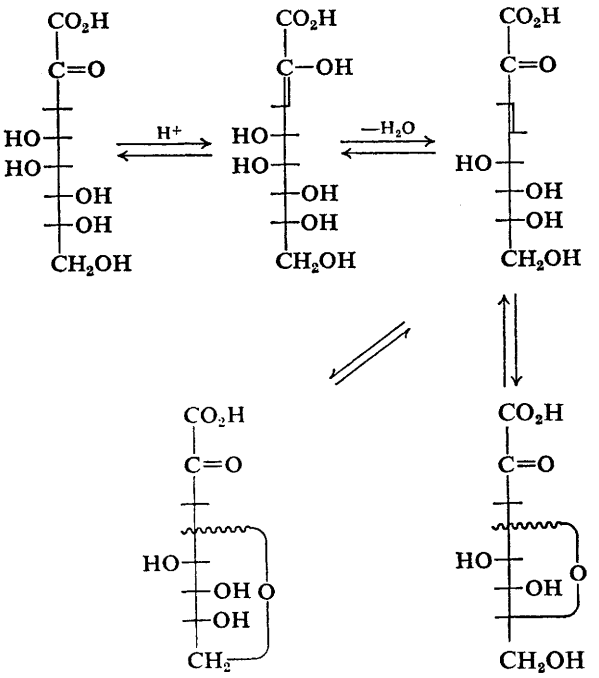
Unsaturated uronic acid esters are referred to in Chapter 14 and mass-spectral studies on uronic acids are noted in Chapter 24.

In aqueous solution at slightly acid pH at 96 °C, D-glucuronic acid and D-galacturonic acid afforded compounds (9)—(20), whereas compounds

[490] A. M. Sepulchre, A. Gateau, and S. D. Gero, *Carbohydrate Res.*, 1972, **24**, 311.
[491] V. I. Kornilov, B. B. Paidak, and Yu. A. Zhdanov, *Zhur. obshchei Khim.*, 1972, **42**, 480.
[492] F. Trigalo and L. Szabo, *European J. Biochem.*, 1972, **25**, 336.
[493] D. Charon and L. Szabo, *European J. Biochem.*, 1972, **29**, 184.
[494] U. Kotera, T. Kodama, Y. Minoda, and K. Yamada, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 1315.



Scheme 55



Scheme 56

(9), (11), (12), (14), (18), and (19) were obtained from pentoses (see Chapter 2). It was considered that many of these compounds are intermediates in colour formation during the heating or ageing of cellulosic materials containing hemicelluloses comprised of pentoses and uronic acids.[63]

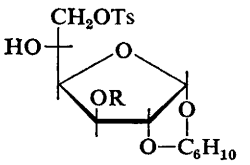
Various fully protected 1-*O*-acylamino-D-glucuronic acids have been prepared by different condensation procedures[495] and several derivatives

[495] G. Roglić and D. Keglević, *Croat. Chem. Acta*, 1972, **44**, 229.

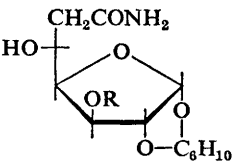
of D-xylofuranurononitrile have been reported as part of a study of uronic acids.[433, 496]

Because of their occurrence in polysaccharides, interest has been shown by two groups of workers in iduronic acid derivatives. Perlin and his co-workers have reported the synthesis of methyl α- and β-D-idopyranosiduronic acids and have studied the conformations of these acids in solution by ^1H and ^{13}C n.m.r. spectroscopy. Considerations of the optical activities of the acids led to the conclusion that the rotation of heparin is consistent with the presence, *inter alia*, of both D-glucuronic acid and L-iduronic acid residues, with the latter predominating. However, the rotation of dermatan sulphate is not consistent with it being a mixed polymer of equal proportions of α-L-idopyranuronosyl and 2-acetamido-2-deoxy-β-D-galactopyranosyl residues.[497] In related work, phenyl α- and β-L-idopyranosiduronic acids have been prepared by the catalytic oxidation of appropriate glycosides.[498] Azido-derivatives of aldohexoses can be oxidized catalytically with oxygen in the presence of platinum to give hexuronic acids without affecting the azido-group.[315]

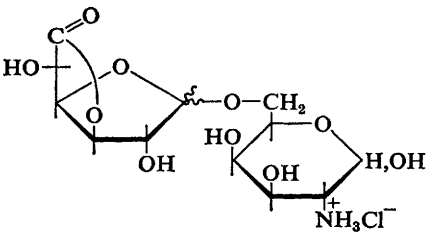
The 6-amido-derivative (337) has been prepared by treating (336) with potassium cyanide in methanol followed by reaction with hydrogen peroxide.[499]



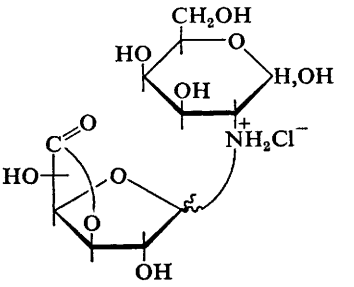
(336) R = Bn or Me



(337)



(338)



(339)

[496] H. Wiedmann, H. W. Oberwalder, E. Wildschek, and H. Schwarz, *Monatsh.*, 1972, **103**, 210.
[497] A. S. Perlin, B. Casu, G. R. Sanderson, and J. Tse, *Carbohydrate Res.*, 1972, **21**, 123.
[498] R. B. Friedman and B. Weissmann, *Carbohydrate Res.*, 1972, **24**, 123.
[499] Yu. A. Zhdanov, Yu. E. Alekseev, H. A. Kurdanov, and G. E. Gutterman, *Zhur. obshchei Khim.*, 1972, **42**, 481.

Disaccharides containing uronic acids are mentioned in Chapter 3 and, in addition, the unusual compounds (338) and (339) have been reported.[500]

Acetolyses of methyl esters of *pseudo*-aldobiouronic acids have been examined.[501]

Ascorbic Acids

A new sub-micro method for determining L-ascorbic acid is noted in Chapter 26.

Dehydro-D-ascorbic acid has been prepared by iodine oxidation of the parent acid [502] and factors affecting the decomposition of aqueous solutions of dehydro-L-ascorbic acid have been studied.[503] The crystal structures of dehydro-L-ascorbic acid and D-iso-ascorbic acid are noted in Chapter 24, and procedures for the separation of L-ascorbic and related acids are mentioned in Chapter 26.

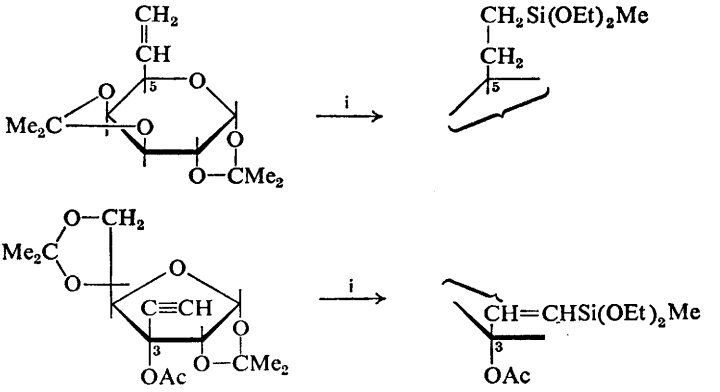
[500] A. Klemer and G. Müller, *Tetrahedron Letters*, 1972, 2313.
[501] V. I. Govorchenko, V. I. Gorbach, and Yu. S. Ovodov, *Khim. prirod. Soedinenii*, 1972, **3**, 258.
[502] W. Weis and H. Staudinger, *Annalen*, 1971, **754**, 152.
[503] J. Velíšek, J. Davídek, and G. Janíček, *Coll. Czech. Chem. Comm.*, 1972, **37**, 1465.

18
Inorganic Derivatives

Carbon-bonded Compounds

Little has been published in this area this year, but attention continued to be devoted to mercury derivatives obtained by addition reactions to unsaturated sugars (see Chapter 14). Photolysis of these compounds has been investigated; for example, methyl 2-(acetoxymercuri)-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside has been shown to give the corresponding 2-deoxy-sugar, thereby establishing a new route to this class of compound.[410] ^{199}Hg–^{1}H N.m.r. studies were reported, but further work will be necessary before the spectroscopic parameters can be fully interpreted. Mass-spectral data for a number of mercury compounds were also recorded.

The addition of diethoxymethylsilane to unsaturated sugar derivatives afforded a means of obtaining a new class of *C*-bonded, silicon-containing sugars (see Scheme 57).[504]



Reagent: i, $MeSiH(OEt)_2$–H_2PtCl_6

Scheme 57

Oxygen-bonded Compounds

This year's literature has revealed how significantly metal ions can influence carbohydrate reactions and how they can be advantageously utilized in

[504] J. Lehmann and H. Schäfer, *Chem. Ber.*, 1972, **105**, 969.

synthesis. Notable examples are described [77] in Chapter 3. Additionally, the synthesis of D-talose by hydroxylation of D-galactal was improved by the use of sodium molybdate [19] and that of 6-deoxy-L-glucose from L-rhamnal by the use of molybdic acid.[416] In the first of an important series of papers, Angyal has shown that such ions as Ca^{2+}, Pb^{2+}, and La^{3+} cause the equilibrium of D-allose solutions to be altered appreciably in favour of the α-isomers. Results with other sugars were also discussed.[505] The mutarotation of β-D-mannopyranose was affected by such ions as Fe^{2+}, Zn^{2+}, and Mn^{2+}; the complexes formed mutarotated faster than the free sugar.[35]

The complexing sites of various transition-metal ions with adenosine 5′-phosphate have been determined by n.m.r. spectroscopy. All the ions used complexed with the phosphate group and the heterocyclic ring, but the binding sites utilized on the latter varied.[506]

Various carbohydrate–ion complexes have been examined; 1 : 1 adducts of D-glucose and maltose with the hydroxides of barium, calcium, and strontium,[58] and related adducts of a series of di- and poly-saccharides [59] have been formed. Sodium iodide complexes of α-cellobiose and α-isomaltose were isolated in crystalline form, thereby offering a means of preparing the hitherto inaccessible α-anomers of these sugars.[507] These complexes were studied by such physical methods as *X*-ray diffraction, which also has been applied to β-D-mannopyranose,$CaCl_2$,$4H_2O$ [508] and hydrated calcium bromide complexes of lactose, α-D-galactopyranose, and *myo*-inositol.[54a]

Various sodium, copper, and mixed sodium–copper chelates of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside [509] and methyl 2,3-di-*O*-methyl-D-glucopyranoside [157] have been described.

An important new silylating agent is mentioned in Chapter 4 and sugar borate esters are referred to in Chapter 26.

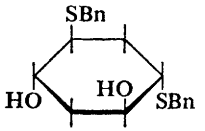
[505] S. J. Angyal, *Austral. J. Chem.*, 1972, **25**, 1957.
[506] A. W. Missen, D. F. S. Natusch, and L. J. Porter, *Austral. J. Chem.*, 1972, **25**, 129.
[507] J. A. Rendleman, jun., *Carbohydrate Res.*, 1972, **21**, 235.
[508] D. C. Craig, N. C. Stephenson, and J. D. Stevens, *Carbohydrate Res.*, 1972, **22**, 494.
[509] T. Nortia, R. Sillanpaa, and E. Avela, *Suomen Kem.* (*B*), 1972, **45**, 188.

19
Cyclitols

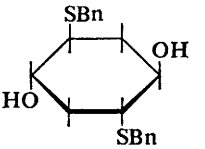
Racemic [510] and optically active [511] 2,3,4,5,6- and 1,2,4,5,6-penta-*O*-acetyl-*sn-myo*-inositols have been prepared and their configurations have been established.

During the attempted conversion of 2,3,4-tri-*O*-acetylxylaryl dichloride (311) into the *xylo*-2,6-heptodiulose derivative (313) by way of the bis-(diazomethyl) ketone (312), ring-closure occurred to give the cyclitol derivative (314) (see Scheme 51).[476]

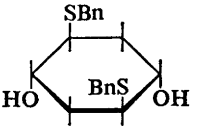
The three dibenzylthiocyclohexanediols (340)—(342) have been obtained by treating a mixture of the diepoxides (343) and (344) with sodium benzylmercaptide.[512] Structures were assigned on the basis of ^{1}H n.m.r. spectroscopy.



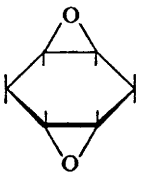
(340)



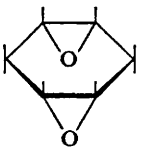
(341)



(342)



(343)



(344)

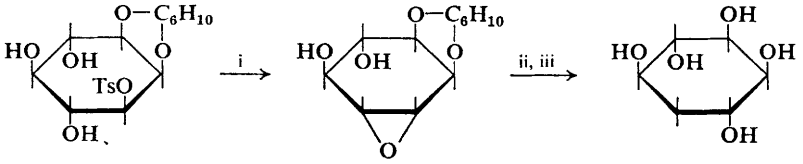
DL-*proto*-Quercitol has been obtained in high yield from 1,2-*O*-cyclohexylidene-3-*O*-toluene-*p*-sulphonyl-DL-*myo*-inositol as illustrated in Scheme 58.[513]

[510] A. E. Stepanov, O. O. Tutorskaya, B. A. Klyashchitskii, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1971, **7**, 1762.

[511] A. E. Stepanov, O. O. Tutorskaya, B. A. Klyashchitskii, V. I. Shvets, and R. P. Evstigneeva, *Zhur. obshchei Khim.*, 1972, **42**, 709.

[512] G. E. McCasland, A. K. M. Anisuzzaman, S. R. Naik, and L. J. Durham, *J. Org. Chem.*, 1972, **37**, 1201.

[513] T. Suami, S. Ogawa, T. Ueda, and H. Uchino, *Bull. Chem. Soc. Japan*, 1972, **45**, 3226.



Reagents: i, NaOMe–MeOH; ii, $LiAlH_4$; iii, H^+

Scheme 58

Various mono-, di-, and tri-*O*-toluene-*p*-sulphonyl derivatives of 1,2-*O*-cyclohexylidene-*myo*-inositol have been treated with sodium methoxide in methanol to give various mono- and di-epoxides.[514] 1,2:4,5-, 1,2:3,4-, and 1,2:5,6-di-, and 1,2:3,4:5,6-tri-*O*-cyclohexylidene derivatives of *myo*-inositol have been prepared.[515]

The reaction between diphenyl chlorophosphate and suitably protected *myo*-inositol derivatives afforded the 1- and 4-mono- and 1,4-di-phosphates of *myo*-inositol.[516]

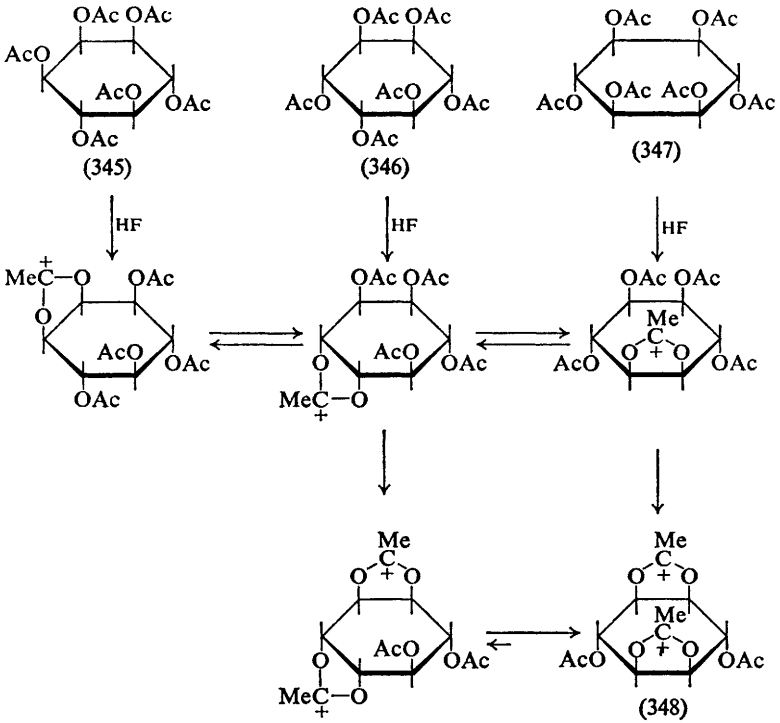
In continuing their investigations on the migration of acyloxonium ions, Paulsen and his colleagues have reported that treatment of the hexa-acetates of *myo*-inositol (345), L-*chiro*-inositol (346), and *muco*-inositol (347) with liquid hydrogen fluoride ultimately gave the *muco*-derivative (348), which exists in a stable boat conformation[219] (see Scheme 59). Monomethyl ethers of inositol penta-acetates were also studied. Derivatives of *cis*-cyclohexane-1,2-diol and *cis*-cyclopentane-1,2-diol were also used to study the formation of cyclic acyloxonium ions promoted by anhydrous hydrogen fluoride.[220]

3-Acetoxy-3-(acetoxymethyl)-5-methylcyclohexane-1,2-dione (349), one of the hydrolytic products of leucogenenol, has been synthesized as illustrated in Scheme 60.[517]

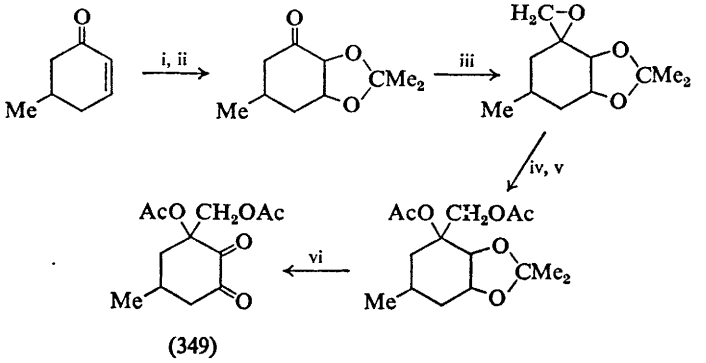
In a very detailed paper,[518] the bromination of several inosamines and inosadiamines with acetyl bromide and acetic anhydride has been studied and the conversion of the mono- and di-bromo-compounds formed into deoxy-compounds was also described. A mechanism for the bromination was proposed involving intermediate acetoxonium and oxazolinium ions.

A series of carbonyl derivatives of cyclopentane has been prepared from (350)[519] and the conversion of various ketals of polyhydroxycyclopentanones into free ketoses by transketalization, for example (351) into (352), has been investigated.[520]

[514] T. Suami, S. Ogawa, S. Oki, and K. Ohashi, *Bull. Chem. Soc. Japan.*, 1972, **45**, 2597.
[515] M. S. Sadovnikova, B. A. Klyashchitskii, V. I. Shvets, and R. P. Evstigneeva, *Zhur. obshchei Khim.*, 1971, **41**, 1855.
[516] S. P. Kozlova, I. S. Pekarskaya, B. A. Klyashchitskii, V. I. Shvets, and R. P. Evstigneeva, *Zhur. obshchei Khim.*, 1972, **42**, 702.
[517] F. A. H. Rice, *Carbohydrate Res.*, 1972, **21**, 65.
[518] T. Suami, S. Ogawa, S. Yanagida, and K. Nojima, *Bull. Chem. Soc. Japan.*, 1972, **45**, 2593.
[519] F. G. Cocu and T. Posternak, *Helv. Chim. Acta*, 1972, **55**, 2828.
[520] F. G. Cocu and T. Posternak, *Helv. Chim. Acta*, 1972, **55**, 2838.

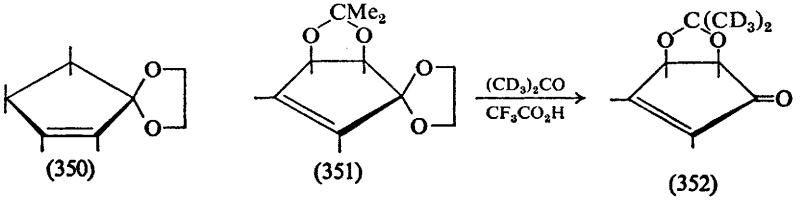


Scheme 59



Reagents: i, $KMnO_4$; ii, Me_2CO–H^+; iii, CH_2N_2; iv, NaOH; v, Ac_2O–py; vi, $(NH_4)_3VO_4$

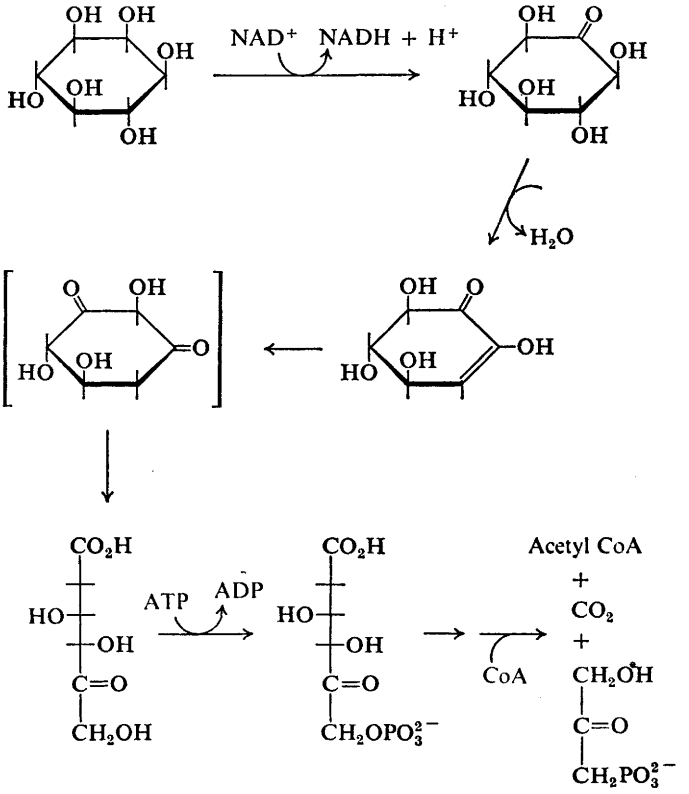
Scheme 60



(350) (351) (352)

The first non-cyclic intermediate in the catabolism of *myo*-inositol by *Aerobacter aerogenes* has been identified as '2-deoxy-5-keto-D-gluconic acid' [521] and the sequence of reactions illustrated (Scheme 61) was suggested for this transformation.[522]

Oxidation of *myo*-inositol gave DL-*epi*-2-inosose, which was reduced electrolytically in hydrogen-type cells with nickel, tantalum, copper, molybdenum, lead, tin, tungsten, cadmium, or mercury cathodes to give



Scheme 61

[521] W. A. Anderson and B. Magasanik, *J. Biol. Chem.*, 1971, **246**, 5653.
[522] W. A. Anderson and B. Magasanik, *J. Biol. Chem.*, 1971, **246**, 5663.

stereoisomeric inositols, among which *myo*-inositol and *epi*-inositol were identified by paper chromatography.[523]

The favoured conformations of the cyclohexanediols and cyclitols have been examined by taking into account non-bonded interactions and restricted rotation of the hydroxy-groups.[524] Calculations using the extended Hückel MO(EHMO) method were performed to determine the conformational energy. Conformations with equatorial hydroxy-groups and having certain orientations of hydroxy-protons were indicated to be most stable.

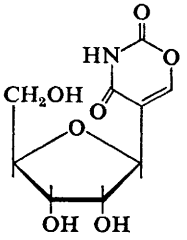
Use has been made of differences in the chemical shifts of equatorial and axial hydroxy-protons at low temperatures for conformational assignments to cyclohexanols by ^{1}H n.m.r. spectroscopy.[525]

[523] J. H. Sohn, C. W. Woo, and C. H. Park, *Daehan Hwahak Hwoejee*, 1972, **16**, 93.
[524] Yu. A. Zhdanov and E. N. Malysheva, *Carbohydrate Res.*, 1972, **24**, 87.
[525] J. Moulines, J.-P. Bats, and M. Petraud, *Tetrahedron Letters*, 1972, 2971.

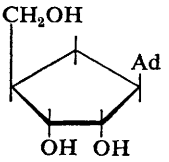
20
Antibiotics

Identical structures (353) have been assigned to minimycin [526] and oxazinomycin.[527] Evidence from a variety of sources, including *X*-ray studies, has confirmed that aristeromycin has the structure (354).[528] Hikizimycin (molecular formula $C_{21}H_{37}N_5O_{14}$) has the partial structure (355).[529] The biosynthesis of arabinofuranosyladenine by *Streptomyces antibioticus* has been studied [530] and a range of aminoacyl derivatives of polyoxin C has been synthesized as part of a study of structure–activity relationships.[531]

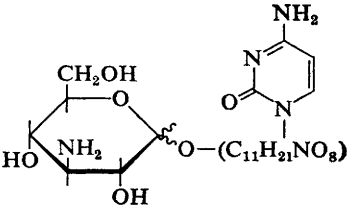
The total syntheses of gougerotin and blasticidin S have been reviewed [532] and details of the former synthesis have been given in two papers by Fox's



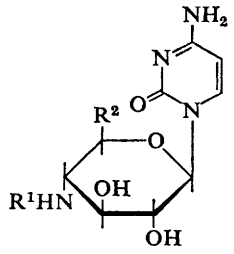
(353)



(354)



(355)



(356) R^1 = H, R^2 = CO_2H

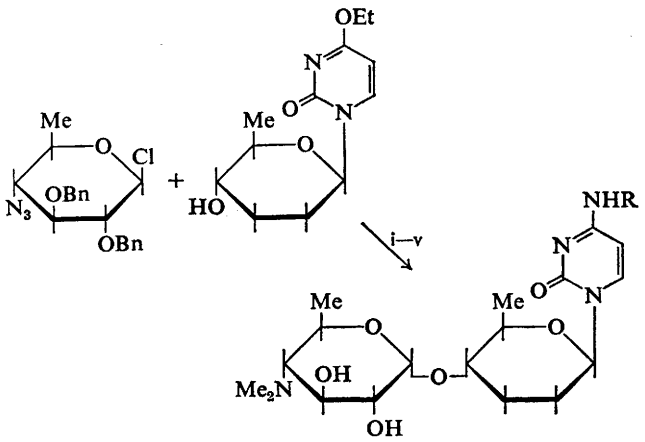
(357) R^1 = ·COCHNHCOCH$_2$NHMe, R^2 = $CONH_2$
|
CH_2OH

(358) R^1 = ·COCHNHCOCH$_2$NHMe, R^2 = CH_2OH
|
CH_2OH

[526] K. Sasaki, Y. Kusakabe, and S. Esumi, *J. Antibiotics*, 1972, **25**, 15.
[527] T. Haneishi, T. Okazaki, T. Hata, C. Tamura, M. Nomura, A. Naito, I. Seki, and M. Arai, *J. Antibiotics*, 1971, **24**, 797.
[528] T. Kishi, M. Muroi, T. Kusaka, M. Nishikawa, K. Kamiya, and K. Mizuno, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 940.
[529] B. C. Das, J. Defaye, and K. Uchida, *Carbohydrate Res.*, 1972, **22**, 293.
[530] P. B. Farmer and R. J. Suhadolnik, *Biochemistry*, 1972, **11**, 1911.
[531] K. Isono, S. Suzuki, and T. Azuma, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1986.
[532] J. J. Fox and K. A. Watanabe, *Pure Appl. Chem.*, 1971, **28**, 475.

group. The first [533] described the synthesis of 'C-substance' (356) and the second [534] its conversion into the complete antibiotic (357). Another paper [534a] from the same laboratory has reported on the synthesis of the gougerotin analogue (358) and asteromycin has been shown to be identical to gougerotin.[535]

The nucleoside component of blasticidin S, namely cytosinine, has been synthesized from 3,4,6-tri-*O*-acetyl-D-glucal.[536] The naturally occurring nucleosides pentopyranines A and C have been identified as 1-(2,3-dideoxy-α-L-*glycero*-pentopyranosyl)- and 1-(3-deoxy-α-L-*threo*-pentopyranosyl)-cytosine, respectively, and are presumed to be formed by a shunt mechanism during the biosynthesis of blasticidin S.[537] A synthesis of the disaccharide nucleoside antibiotic plicacetin (359), a close relative of amicetin, has now been accomplished [538] by the synthesis of cytosamine (360) (Scheme 62); previously (360) had been converted into (359), thus formally completing the synthesis.



(359) R = ·$COC_6H_4NH_2$-*p*
(360) R = H

Reagents: i, base; ii, H_2–Pt/C, iii, HCHO–H_2–Pd/C; iv, NH_3 (liq.)–EtOH; v, H_2–Pd/C–H_3O^+

Scheme 62

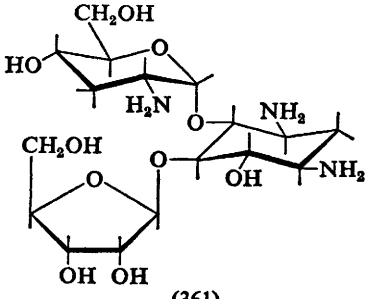
Much work continued to be carried out on the amino-glycoside antibiotics, especially by the modification of natural molecules. The chemistry

[533] K. A. Watanabe, I. M. Wempen, and J. J. Fox, *Carbohydrate Res.*, 1972, **21**, 148.
[534] K. A. Watanabe, E. A. Falco, and J. J. Fox, *J. Amer. Chem. Soc.*, 1972, **94**, 3272.
[534a] K. A. Watanabe, E. A. Falco, and J. J. Fox, *J. Org. Chem.*, 1972, **37**, 1198.
[535] T. Ikeuchi, F. Kitame, M. Kikuchi, and N. Ishida, *J. Antibiotics*, 1972, **25**, 548.
[536] T. Kondo, H. Nakai, and T. Goto, *Tetrahedron Letters*, 1972, 1881.
[537] H. Seto, N. Otake, and H. Yonehara, *Tetrahedron Letters*, 1972, 3991.
[538] C. L. Stevens, J. Němec, and G. H. Ransford, *J. Amer. Chem. Soc.*, 1972, **94**, 3280.

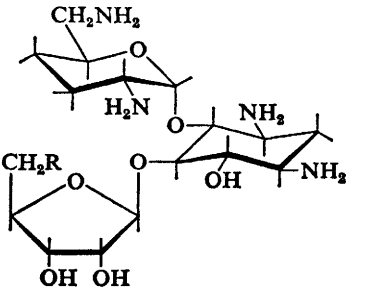
of this class of antibiotic has been reviewed with emphasis on the gentamicins.[539] Syntheses of garosamine and gentosamine [294] (Vol. 5, p. 130) and of epipurpurosamine C [540] (see Vol. 5, p. 68) have been described in full.

3′- and 4′-*O*-Methylneamines have been prepared by standard routes [541] and enzymic transglucosylation of neamine gave 5-*O*-D-glucosylneamine of unspecified anomeric linkage.[542] Paromamine and its isomer 6-*O*-(2-amino-2-deoxy-α-D-glucosyl)-2-deoxystreptamine have been synthesized by condensation of racemic mono-*O*-cyclohexylidene-*NN*′-diethoxycarbonyl-2-deoxystreptamine with 3,4,6-tri-*O*-acetyl-*N*-(4-methoxybenzylidene)-α-D-glucosaminyl bromide.[543]

Structure (361) has been established for lividomycin B, which was found to be identical with the product from Barry degradation of lividomycin A.[544] The 5″-phosphate of lividomycin A has been synthesized and was shown



(361)



(362) R = OH
(363) R = H

to be identical with an enzymically inactivated material.[545] 5″-Deoxy-derivatives of lividomycin A [546] and B [547] have been reported, and 3′,4′-di- and 3′,4′,5″-tri-deoxy-derivatives of ribostamycin [(362) and (363), respectively], have been synthesized.[548]

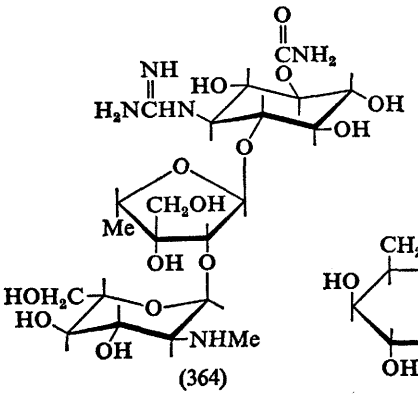
Full details have been given of the syntheses of 3′-deoxy- [549] and 3′-*O*-methyl-kanamycin A.[550] Enzymically inactivated products of kanamycin A,

[539] D. J. Cooper, *Pure Appl. Chem.*, 1971, **28**, 455.
[540] R. D. Guthrie and G. J. Williams, *J.C.S. Perkin I*, 1972, 2619.
[541] S. Umezawa, T. Jikihara, and T. Tsuchiya, *J. Antibiotics*, 1972, **25**, 322.
[542] T. Endo and D. Perlman, *J. Antibiotics*, 1972, **25**, 681.
[543] S. Umezawa, T. Miyazawa, and T. Tsuchiya, *J. Antibiotics*, 1972, **25**, 530.
[544] T. Mori, Y. Kyotani, I. Watanabe, and T. Oda, *J. Antibiotics*, 1972, **25**, 149.
[545] H. Yamamoto, S. Konda, K. Maeda, and H. Umezawa, *J. Antibiotics*, 1972, **25**, 485.
[546] H. Yamamoto, S. Konda, K. Maeda, and H. Umezawa, *J. Antibiotics*, 1972, **25**, 487.
[547] S. Umezawa, I. Watanabe, T. Tsuchiya, H. Umezawa, and M. Hamada, *J. Antibiotics*, 1972, **25**, 617.
[548] S. Umezawa, T. Tsuchiya, D. Ikeda, and H. Umezawa, *J. Antibiotics*, 1972, **25**, 613.
[549] S. Umezawa, Y. Nishimura, H. Hineno, K. Watanabe, S. Koike, T. Tsuchiya, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 2847.
[550] H. Umezawa, T. Tsuchiya, R. Muto, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 2842.

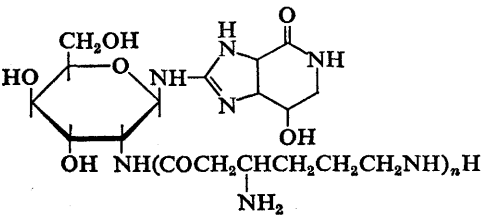
paromamine, and dehydrostreptomycin have been identified as the 3′-phosphates for the first two antibiotics and as the 3″-phosphate for the third antibiotic.[551] It has been established that 3′,4′-dideoxykanamycin B is inactivated enzymically either by adenylation at the 2″-hydroxy-group [552] or as the 2″-guanylate or 2″-inosinate derivatives,[553] depending on which nucleoside triphosphate is present. An enzyme in a resistant strain of *Pseudomonas aeruginosa* has been shown to inactivate 3′,4′-dideoxykanamycin B by 6′-*N*-acetylation.[554]

It has been suggested that τ of H-1 in the n.m.r. spectra of kanamycins and related compounds can be used for assigning the absolute configurations at C-4 and C-6; for 4-linkages, τ of H-1 was observed in the range 4.62—4.72, whereas for 6-linkages it appeared in the range 4.86—4.95 (all in D_2O).[555]

Antibiotic 2230-C has been firmly established as a mannosylparomomycin.[556] A study of the Cupra B and TACu complexing of bluensomycin, deamidinocarbamoylbluensomycin, and other related compounds has provided evidence that (364) represents the structure of bluensomycin.[557]



(364)



F $n = 1$ β $n = 5$
E $n = 2$ α $n = 6$
D $n = 3$ χ $n = 7$
C $n = 4$

(365)

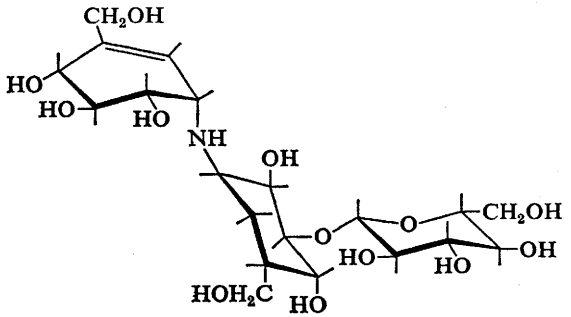
[551] H. Naganawa, S. Kondo, K. Maeda, and H. Umezawa, *J. Antibiotics*, 1971, **24**, 823.
[552] M. Yagisawa, H. Naganawa, S. Kondo, M. Hamada, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1971, **24**, 911, 913.
[553] M. Yagisawa, H. Naganawa, S. Kondo, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1972, **25**, 492.
[554] M. Yagisawa, H. Naganawa, S. Kondo, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1972, **25**, 495.
[555] A. Hasegawa, D. Nishimura, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1043.
[556] T. Mori, Y. Kyotani, I. Watanabe, and T. Oda, *J. Antibiotics*, 1972, **25**, 317.
[557] C. B. Barlow and L. Anderson, *J. Antibiotics*, 1972, **25**, 281.

Destomycin has been shown to be 5-*O*-(2-deoxy-3-*N*-methylstreptaminyl)-3,2-*O*-(6-amino-6-deoxyheptopyranosyl)-β-D-talopyranoside [558] and it has been confirmed that the streptothricins have the structures (365).[559] The sugar moiety of antibiotic YA56 has been established as 6-deoxy-2-*O*-(3-*O*-carbamoyl-D-mannosyl)-L-gulose.[560] A mutant strain of *S. caelestis*, a micro-organism that produces celesticetin, gave rise to 7-*O*-demethylcelesticetin.[561]

Full details of the total synthesis of kasugamycin have been given [562] (*cf.* Vol. 2, p. 176).

Stereochemical aspects of the macrolide antibiotics [563] and the chemistry and conformation of erythromycin [564] have been reviewed; further studies on the biosynthesis of these antibiotics have been reported.[565] Rosamicin, a new macrolide antibiotic, has been shown to contain D-desosamine.[566] The conformation of the macrolide leucomycin in solution has been studied by several physical techniques.[567]

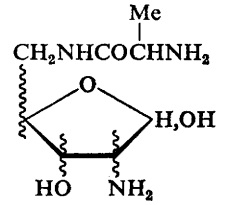
Validamycin A has been shown to have the novel structure (366) [568] and a notable feature is the presence of an unsaturated cyclitol moiety.[568a] Prumycin, a new anti-fungal antibiotic, has been shown to have the structure (367) [569] and sibirosamine (from sibiromycin) to have



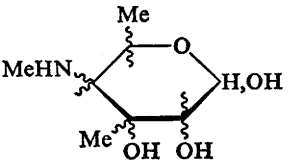
(366)

[558] S. Kondo, *Keio Gijuku Daigaku Kogakubu Kenkyu Hokuku, Bessatsu*, 1971, **9**, 71.
[559] A. S. Khokhlov and K. I. Shutova, *J. Antibiotics*, 1972, **25**, 501.
[560] Y. Ito, Y. Ohashi, S. Kawabe, H. Abe, and T. Okuda, *J. Antibiotics*, 1972, **25**, 543.
[561] A. D. Argoudelis, J. H. Coats, P. G. Lemaux, and O. K. Sebek, *J. Antibiotics*, 1972, **25**, 445.
[562] Y. Suhara, F. Sasaki, G. Koyama, K. Maeda, H. Umezawa, and M. Ohno, *J. Amer. Chem. Soc.*, 1972, **94**, 6501.
[563] W. D. Celmer, *Pure Appl. Chem.*, 1971, **28**, 413.
[564] T. J. Perun, *Drug. Action Drug Resist. Bact.*, 1971, **1**, 123.
[565] J. R. Martin, T. J. Perun, and R. S. Egan, *Tetrahedron*, 1972, **28**, 2937.
[566] H. Reimann and R. S. Jaret, *J.C.S. Chem. Comm.*, 1972, 1270.
[567] S. Omura, A. Nakagawa, N. Yagisawa, Y. Suzuki, and T. Hata, *Tetrahedron*, 1972, **28**, 2839.
[568] S. Horii and Y. Kameda, *J.C.S. Chem. Comm.*, 1972, 747.
[568a] Y. Kameda and S. Horii, *J.C.S. Chem. Comm.*, 1972, 746.
[569] S. Omura, M. Tishler, M. Katagiri, and T. Hatz, *J.C.S. Chem. Comm.*, 1972, 633.

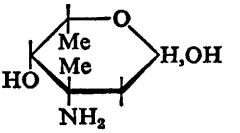
the structure (368).[570] Two groups have reported on the structure of vancosamine,[316, 317, 317a] obtained on hydrolysis of the antibiotic vancomycin (from *Streptomyces orientalis*) with acid,[457] but only the Sussex group were able to establish the absolute configuration as (369).



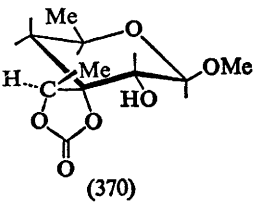
(367)



(368)



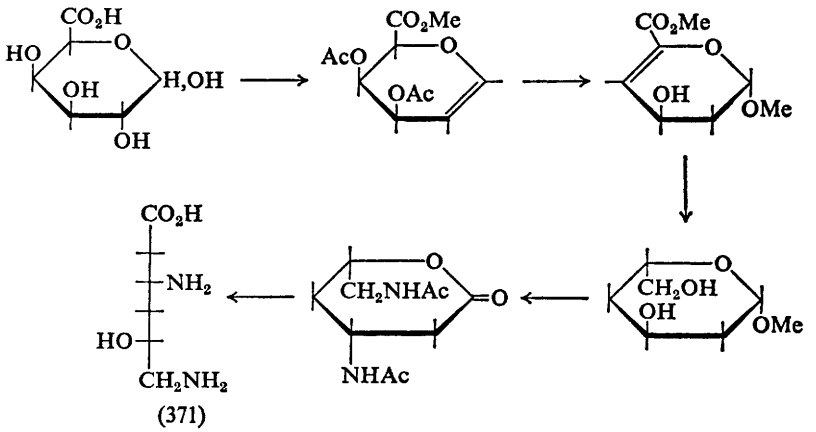
(369)



(370)

The four possible structures for methyl β-D-aldgaroside have been synthesized and comparisons with the natural sugar showed that it has the (*S*)-configuration (370) at the asymmetric carbon atom of the branched chain.[571]

Unsaturated carbohydrates have been used in novel syntheses of a component (371) (Scheme 63) of the antibiotic negamycin and its enantiomer

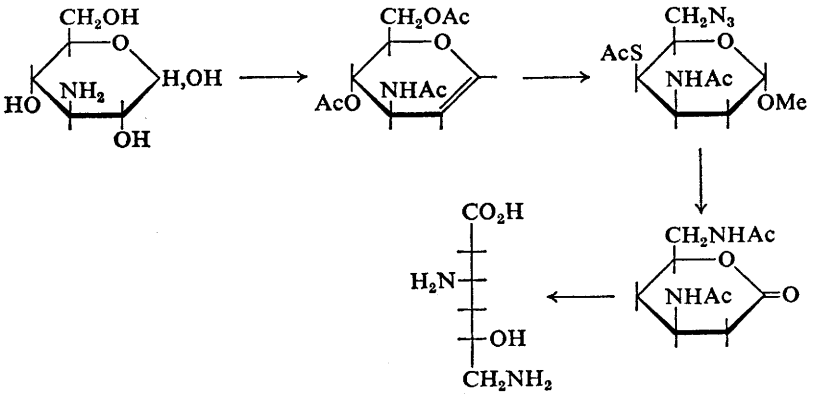


(371)

Scheme 63

[570] A. S. Mezentsev, V. V. Kulyaeva, L. M. Rubasheva, M. G. Brazhnikova, O. S. Anisimova, T. V. Vlasova, and Yu. N. Sheinker, *Khim. prirod. Soedinenii*, 1971, 650.
[571] H. Paulsen and H. Redlich, *Angew. Chem. Internat. Edn.*, 1972, **11**, 1021.

(Scheme 64). In both schemes, the chemical transformations are standard [572] and the syntheses were completed by condensing the final products with δ-hydroxy-β-lysines.



Scheme 64

Studies on the synthesis of nojirimycin and its 6-deoxy-derivative have been described.[402] The lactam (201) derived from nojirimycin was demonstrated to be a potent inhibitor of β-glucuronidase.[403]

[572] S. Shibahara, S. Kondo, K. Maeda, H. Umezawa, and M. Ohno, *J. Amer. Chem. Soc.*, 1972, **94**, 4353.

21
Nucleosides

Both parts of a book entitled 'Organic Chemistry of the Nucleic Acids' have been translated into English.[573] *N*-[9-(β-D-Ribofuranosylpurin-6-yl)-*N*-methylcarbamoyl]threonine has been isolated from *Escherichia coli* t-RNA^{Thr}.[574] Some thirty-five modified nucleosides found in t-RNA and DNA have been described in detail in a book [575] that contains a wealth of associated knowledge on these compounds. The cytosine nucleosides pentopyranine A and C have been identified as 1-(2,3-dideoxy-α-L-*glycero*-pentopyranosyl)- and 1-(3-deoxy-α-L-*threo*-pentopyranosyl)-cytosines.[537] These are the first naturally occurring nucleosides with the α-L-configuration to be isolated and are presumed to be products of a shunt pathway in the biosynthesis of blasticidin S.

Various nucleoside antibiotics [526, 529, 535] are described in Chapter 20.

Synthesis

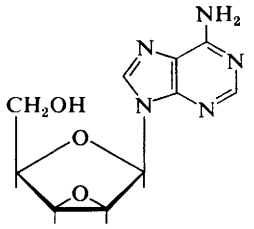
Among compounds that have been synthesized by conventional procedures are polynucleotides containing 2-chloro-2-deoxy-D-ribose,[576] 5′-deoxy-5′-fluoro-derivatives of uridine, cytidine [577] (the fluorine atom was introduced into the intact nucleosides), 6-azauridine, and D-ribofuranosylthymine,[578] uridinyl-(3′ → 5′)-uridine,[579] 2-fluoro-9-β-D-ribofuranosylpurine [580] (prepared from 6-benzylthio-2-fluoronebularine), a number of 5′-butylpyrimidines,[581] 5′-*S*-butyladenosine [581a] [and other 5′-CH_2SCH_2R derivatives, where R = $CH(OH)CH_2OH$, CH_2NH_2, CH_2NHAc, or CH_2CO_2H), xylose, 2-deoxyribose, and arabinose analogues of 8-aza-adenosine [582]

[573] 'Organic Chemistry of the Nucleic Acids', ed. N. K. Kochetkov and E. I. Budovskii, Plenum Press, Part A, 1971, and Part B, 1972.
[574] F. Kimura-Harada, D. L. van Minden, J. A. McCloskey, and S. Nishimura, *Biochemistry*, 1972, **11**, 3910.
[575] R. H. Hall, 'The Modified Nucleosides of Nucleic Acids', Columbia University Press, New York, 1972.
[576] J. Hobbs, H. Sternbach, M. Sprinzl, and F. Eckstein, *Biochemistry*, 1972, **11**, 4336.
[577] M. Schütt, G. Kowollik, G. Etzold, and P. Langen, *J. prakt. Chem.*, 1972, **314**, 251.
[578] G. Kowollik, G. Demirow, M. Schütt, and P. Langen, *Z. Chem.*, 1972, **12**, 106.
[579] A. Hodgson and A. S. Jones, *Tetrahedron Letters*, 1972, 5349.
[580] M. Kawana, R. J. Rousseau, and R. K. Robins, *J. Medicin. Chem.*, 1972, **15**, 214.
[581] E. H. Hamamura, K. Sato, and J. G. Moffatt, *J. Medicin. Chem.*, 1972, **15**, 1061.
[581a] J. Hildesheim, R. Hildesheim, and E. Lederer, *Biochemie*, 1972, **54**, 431.
[582] J. A. Montgomery and H. J. Thomas, *J. Medicin. Chem.*, 1972, **15**, 105.

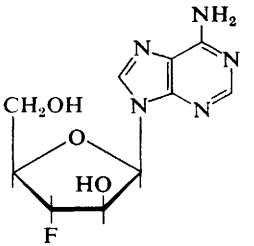
(prepared from *N*-nonanoyl-8-aza-adenine and the appropriate pentofuranosyl halides), 1,2,3-triazole analogues of pyrazomycin,[583] 3-β-D-ribofuranosyl- and 2-deoxy-D-ribofuranosyl-γ-triazolo[4,5-*d*]pyrimidines,[584] 7-amino-3-α-D-arabinofuranosyl-γ-triazolo-[4,5-*d*]pyrimidine and related nucleosides,[585] 7-(β-D-ribofuranosyl)imidazo[4,5-*d*]-*vic*-triazin-4-one,[586] a range of D-glucosyl and D-ribosyl guanine nucleosides,[587] nucleosides containing unsaturated pyranosyl moieties,[427] and purine derivatives of 3-amino-2,3-dideoxypyranosyl sugars.[425]

Oxazoline intermediates have been used for stereospecific syntheses of some alkyl-5-amino-1-glycofuranosylimidazole 4-carboxylates related to intermediates in the *de novo* biosynthesis of purine nucleosides.[588]

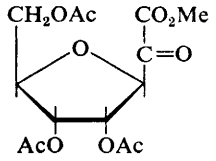
9-(3-Deoxy-3-fluoro-β-D-arabinofuranosyl)adenine (373) has been prepared by treating (372) with potassium hydrogen fluoride in ethylene glycol.[589] 9-(3-Amino-3-deoxy-β-D-ribofuranosyl)-6-methylamino-9-*H*-purine, a major metabolite of puromycin aminonucleoside in rabbit urine, has been synthesized.[590] Compound (374) has been converted into the nucleoside antibiotic pyrazomycin (375).[591]



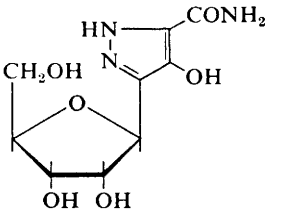
(372)



(373)

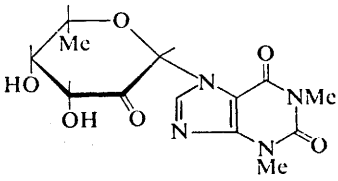


(374)



(375)

[583] O. Makabe, S. Fukatsu, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 2577.
[584] W. Hutzenlaub, R. L. Tolman, and R. K. Robins, *J. Medicin. Chem.*, 1972, **15**, 879.
[585] C. W. Smith, R. W. Sidwell, R. K. Robins, and R. L. Tolman, *J. Medicin. Chem.*, 1972, **15**, 883.
[586] M. Kawana, G. A. Ivanovics, R. J. Rousseau, and R. K. Robins, *J. Medicin. Chem.*, 1972, **15**, 841.
[587] W. Pfleiderer, M. Shanshal, and K. Eistetter, *Chem. Ber.*, 1972, **105**, 1497.
[588] D. H. Robinson and G. Shaw, *Experientia*, 1972, **28**, 763.
[589] K. Miyai, R. K. Robins, and R. L. Tolman, *J. Medicin. Chem.*, 1972, **15**, 1092.
[590] H. T. Nagasawa, F. N. Shirota, and C. S. Alexander, *J. Medicin. Chem.*, 1972, **15**, 177.
[591] J. Farkaš, Z. Flegelová, and F. Šorm, *Tetrahedron Letters*, 1972, 2279.



(376)

7-(6-Deoxy-β-L-*lyxo*-hexopyranosulosyl)theophylline (376) inhibited cellular growth, whereas the corresponding 2′-hydroxy-analogue was inactive.[592]

Methods for partial detritylation of 3′,5′- and 2′,5′-di-*O*-trityluridine have been investigated, and 2′- and 3′-*O*-trityluridine have been prepared.[593] 4-Aminophenyl ethers of pyrimidine nucleosides have been formed as derivatives that may be linked to proteins following diazotization.[594]

Nucleosides of 4(5)-nitro- and 4(5)-amino-imidazole have been prepared by condensation of acylglycosyl halides with mercury(II) acetate complexes of 4(5)-nitroimidazole in non-polar solvents.[595] 1-β-D-Ribofuranosyl-pyrimidine derivatives have been prepared from 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride and the mercuri-salt of 2-hydroxypyrimidine;[596] the L-isomer was prepared in similar fashion. An isomeric pyranosyl nucleoside was also obtained from the sodium salt of pyrimid-2-one and 2-*O*-toluene-*p*-sulphonyl-D-arabinose.

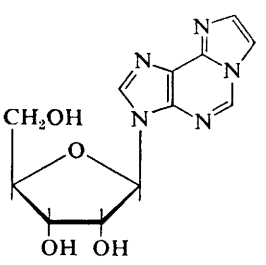
A new procedure for the synthesis of pyrimidine nucleosides involved heating the peracetylated sugar, the trimethylsilylated base, and a Lewis acid (such as boron trifluoride) in an aprotic solvent (such as HMPT).[597]

Isomers of 6-(D-ribosyl)aminopurine were formed in yields up to 74% when a dry mixture of adenine and D-ribose was heated; similar derivatives were also formed from the primary amino-group of adenosine and other bases. When Mg^{2+} and inorganic polyphosphates were present, the natural nucleosides were also obtained.[598]

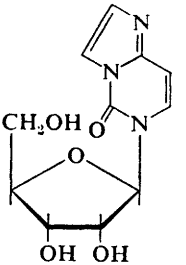
Compounds (377) and (378) are fluorescent adenosine and cytidine derivatives, respectively, formed by treating the nucleoside with chloroacetaldehyde under mildly acidic conditions.[599]

Bis(2-thiouracil)mercury on treatment with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride gave a di- and two mono-N-substituted products; the latter two compounds were converted into 2-thio- and 2-thioiso-uridines.[600]

[592] K. Antonakis, *Carbohydrate Res.*, 1972, **24**, 229.
[593] G. Kowollik, K. Gaertner, and P. Langen, *Tetrahedron Letters*, 1972, 3345.
[594] S. David and J.-C. Fischer, *Bull. Soc. chim. France*, 1972, 3610.
[595] H. Gugliemi and H. Vergin, *Annalen*, 1972, **761**, 67.
[596] H. Pischel, A. Holý, and G Wagner, *Coll. Czech. Chem. Comm.*, 1972, **37**, 3475.
[597] T. Ogawa, M. Yasui, and M. Matsui, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 913.
[598] W. D. Fuller, R. A. Sanchez, and L. E. Orgel, *J. Mol. Biol.*, 1972, **67**, 25.
[599] J. R. Barrio, J. A. Secrist, and N. J. Leonard, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 597.
[600] B. C. Pal, *J. Org. Chem.*, 1971, **36**, 3026.



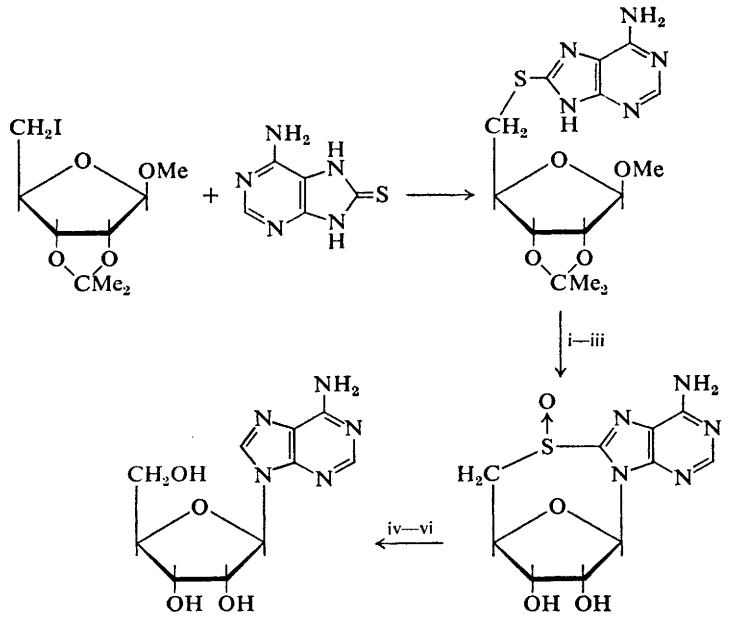
(377)



(378)

A new approach to the synthesis of purine β-D-nucleosides (Scheme 65) represented an interesting development in this area.[601]

Further evidence has accrued of the usefulness of the silyl ether method of nucleoside synthesis;[602] guanine, thioguanine, and other related nucleosides, particularly α-2′-deoxythioguanosine (a compound with antitumour activity), were prepared.



Reagents: i, Ac_2O–AcOH–H_2SO_4; ii, MeO^-; iii, NBS; iv, Bz_2O; v, MeOH–NH_3; vi, Ni–H_2

Scheme 65

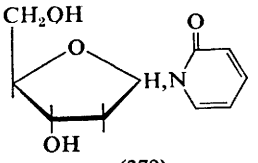
[601] Y. Mizuno, C. Kaneo, kY. Oikawa, T. Ikeda, and T. Itoh, *J. Amer. Chem. Soc.*, 1972, **94**, 4737.

[602] W. W. Lee, A. P. Martinez, L. Goodman, and D. W. Henry, *J. Org. Chem.*, 1972, **37**, 2923.

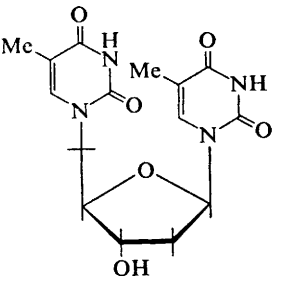
The anomeric nucleosides (379) and their 5′-phosphates have been prepared and characterized.[603]

Condensation of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide with either adenine or 8-chloro- or 8-iodo-adenine gave 3- and 9-substituted products in yields of 25 and 18%; 22 and 12%; and 30 and 5%, respectively.[604] The results were interpreted in terms of steric shielding at position 9 by the increasing size of the group on the adjacent position.

Further studies have been reported on such 'double-headed' nucleosides as (380) (Vol. 4, pp. 137 and 166), but the authors prefer to describe the compounds as 'abbreviated dinucleosides'.[605]



(379)



(380)

Esters and other Derivatives

The synthesis and biological activity of several 6-substituted 9-β-D-ribofuranosylpurine 3′,5′-cyclic phosphates have been described[606] and 9-β-D-arabinofuranosyladenine 3′,5′-cyclic phosphate has been prepared from adenosine 3′,5′-cyclic phosphate.[606a] Nucleoside 5′-phosphates afforded nucleoside 3′,5′-cyclic phosphates when heated with triphenylphosphine and 2,2′-dipyridyl disulphide.[607] 3-β-D-Ribofuranosyluracil 2′,3′-cyclic phosphate and corresponding 6-methyl- and 5-bromo-6-methyl-derivatives have been prepared.[608]

All four D-ribonucleoside 3′,5′-cyclic phosphates can be separated by t.l.c. on alumina.[609] 4-Nitrophenyl phosphate has been recommended as a phosphorylating agent for nucleosides,[610] and trichloromethylphosphonic acid esters have been used to phosphorylate nucleosides containing *cis*-diol groups.[611]

[603] U. Séquin and C. Tamm, *Helv. Chim. Acta*, 1972, **55**, 1196.
[604] C. L. Schmidt and L. B. Townsend, *J. Org. Chem.*, 1972, **37**, 2300.
[605] M. W. Logue and N. J. Leonard, *J. Amer. Chem. Soc.*, 1972, **94**, 2842.
[606] R. B. Meyer, D. A. Shuman, R. K. Robins, R. J. Bauer, M. K. Dimmitt, and L. N. Simon, *Biochemistry*, 1972, **11**, 2704.
[606a] T. A. Khwaja, R. Harris, and R. K. Robins, *Tetrahedron Letters*, 1972, 4681.
[607] T. Mukaiyama and M. Hashimoto, *J. Amer. Chem. Soc.*, 1972, **94**, 8528.
[608] R. W. Bald and A. Holý, *Coll. Czech. Chem. Comm.*, 1971, **36**, 3657.
[609] K. Potter and H. Yamazaki, *J. Chromatog.*, 1972, **68**, 296.
[610] T. Hata and K.-J. Chong, *Bull. Chem. Soc. Japan*, 1972, **45**, 654.
[611] A. Holý, *Tetrahedron Letters*, 1972, 157.

2′- and 3′-*O*-Palmitoyl, -stearoyl, and -benzoyl esters of azacytidine have been tested for immunosuppressive and anti-leukaemic activity in mice.[612] Treatment of thymidine with benzoic acid (or 4-substituted benzoic acids), diethyl azodicarboxylate, and triphenylphosphine in HMPT afforded 5′-*O*-aroyl esters. Similar reactions in dioxan (and also with acetic acid) gave 5′-aroyl (or 5′-acetyl) derivatives and traces of 3′,5′-disubstituted products.[613]

The mass and n.m.r. spectra of some phosgenated nucleosides have been described.[614]

Diazotizable ribo- and deoxyribo-nucleotide derivatives have been prepared by condensing 4-nitrophenyl-2-ethylphosphoric acid with suitably protected derivatives of adenosine, guanosine, cytosine, and uridine. Following removal of the protecting groups, reduction, and diazotization, the nucleoside derivatives were coupled with bovine serum albumin.[615]

The 3′- and 5′-phosphates have been prepared from 2′-azido-2′-deoxyuridine as suitable intermediates for the preparation of 2′-amino-2′-deoxyuridine oligonucleotides. Reduction of the azides gave the corresponding amino-nucleotides in crystalline form.[616]

The 5′-amido-analogue of adenosine 3′,5′-cyclic phosphate has been prepared from 5′-toluene-*p*-sulphonyladenosine by way of the 5′-amino-5′-deoxy-compound and subsequent *N*-(dinitrophenyl)phosphorylation.[617] Uridine 5′-(L-arabinofuranosyl pyrophosphate) and uridine 5′-(L-arabinopyranosyl pyrophosphate) have been synthesized and compared with the UDP-L-arabinose formed from UDP-α-D-xylopyranose and UDP-α-D-glucuronic acid by plant (*Phaseolus aureus*) particulate fractions. It was concluded that the product of the enzymic reactions has a pyranose ring-form.[618]

Various adenosine 2′(3′)-*O*-peptidyl derivatives have been synthesized by condensation of appropriate 2′(3′)-aminoacyladenosines with 5-chloro-8-hydroxyquinoline esters of amino-acids.[619] These compounds were required as substrates for a study of the transpeptidation reactions on ribosomes.

Nucleoside 5′-carboxy-derivatives, when treated with amino-acid benzyl esters in the presence of DCC followed by removal of the protecting groups, afforded nucleoside amino-acid derivatives.[620] Glycine, alanine, phenylalanine, glutamic acid, and lysine were among the amino-acids investigated.

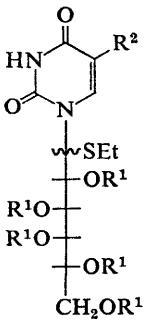
[612] D. T. Warner, G. L. Neil, A. J. Taylor, and W. J. Wechter, *J. Medicin. Chem.*, 1972, **15**, 790.
[613] O. Mitsunobu, J. Kimura, and Y. Fujisawa, *Bull. Chem. Soc. Japan*, 1972, **45**, 245.
[614] H. Seliger, *Tetrahedron Letters*, 1972, 4043.
[615] S. David and A. Lubineau, *Bull. Soc. chim. France*, 1972, 1569.
[616] D. Wagner, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, 1972, **37**, 1876.
[617] A. Murayama, B. Jastorff, F. Cramer, and H. Hettler, *J. Org. Chem.*, 1971, **36**, 3029.
[618] G. O. Aspinall, I. W. Cottrell, and N. K. Matheson, *Canad. J. Biochem.*, 1972, **50**, 574.
[619] S. Chládek, *J. Org. Chem.*, 1972, **37**, 2863.
[620] M. Kawana, R. J. Rousseau, and R. K. Robins, *J. Org. Chem.*, 1972, **37**, 288.

Phosphoramidates resulted when nucleoside azides were treated with triphenyl phosphite.[621]

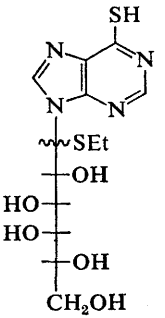
The three monobenzyl ethers of adenosine have been described[622] and a facile, one-stage synthesis of 2′- and 3′-*O*-benzyl ethers of adenosine, inosine, guanosine, cytidine, and uridine has been developed.[164]

Nucleosides containing Acyclic Sugars

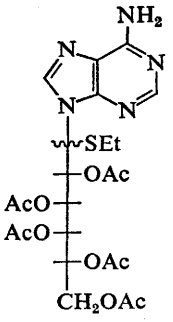
The acyclic nucleoside analogue (381) derived from uracil and D-galactose, and the corresponding derivatives from D-xylose, D-arabinose, and D-glucose, have been prepared[623] and so have the 6-mercaptopurine derivative (382) and its corresponding D-glucose isomer.[624] Whereas under conditions of acetolysis (381) afforded (383), similar treatment of (384) resulted in scission of the 1-substituent to give (385).[625]



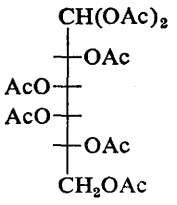
(381) R^1 = H, R^2 = H or Me
(383) R^1 = Ac, R^1 = H or Me



(382)



(384)



(385)

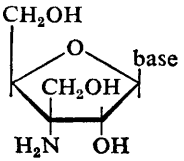
[621] W. Freist, K. Schattka, F. Cramer, and B. Jastorff, *Chem. Ber.*, 1972, **105**, 991.
[622] A. Myles and W. Pfleiderer, *Chem. Ber.*, 1972, **105**, 3327.
[623] D. Horton and S. S. Kokrady, *Carbohydrate Res.*, 1972, **24**, 333.
[624] M. L. Wolfrom, P. McWain, H. B. Bhat, and D. Horton, *Carbohydrate Res.*, 1972, **23**, 296.
[625] M. L. Wolfrom, H. B. Bhat, P. McWain, and D. Horton, *Carbohydrate Res.*, 1972, **23**, 289.

Nucleosides containing Branched-chain Sugar Components

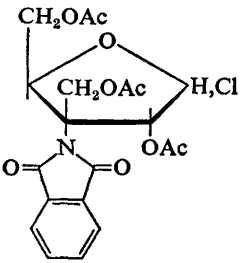
Novel nucleosides derived from 5′-*C*-methyladenosine have been described.[626] Purine nucleosides have been prepared by a fusion procedure from the peracetates of L-mycarose and L-cladinose using dichloroacetic acid as a catalyst.[627]

A range of nucleosides of general type (386) has been prepared from the key intermediate (387), which was obtained from (388).[628]

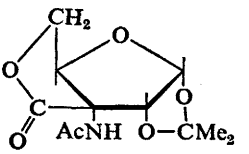
Branched-chain nucleoside derivatives such as (389) and (390) have been prepared from the corresponding glycosyl chloride.[629]



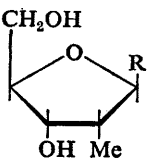
(386)



(387)



(388)



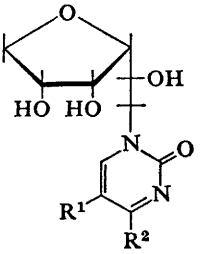
(389) R = adenyl
(390) R = thymyl

Condensation of nitroethane with periodate-oxidized uridine gave products from which a number of stereoisomeric 3-amino-3-deoxy-3-*C*-methylpyranosyl nucleosides were subsequently obtained.[630] The main product was the D-*gluco*-isomer (40%) and the D-*galacto*-, D-*manno*-, and D-*allo*-compounds were also obtained. Configurational assignments were made on the basis of n.m.r. spectroscopy and chemical evidence.

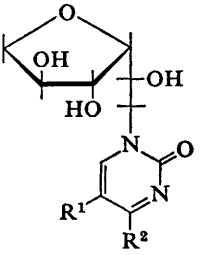
C-Nucleosides

3,6-Anhydro-1-deoxy-1-(pyrimidin-1-yl)-D-mannitols[631] (*e.g.* 391) and 1,4-anhydro-6-deoxy-6-(pyrimidin-1-yl)-D-glucitols[631a] (*e.g.* 392) have been

[626] H. Follmann and T. Walker, *European Biophys. Congress Proc.*, 1971, **1**, 285.
[627] S. Koto, S. Zen, S. Omura, and T. Hata, *Bull. Chem. Soc. Japan*, 1972, **45**, 532.
[628] H. Yanagisawa, M. Kinoshuta, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1971, **44**, 3399.
[629] J. J. K. Novák, J. Šmejkal, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1971, **36**, 3670.
[630] F. W. Lichtenthaler and H. Zinke, *J. Org. Chem.*, 1972, **37**, 1612.
[631] G. Giovanninetti, L. Nobile, M. Amorosa, and J. Defaye, *Carbohydrate Res.*, 1972, **21**, 320.
[631a] L. Nobile, G. Giovanninetti, T. P. Balbi, M. Amorosa, and J. Defaye, *Carbohydrate Res.*, 1972, **24**, 489.

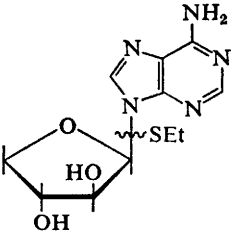


(391)

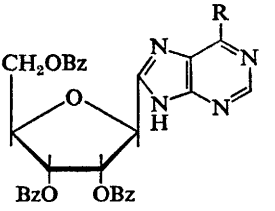


(392)

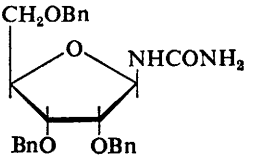
$R^1 = H, R^2 = OH$
$R^1 = Me, R^2 = OH$
$R^1 = H, R^2 = NH_2$



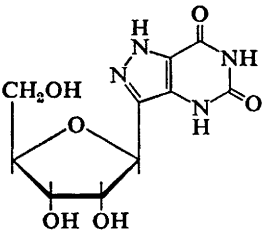
(393)



(394) R = CN, $CONH_2$, CO_2Me, or CO_2Et



(395)



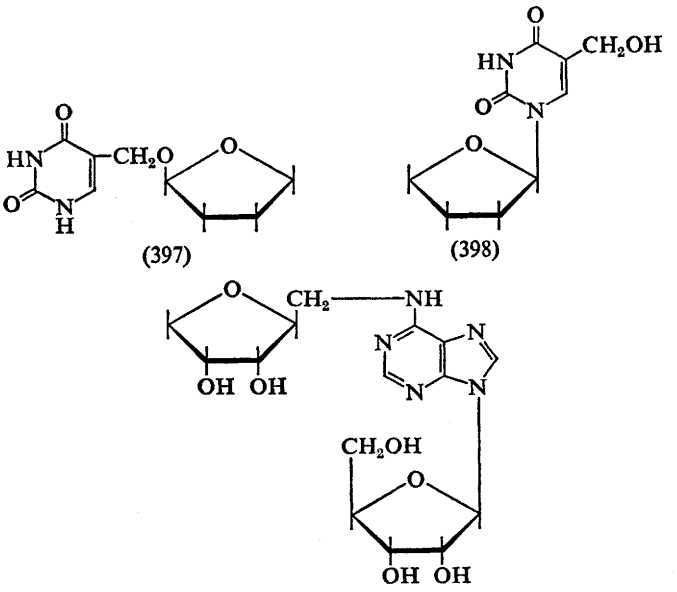
(396)

prepared as homologues of nucleosides. Other *C*-nucleosides to be reported were (393)[632] and (394).[633]

As part of a study directed towards a synthesis of formycin, compound (395) has been prepared from D-glucose in nineteen steps. Since (395) had been converted into oxaformycin (396) in six steps in previous work, this formally constitutes a total synthesis of oxaformycin.[634]

Nucleoside analogues of 5-hydroxymethyluracil [*e.g.* (397) and (398)] have been prepared by treating 2,3-dihydrofuran and 2-chlorotetrahydrofuran,

[632] J. Defaye and Z. Machon, *Carbohydrate Res.*, 1972, **24**, 235.
[633] J. Igolen, T. Huynh Dinh, A. Kolb, and C. Perreur, *Chim. Ther.*, 1972, **7**, 207.
[634] T. Ogawa, Y. Kikuchi, M. Matsui, H. Ohrui, H. Kuzuhara, and S. Emoto, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1825.



(397) (398)

(399)

respectively, with 5-hydroxymethyluracil; related tetrahydropyranyl derivatives were also prepared.[635]

Compound (399) was tested for cytokinin activity and showed appreciable activity at a concentration of 10^{-5} mol l^{-1} in a typical bioassay of tobacco.[253]

A novel synthesis of acyclic C-nucleosides involved fusion of 2-trimethylsilylpyridine with 2,4:3,5-di-*O*-benzylidene-*aldehydo*-D-ribose to afford an equimolar mixture of D-*altro*- and D-*allo*-derivatives.[636] Similar reactions with 6-*aldehydo*-sugars gave 'reverse' *C*-nucleosides.[637] The synthesis of 'reversed' 1,3,4-oxadiazoyl *C*-nucleosides[341] has been referred to in Chapter 10.

An interesting series of C-linked nucleosides has been produced by treating sugar bromophenylhydrazines with acetylenes (see Scheme 12).[147]

2,5-Anhydro-3,4,6-tri-*O*-benzyl-L-talose dimethyl diacetal and related compounds have been prepared as intermediates for use in syntheses of *C*-nucleosides.[183, 183a]

Anhydronucleosides

Adenine 8,2′-cyclonucleosides have been prepared using diphenyl carbonate.[638] For example, 8-mercaptoadenosine on heating at 150 °C

[635] R. Brossmer and V. Eschenfelder, *Annalen*, 1972, **762**, 160.
[636] T. Ogawa, M. Yasui, and M. Matsui, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1443.
[637] T. Ogawa, M. Yasui, and M. Matsui, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1445.
[638] M. Ikehara and S. Tezuka, *Tetrahedron Letters*, 1972, 1169.

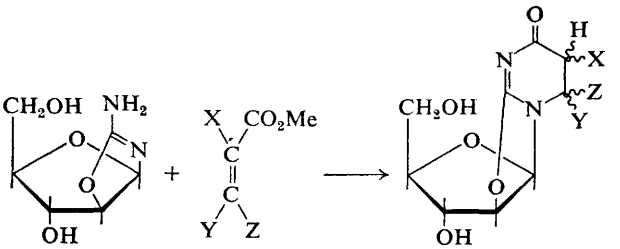
for 30 min in DMF containing diphenyl carbonate and sodium bicarbonate yielded the cyclonucleoside, which was precipitated from solution with methanol.

8-Bromo-2′-toluene-*p*-sulphonyladenosine 5′-monophosphate was cyclized by treatment with either hydrogen sulphide in pyridine or sodium hydrogen sulphide in aqueous DMF to afford 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine.[639] The reactions of 8,2′-anhydro-8-oxy-9-β-D-arabinofuranosyladenine with various nucleophiles have been described.[640]

The photodimerization of 3′,5′-di-*O*-acetylanhydrouridine to give (400) has been reported.[641]

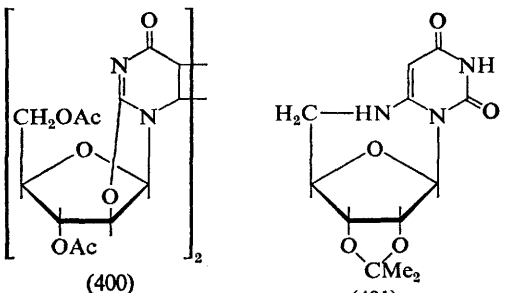
When 2-amino-β-D-arabinofurano[1′,2′:4,5]-2-oxazoline was allowed to react with αβ-unsaturated esters, the previously unknown O^2,2′-anhydro-5,6-dihydrouridines were produced in moderate yield [642] (Scheme 66).

The N-bridged nucleoside derivative (401) has been prepared and characterized.[643]



X = Y = Z = H
X = Me, Y = Z = H
X = H, Y = CO_2Me, Z = H

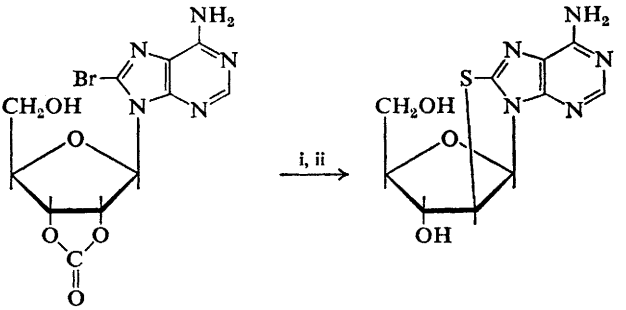
Scheme 66



(400) (401)

[639] M. Ikehara and S. Uesugi, *Tetrahedron*, 1972, **28**, 3687.
[640] M. Ikehara and Y. Ogiso, *Tetrahedron*, 1972, **28**, 3695.
[641] K. K. Ogilvie and J. W. Berestiansky, *Tetrahedron Letters*, 1972, 4667.
[642] C. M. Hall, G. Slomp, S. A. Mizsak, and A. J. Taylor, *J. Org. Chem.*, 1972, **37**, 3290.
[643] K. Isono and T. Azuma, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 193.

8,2′-Thioanhydroguanosine has been prepared and converted into 2′-deoxyguanosine,[644] and a series of 8,2′-thioanhydropurine nucleosides has been prepared as illustrated in Scheme 67.[645]

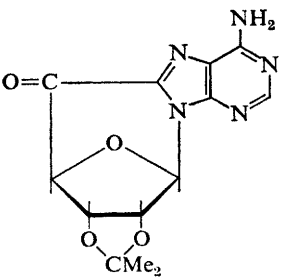


Reagents: i, NaSH–DMF; ii, H_2NCSNH_2

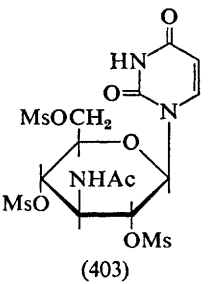
Scheme 67

The unusual cyclonucleoside (402) having a carbon–carbon bond between the sugar and the base has been reported.[646] Reactions of 2,2′-anhydro-orotidine ethyl ester have been investigated[647] and 2,2′-anhydro-1-β-D-arabinofuranosylcytosine has been prepared by treatment of β-D-ribofuranosylcytosine (cytidine) with Vilsmeier–Haack reagents; prolonged reaction with the reagent also caused chlorination at C-5′ and C-2′ (following ring-opening).[648]

An extensive set of reactions of mesylated 1-(3-acetamido-3-deoxy-β-D-glucopyranosyl)uracils has been reported; for example, (403) was converted into (404) and (405) on treatment with ethoxide ion.[649]

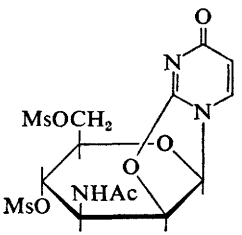


(402)

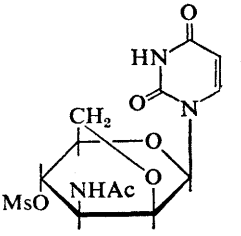


(403)

[644] K. K. Ogilvie, L. Slotin, J. B. Westmore, and D. Lin, *Canad. J. Chem.*, 1972, **50**, 1100.
[645] K. K. Ogilvie, L. Slotin, J. B. Westmore, and D. Lin, *Canad. J. Chem.*, 1972, **50**, 2249.
[646] P. J. Harper and A. Hampton, *J. Org. Chem.*, 1972, **37**, 795.
[647] C. V. Z. Smith, R. K. Robins, and R. L. Tolman, *J. Org. Chem.*, 1972, **37**, 1418.
[648] K. Kikugawa and M. Ichino, *J. Org. Chem.*, 1972, **37**, 284.
[649] K. A. Watanabe, M. P. Kotick, M. Kunori, R. J. Cushley, and J. J. Fox, *J. Org. Chem.*, 1971, **36**, 4105.



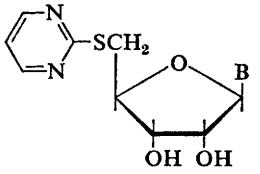
(404)



(405)

Reactions

Unprotected nucleosides can be converted into 5′-deoxy-derivatives by treatment with 2-mercaptopyrimidine and DMF dineopentyl diacetal [to afford (406)] followed by Raney nickel desulphurization.[650]



(406)

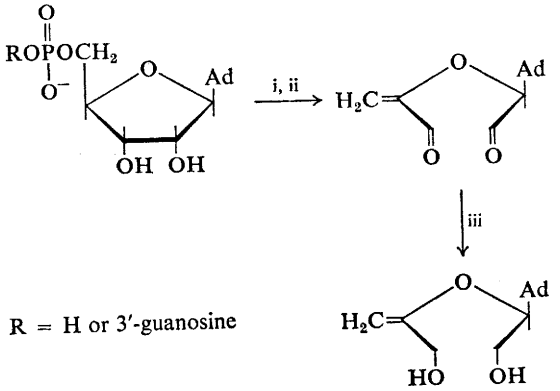
2′,3′-*O*-Isopropylideneadenosine has been oxidized to the corresponding 5′-aldehyde using a mixture of DMSO, pyridine, trifluoroacetic acid, and DCC (the Pfitzner–Moffatt reagent). Treatment of the 5′-aldehyde with methylmagnesium iodide and removal of the protecting groups afforded a mixture of 9-(6-deoxy-β-D-allofuranosyl)- and 9-(6-deoxy-α-L-talofuranosyl)-adenines.[651] 5′-Aldehydo-derivatives of adenosine (309) and uridine have also been prepared by photolysis of suitably protected 5′-azido-derivatives, *e.g.* (308).[473]

1-β-D-Arabinofuranosylcytosine has been methylated with dimethyl sulphate and potassium hydroxide to give all the possible methylated derivatives, which were isolated by chromatography.[652] Methods for O′-alkylation of cytosine and uracil, which avoided N-3 alkylation, have been developed. The reactions were performed at pH > 13 with dialkyl sulphates.[653]

Treatment of cytidine with diphenyl carbonate and sodium bicarbonate in hot DMF resulted in inversion of configuration at C-2′ and the formation of 1-β-D-arabinofuranosylcytosine.[654]

Models for the sequence analysis of polynucleotides have used the reactions illustrated in Scheme 68. The advantage of this β-elimination

[650] A. Holý, *Tetrahedron Letters*, 1972, 585.
[651] P. Howgate and A. Hampton, *Carbohydrate Res.*, 1972, **21**, 309.
[652] E. Darzynkiewicz, J. T. Kuśmierek, and D. Shugar, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1734.
[653] J. T. Kuśmierek and D. Shugar, *Acta Biochim. Polon.*, 1971, **18**, 413.
[654] K. K. Ogilvie, *Carbohydrate Res.*, 1972, **24**, 210.



Reagents: i, $NaIO_4$, pH 8.4; ii, heat; iii, $NaBH_4$

Scheme 68

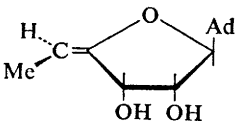
reaction over previously developed methods is that it provided a means (by reduction with sodium borotritide) of selectively labelling the nucleoside after it has been released from the chain.[655]

The O→N-shift of theobromine-*O*-glucoside in the presence of mercuric bromide occurred only if all traces of acid and water were removed. Otherwise glycosyl cleavage occurred.[656]

In the first of a series of papers on the interconversion of hexofuranosyl nucleosides, a 9-α-D-mannofuranosyladenine nucleoside was converted into the corresponding β-L-*gulo*-derivative by means of a sulphonyloxy displacement at C-5. Various 5′,6′-unsaturated nucleosides were also formed.[657] However, attempts to convert 9-β-D-glucofuranosyladenine derivatives into the corresponding α-L-idosyl derivatives by a similar procedure were unsuccessful.[658] 9-α-L-Idofuranosyladenine was prepared ultimately by treatment of suitable adenine derivatives with 1,2,3,6-tetra-*O*-acetyl-5-*O*-benzoyl-L-idofuranose. Instead of nucleophilic displacement occurring when (2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-α-L-rhamnofuranosyl)adenine was treated with sodium benzoate in DMF, elimination to give both the 4′,5′- and 5′,6′-olefins [(235) and (234), respectively] was favoured.[437] The former olefin could not be deacetonated to give (407), which was required as an analogue of the unsaturated nucleoside antibiotic decoyinine.

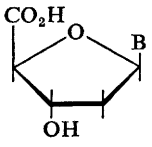
Treatment of such nucleosides as (408) with DMF dineopentyl diacetal in DMF at 80—90 °C yielded decarboxylated, unsaturated nucleosides such as (409) in both the purine and pyrimidine series.[659]

[655] D. E. Schwartz and P. T. Gilham, *J. Amer. Chem. Soc.*, 1972, **94**, 8921.
[656] J. A. Elvidge, G. T. Rogers, and T. L. V. Ulbricht, *J. Heterocyclic Chem.*, 1971, **8**, 1039.
[657] L. M. Lerner, *J. Org. Chem.*, 1972, **37**, 470.
[658] L. M. Lerner, *J. Org. Chem.*, 1972, **37**, 473.
[659] J. Žemlička, R. Gasser, J. V. Freisler, and J. P. Horwitz, *J. Amer. Chem. Soc.*, 1972, **94**, 3213.

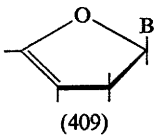


(407)

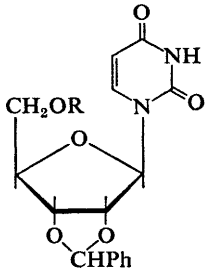
2′-Deoxyuridine was synthesized by treatment of (410) with NBS followed by hydrogenation and debenzoylation.[660] Similar treatment of (411) gave a cyclonucleoside, whereas (412) gave the expected deoxy-product. The reactions were rationalized in terms of 2,2′-anhydronucleoside intermediates.[660a]



(408)



(409)



(410) R = H
(411) R = Ac
(412) R = Me

A full report has been presented on the introduction of chloro- or bromo-groups into the sugar moiety of nucleosides by the use of carbon tetrahalides and triphenylphosphine in DMF or dimethylacetamide.[661] In particular, the replacement of primary hydroxy-groups was straightforward. Unexpected points to emerge from this study were that replacement of a secondary hydroxy- by a chloro-group occurred mainly with inversion of configuration, whereas retention was observed with bromination. With pyrimidine nucleosides having a *cis*-2′,3′-diol system, selective chlorination occurred at C-2′ with retention of configuration.

Physical Measurements and Conformational Studies

A new convention has been suggested to replace previous descriptions of furanose-ring conformations in nucleosides, which are based on calculated three- and four-atom planes and the torsion angles about the furanose-ring bonds. The new description is based on the concept of pseudorotation and allows unequivocal assignment of the exact conformation of the furanoid ring in terms of the phase angle of pseudorotation (P) and the degree of pucker (τ_m).[662]

[660] M. M. Ponpipom and S. Hanessian, *Canad. J. Chem.*, 1972, **50**, 246.
[660a] M. M. Ponpipom and S. Hanessian, *Canad. J. Chem.*, 1972, **50**, 253.
[661] J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, 1972, **37**, 2289.
[662] C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1972, **94**, 8205.

Bond lengths, bond angles, and torsion angles of nucleosides and nucleotides based on *X*-ray data have been reviewed.[663] Molecular orbital calculations have predicted that the nucleosides of adenine, cytosine, purine, and xanthine should, in general, have a higher dipole moment in the *anti* than in the *syn* form, whereas the reverse should be true for the nucleosides of guanine and hypoxanthine.[664]

Factors determining the *syn* or *anti* conformations of nucleosides have been reviewed [665] and c.d. measurements have been used in conformational studies of dinucleoside 5′,5′-pyrophosphates of various purines [666] and of other nucleosides and dinucleoside monophosphates.[667] From c.d. studies of various purine nucleosides and their 5′-monophosphates, it was observed [668] that (i) purine nucleosides having bulky 8-substituents have *syn* conformations, (ii) introduction of a 5′-phosphate group into a *syn* nucleoside causes drastic changes in the c.d. curves, (iii) introduction of a 5′-phosphate group into an *anti* nucleoside does not change the c.d. in the B-band region, and (iv) introduction of a 5′-phosphate group produces changes in the c.d. band at 200—220 nm for both *syn* and *anti* nucleosides.

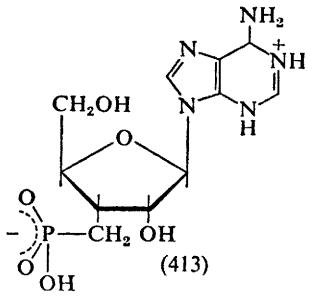
Various furanosyl nucleoside 5′-carboxylates have been examined, particularly with regard to the rotamer states about the C—N bond, by n.m.r., o.r.d., and i.r. spectroscopy.[669] The influence of the 2′-hydroxy-group on the conformations of nucleic acids has been investigated by studying the conformations of six adenine dinucleoside monophosphates in solution by means of n.m.r., c.d., and u.v. hypochromicity. It appeared that the influence of the 2′-hydroxy-group on the conformation of D-ribose-containing dimers is exerted through steric, and not through hydrogen-bonding, effects.[670]

An n.m.r. study of the influences of aqueous sodium perchlorate and temperature on the conformations of uracil nucleosides and nucleotides has been reported.[671] The following conformational features were reported: (i) a sugar-base torsion angle corresponding to an *anti* conformation, (ii) a D-ribose ring which is a rapid, time-averaged blend of conformers, and (iii) an exocyclic hydroxymethyl group that favours the *gauche–gauche* rotamer.

The rotational isomerism of certain 3-β-D-glucopyranosyl-6-methyl-uracils, which has led previously to misinterpretation of n.m.r. data, has been discussed.[672]

[663] S. Arnott and D. W. L. Hukins, *Biochem. J.*, 1972, **130**, 453.
[664] H. Berthod and B. Pullman, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 125.
[665] W. Saenger, *European Biophys. Congress Proc.*, 1971, **1**, 289.
[666] M. Ikehara, S. Uesugi, and K. Yoshida, *Biochemistry*, 1972, **11**, 837.
[667] A. Rabczenko and D. Shugar, *Acta Biochim. Polon.*, 1971, **18**, 387.
[668] M. Ikehara, S. Uesugi, and K. Yoshida, *Biochemistry*, 1972, **11**, 830.
[669] H.-J. Fritz, R. Machat, and R. S. Schmidt, *Chem. Ber.*, 1972, **105**, 642.
[670] N. S. Kondo, K. N. Fang, P. S. Miller, and P. O. P. Ts'o, *Biochemistry*, 1972, **11**, 1991.
[671] T. Schleich, B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *Biochemistry*, 1972, **11**, 137.
[672] K. A. Watanabe, S. S. Saluja, B. A. Otter, and J. J. Fox, *Tetrahedron Letters*, 1972, 3911.

The crystal structures of 6,7-dimethyl-lumazine-*N*-1-β-D-ribofuranoside,[673] (353),[527] (354),[528] and the adenosine 3′-phosphate analogue (413)[674] have been determined. Crystal structures of other nucleosides are referred to in Chapter 24.



(413)

An extensive paper has been published on the u.v. and c.d. spectral characteristics of the eight anomeric pentofuranosyl adenines.[675] Almost enantiomeric c.d. curves were obtained for those isomers with enantiomeric relationships at C-1′, C-2′, and C-3′ (*e.g.* α-*xylo* and β-*arabino*).

A c.d. study of the effects of magnesium perchlorate and temperature on the conformations of uridine 5′-phosphate, uridine 3′-phosphate, uridine, and uridyl-(3′→5′)-uridine in solution has been reported.[676]

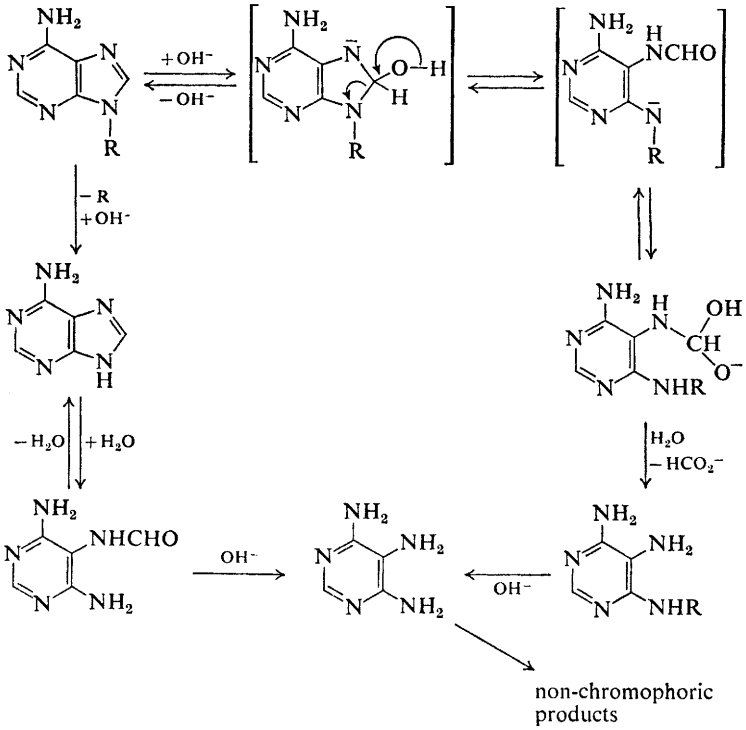
The complexing sites of various transition-metal ions with adenosine 5′-phosphate have been determined by n.m.r. methods. All the ions complexed with the phosphate group and the heterocyclic system, but the location of the binding sites on the adenine ring varied.[506] N.m.r. evidence has been presented for a 1 : 1 guanosine–Ca^{2+} ion complex, which is believed to be the first alkaline earth–guanine base complex; Mg^{2+} ions and other nucleosides did not complex.[677] The complexes formed by adenosine and Cu^{2+} ions have been studied at various pH values. In acid solution, a complex was formed between the copper ions and the base, but the D-ribose hydroxy-groups were shown to be involved at high pH values.[678]

The effects of sugars and substitution on the base on the acid solvolysis of adenine nucleosides have been examined and thermodynamic parameters for the various compounds have been determined. It was presumed that protonation of the imidazole, rather than the sugar, moiety is the first step in the solvolysis.[679] The pH–rate profiles, substituent effects, and other parameters obtained from a kinetic investigation of the acid hydrolyses (in the

[673] W. Saenger, G. Ritzmann, and W. Pfleiderer, *European J. Biochem.*, 1972, **29**, 440.
[674] S. M. Hecht and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1972, **94**, 4314.
[675] J. S. Ingwall, *J. Amer. Chem. Soc.*, 1972, **94**, 5487.
[676] C. Formoso, *Biochemistry*, 1972, **11**, 4031.
[677] F. Jordan and B. Y. McFarquhar, *J. Amer. Chem. Soc.*, 1972, **94**, 6557.
[678] G. Boivin and M. Zador, *Canad. J. Chem.*, 1972, **50**, 3117.
[679] E. R. Garrett and P. J. Mehta, *J. Amer. Chem. Soc.*, 1972, **94**, 8532.

range pH 6 to $H_0 = -6$) of deoxycytidine, deoxyuridine, and their 5-bromo- and 5-methyl derivatives were consistent with a mechanism involving the formation of mono- and di-cations of the nucleoside followed by rupture of the *N*-glycosyl bond.[680]

7-β-D-Ribofuranosylpurines were found, with few exceptions, to hydrolyse faster than the 9-substituted isomers[681] and kinetic studies were consistent with an *A*-1 mechanism.[681, 682] The influence of charge-transfer interactions on the hydrolyses was also explored, but such compounds as hexamethylbenzene had only slight effects on the rates of reaction. An *A*-1 mechanism was also favoured for the hydrolysis for guanosine and 7-methylguanosine by perchloric acid.[683] The alkaline solvolysis of adenine nucleosides was indicated to proceed by the two parallel routes illustrated in Scheme 69. Electron-withdrawing substituents on the adenine moiety



Scheme 69

[680] R. Shapiro and M. Danzig, *Biochemistry*, 1972, **11**, 23.
[681] R. P. Panzica, R. J. Rousseau, R. K. Robins, and L. B. Townsend, *J. Amer. Chem. Soc.*, 1972, **94**, 4708.
[682] L. Hevesi, E. Wolfson-Davidson, J. B. Nagy, O. B. Nagy, and A. Bruylants, *J. Amer. Chem. Soc.*, 1972, **94**, 4715.
[683] J. A. Zoltewicz and D. F. Clark, *J. Org. Chem.*, 1972, **37**, 1193.

increased the rates of solvolyses, whereas electron-donating substituents had the opposite effect.[684]

The mass spectra of trifluoroacetyl derivatives of ribonucleosides and deoxyribonucleosides have been examined.[685] The most useful ions for determining the molecular mass and composition arose from a series of elimination reactions from the molecular ion involving trifluoroacetyl moieties. Breakage of the glycosidic bond, with retention of charge on the base, produced major ions representative of the base and its substituents. The mass spectra of a series of pyrimidine cyclonucleosides possessing 2,2′-, 2,3′-, 2′,6-, and 5′,6-anhydro-rings have been obtained.[686] 2′- and 3′-Linked compounds could be distinguished readily from 5′,6-isomers, but not from each other. Other mass-spectral studies are reported in Chapter 24.

It has been established that 3- and 5-substituted compounds can be readily distinguished by comparison of the ^{1}H and ^{13}C n.m.r. spectra of 1-β-D-ribofuranosyl-1,2,4-triazoles.[687]

[684] E. R. Garrett and P. J. Mehta, *J. Amer. Chem. Soc.*, 1972, **94**, 8542.
[685] W. A. Koenig, L. C. Smith, P. F. Crain, and J. A. McCloskey, *Biochemistry*, 1971, **10**, 3968.
[686] S. Tsuboyama and J. A. McCloskey, *J. Org. Chem.*, 1972, **37**, 166.
[687] G. P. Kreishman, J. T. Witkowski, R. K. Robins, and M. P. Schweizer, *J. Amer. Chem. Soc.*, 1972, **94**, 5894.

22
Oxidation and Reduction

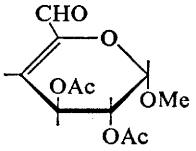
Periodate Oxidation

An automated determination of iodate in periodate-oxidation mixtures has been described.[688] The excess of periodate was masked with molybdate at pH 3 and the iodate was then treated with iodide to form tri-iodide, which was measured spectrophotometrically.

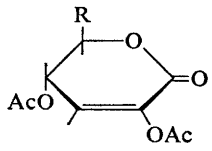
Hypoiodite has been shown to oxidize aldehydes or polyaldehydes formed by periodate oxidation of monosaccharides or polysaccharides to acids or polyacids in much higher yields than bromine in the presence of strontium carbonate.[689]

DMSO-based Oxidations

The oxidation of a number of partially acylated sugar derivatives with DMSO in the presence of sulphur trioxide and triethylamine has been described in detail.[690] Oxidation–elimination reactions leading to the formation of such products as (414)—(416) have been studied with



(414)

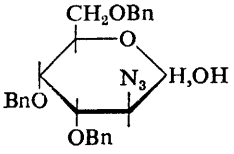


(415) R = CH_2OAc
(416) R = H

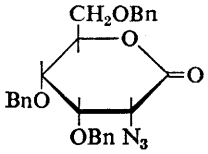
considerable variation in the stereochemistry of the substrates. It was found that the overall rates and extent of the oxidation–elimination sequence are strongly dependent on the concentrations of sulphur trioxide and triethylamine, and on the order of mixing the reagents. Under the reaction conditions described, the rates were largely insensitive to the position of the hydroxy-group and to the initial, relative disposition of the proton and acyloxy-group eliminated. Although a benzoyloxy- (but not benzyloxy-) group was readily eliminated in some instances, in others the initial oxidation step could not be effected.

[688] S. A. Barker, P. V. Peplow, and P. J. Somers, *Carbohydrate Res.*, 1972, **22**, 201.
[689] P. V. Peplow and P. J. Somers, *Carbohydrate Res.*, 1972, **22**, 281.
[690] D. M. Mackie and A. S. Perlin, *Carbohydrate Res.*, 1972, **24**, 67.

Oxidation of (417) with DMSO–acetic anhydride resulted in the formation of the lactone (418), which differs in the configuration at C-2.[691] Methyl α-D-*arabino*-hexopyranosid-2-ulose has been prepared by oxidation of methyl 4,6-*O*-benzylidene-3-*O*-(tetrahydropyran-2-yl)-α-D-glucopyranoside with DMSO–acetic anhydride and removal of protecting groups with trifluoroacetic acid.[692]



(417)



(418)

1,6-Di-*O*-benzyl-2,5-*O*-methylene-D-mannitol, which possesses two equivalent hydroxy-groups, afforded crystalline 1,6-di-*O*-benzyl-2,5-di-*O*-methylene-D-*arabino*-hex-3-ulose in high yield on oxidation with DMSO–acetic anhydride. No epimerization of the α-hydroxy-group occurred.[693] Oxidation–reduction sequences for inverting the configuration at a single carbon atom have been used in syntheses of L-psicose [25] and D-allose [24, 694] (see also Chapter 2).

Oxidation of methanolysates of permethylated polysaccharides with DMSO-based oxidants produced anomeric mixtures of dicarbonyl sugars that were characterized as crystalline 2,4-dinitrophenylhydrazones; these derivatives were diagnostic of the linkages present in the parent polysaccharide [343] (see Chapter 9).

Methyl 4-*O*-ethyl-3-*O*-methyl-β-D-xylopyranoside has been used as a model for studies of the oxidative degradation of polysaccharides.[695]

Electrochemical Procedures

The electrochemical oxidation of sugars has been reviewed.[71]

Replacing the nickel cathode in the electrochemical oxidation of 3,4:5,6-di-*O*-isopropylidene-L-sorbose by a chromium–copper cathode increased the yield of 3,4:5,6-di-*O*-isopropylidene-L-*xylo*-hexulosonic acid and with a platinum–titanium anode an 86% yield of the product was obtained.[696] A similar electrochemical oxidation using a nickel hydroxide electrode has been reported.[697]

Oxidation of *myo*-inositol gave DL-*epi*-2-inosose, which was reduced electrolytically in hydrogen-type cells with nickel, tantalum, copper,

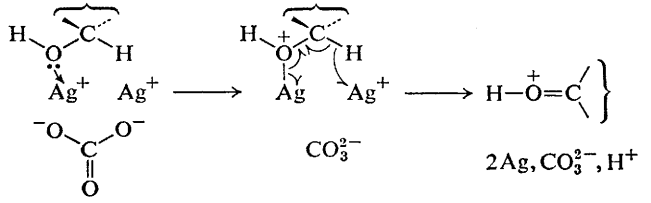
[691] H. Kuzuhara, N. Oguchi, H. Ohrui, and S. Emoto, *Carbohydrate Res.*, 1972, **23**, 217.
[692] A. N. de Belder and E. Wirén, *Carbohydrate Res.*, 1972, **24**, 166.
[693] T. B. Grindley, J. W. Bird, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1972, **24**, 212.
[694] R. Fuertes and M. Cesar, *Bol. Soc. quim. Peru*, 1971, **37**, 161.
[695] L. Kenne and S. Svensson, *Acta Chem. Scand.*, 1972, **26**, 2144.
[696] A. I. Borisov, I. A. Avrutskaya, and M. Ya. Fioshin, *Elektrokhimiya*, 1971, **7**, 1877.
[697] G. Vértes, G. Horányi, and F. Nagy, *Tetrahedron*, 1972, **28**, 37.

molybdenum, lead, tin, tungsten, cadmium, or mercury cathodes to give inositol stereoisomers, among which *myo*- and *epi*-inositols were identified by paper chromatography.[523]

Other Oxidations

Lead tetra-acetate in pyridine was found to be a convenient reagent for the oxidation of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose to 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose.[698] This procedure had practical advantages over DMSO-based oxidation procedures, but its use may be limited.

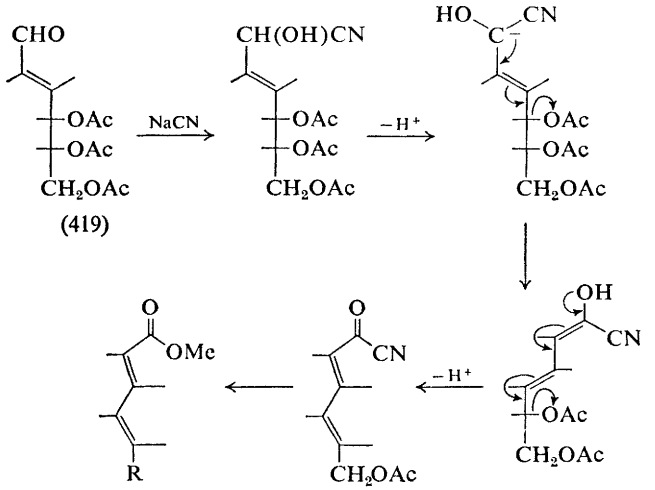
Silver carbonate–Celite in boiling benzene has been used to oxidize 2,3-*O*-isopropylidene-D-ribofuranose, 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose, 2,3:5,6-di-*O*-isopropylidene-D-allofuranose, and 3,4-*O*-isopropylidene-L-arabinopyranose into the corresponding aldonolactones in good to excellent yields.[699] When the latter acetal was treated with the reagent in boiling methanol, descent of the series to give 2,3-*O*-isopropylidene-L-erythrose occurred. Other applications[29, 30] of silver carbonate–Celite for descending the series have been described in Chapter 2. A mechanism (Scheme 70) has been suggested for oxidations employing silver carbonate.[700]



Scheme 70

Sodium metaperiodate–ruthenium dioxide has been used to oxidize hydroxy-lactones to keto-lactones (under neutral conditions) and lactones, by way of γ- or δ-hydroxy-carboxylates, to keto-carboxylates.[701] The scope and limitations of the reagent were discussed. A number of carbohydrate examples were reported in a review dealing with the applications of ruthenium tetroxide in organic chemistry.[702] The 2,3-dideoxy-hex-2-enose triacetate (419) was oxidized by a mixture of sodium cyanide, acetic acid, and manganese dioxide in methanol (the Corey procedure) to give a mixture of (420) and (421). The reaction pathway illustrated (Scheme 71) was suggested for this conversion.[703]

[698] D. J. Ward, W. A. Szarek, and J. K. N. Jones, *Carbohydrate Res.*, 1972, **21**, 305.
[699] S. Morgenlie, *Acta Chem. Scand.*, 1972, **26**, 2518.
[700] M. Fetizon, M. Golfier, and P. Mourgues, *Tetrahedron Letters*, 1972, 4445.
[701] H. Gopal, T. Adams, and R. M. Moriarty, *Tetrahedron*, 1972, **28**, 4259.
[702] J. L. Courtney and K. F. Swansborough, *Rev. Pure Appl. Chem.*, 1972, **22**, 47.
[703] S. Y. K. Tam and B. Fraser-Reid, *Tetrahedron Letters*, 1972, 3151.



Scheme 71

Oxidation of methyl α-D-glucopyranoside, -galactopyranoside, and -mannopyranoside with potassium ferrate in 0.1 mol l^{-1} sodium carbonate at 50 °C afforded the corresponding 6-aldehydo-derivatives, which were isolated following conversion into carboxylic acid derivatives.[704]

The diketone (422) was presumably the intermediate in formation of the mixture of acids (423) resulting from oxidation of methyl β-D-glucopyranoside with oxygen in aqueous alkaline solution.[705]

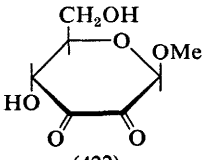
Oxidations of sugars with oxygen in aqueous alkali,[45, 46] with cupric ions,[47] and with vanadium(v) in sulphuric acid [72] have been described in Chapter 2. An oxidative method using vanadium(v) for the analysis of ketoses in the presence of aldoses has also been described.[706]

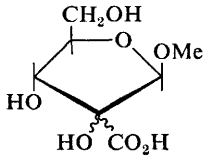
t-Amyl hydroperoxide in the presence of molybdenum chloride has been used for oxidizing secondary hydroxy-groups in the sugar series;[707] for example, 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose was oxidized to the corresponding D-*ribo*-hexofuranos-3-ulose.

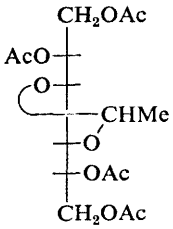
The ring expansion of hexosuloses by Baeyer–Villiger oxidation has been mentioned [479] in Chapter 16.

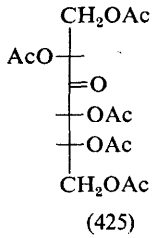
Oxidation with the chromium trioxide–acetic acid reagent has been extended to the synthesis of hex-3-uloses from acetylated hexitol acetals;

[704] J. N. BeMiller, V. G. Kumari, and S. D. Darling, *Tetrahedron Letters*, 1972, 4143.
[705] B. Ericsson, B. O. Lindgren, O. Theander, and G. Petersson, *Carbohydrate Res.*, 1972, **23**, 323.
[706] P. Malangeau, M. Guernet, and Vinh Chon Thanh, *Ann. pharm. franç.*, 1972, **30**, 569.
[707] G. A. Tolstikov, U. M. Gemilev, and V. P. Yurjev, *Zhur. obshchei Khim.*, 1972, **42**, 1611.


(422)


(423)


(424)


(425)

for example, (424) was converted into (425), which was isolated as a crystalline di-*O*-isopropylidene derivative following deacetylation and acetonation.[708] Similarly, L-*lyxo*- and L-*xylo*-hex-3-uloses were obtained by oxidation of the acetates of 3,4-*O*-ethylidene-L-iditol and 2,4-*O*-benzylidene-D-glucitol, respectively. The stereochemistry of spiro-ketals derived from ketoses was also considered. The chromium trioxide–acetic acid reagent has also been used to oxidize pyranosidic 6-deoxy- and 2-acetamido-2-deoxy-sugars having the aglycone in the equatorial orientation to the corresponding hex-5-ulosonates.[709] The anomeric configurations of oligo- and poly-saccharides can be determined by this method, which also holds promise for the selective degradation of polysaccharides.

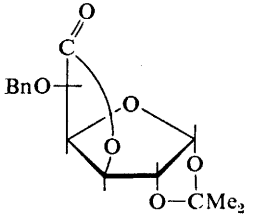
Reduction

Treatment of the lactone (426) with sodium borohydride in DMSO afforded the unsaturated lactone (427) in 82% yield.[710]

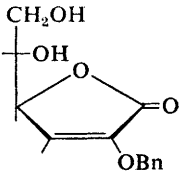
Reduction with aqueous borohydride of the benzyl β-D-glycoside derivative (428) and subsequent acetylation gave the acetates (430) and (429) as the major and minor products, respectively. The α-glycoside (431), however, afforded (432) as the major product and (433) as the minor product.[711]

Sodium borohydride in either DMSO or HMPT has been shown to be a convenient means of converting the 6-sulphonates (434)—(436) into the corresponding 6-deoxy-sugars (437)—(439), respectively.[712] Secondary acyloxy- and sulphonyloxy-groups were unaffected by the reagent.

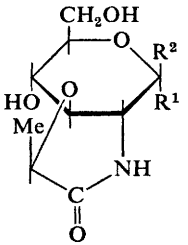
[708] S. J. Angyal and M. E. Evans, *Austral. J. Chem.*, 1972, **25**, 1495.
[709] J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1972, **26**, 661.
[710] H. Weidmann, W. Timpe, and N. Wolf, *Carbohydrate Res.*, 1972, **25**, 67.
[711] P. Sinaÿ and J.-M. Beau, *Carbohydrate Res.*, 1972, **24**, 95.
[712] H. Weidmann, N. Wolf, and W. Timpe, *Carbohydrate Res.*, 1972, **24**, 184.



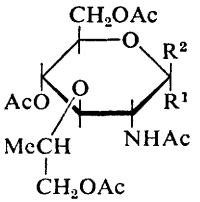
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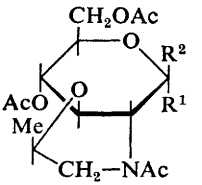
(427)



(428) R^1 = H, R^2 = OBn
(431) R^1 = OBn, R^2 = H

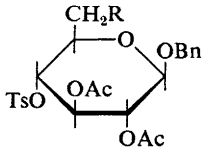


(429) R^1 = H, R^2 = OBn
(432) R^1 = OBn, R^2 = H

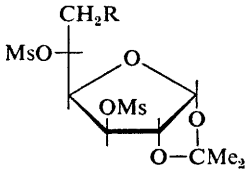


(430) R^1 = H, R^2 = OBn
(433) R^1 = OBn, R^2 = H

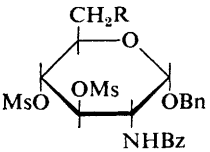
Reduction of the unsaturated sugar derivatives (440) and (442) with lithium aluminium hydride afforded the alcohols (441) and (443), respectively, with loss of the aglycone, whereas similar reduction of (444) and



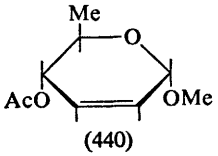
(434) R = OTs
(437) R = H



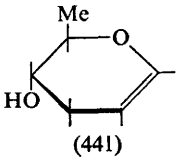
(435) R = OMs
(438) R = H



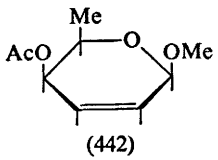
(436) R = OMs
(439) R = H



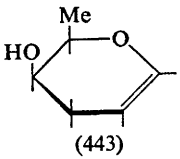
(440)



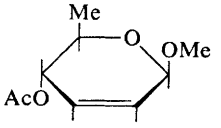
(441)



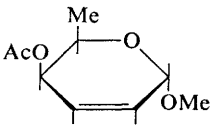
(442)



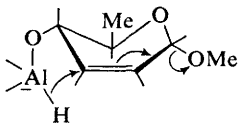
(443)



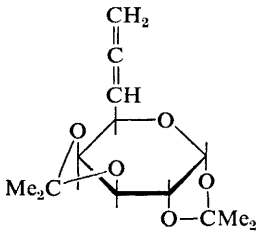
(444)



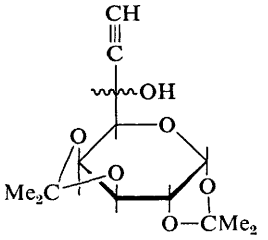
(445)



(446)



(447)



(448)

(445) gave only products of straightforward deacetylation.[713] The results were rationalized by assuming that intramolecular displacement of the glycosidic methoxy-group can occur from such intermediates as (446). The allenic derivative (447) was obtained as a minor product on reduction of (448) with lithium aluminium hydride.[482]

Branched-chain 4-deoxy-4-methyl-sugars have been prepared by desulphurization of the corresponding 4-*C*-thiomethylene derivatives with Raney nickel [453] (see also Chapter 15).

[713] O. Achmatowicz, jun. and B. Szechner, *Tetrahedron Letters*, 1972, 1205.

23

N.M.R. Spectroscopy and Conformational Features of Carbohydrates

A general review of the n.m.r. spectroscopy of carbohydrates for the period 1968—1970 has been published,[714] and special emphasis has been placed on instrumental techniques in another review.[715] Recent developments in the conformational analysis of carbohydrates have been reviewed.[716]

Theoretical studies on the conformations of aldopyranoses have been carried out, and the potential energies of eight aldopentopyranoses in the ^{C1}(D) and ^{1C}(D) conformations have been calculated. Conformational entropies have also been computed for all the aldohexoses and aldopentoses, and these values have been used to obtain the free energies. The calculated free energy values agreed well with the experimental data after adding a value of 0.4 kcal mol^{-1} for the anomeric effect. Calculated values of the percentage of α-anomers present in equilibrium mixtures agreed fairly well with those assigned from n.m.r. studies. The data obtained also indicated that all the aldohexopyranoses, except α-D-altrose and α-D-idose, exist only in ^{C1}(D) conformations, whereas all the aldopentopyranoses, except D-xylose, exist in a ^{C1}(D)$\rightleftarrows^{1C}$(D) equilibrium in solution.[717] EHT and CNDO calculations have been made on the conformations of α-D-glucopyranose; both methods showed the ^{C1}(D) conformation to be the most stable.[718]

The anomeric effect has been reviewed[719] and studied by *ab initio* molecular theory.[720] Theoretical predictions and experimental data on the conformations and bond lengths of pyranoses and methyl pyranosides were compared. Agreement of theoretical results with *X*-ray data was surprisingly good when consideration was given to experimental errors, the limitations of the theoretical model, and the differences expected in the structures of the crystal and the isolated molecule.

In the free-radical additions of bisulphite to methyl 6-deoxy-hex-5-

[714] T. D. Inch, *Ann. Report N.m.r. Spectroscopy*, 1972, **5A**, 305.
[715] B. Coxon, *Adv. Carbohydrate Chem. and Biochem.*, 1972, **27**, 7.
[716] R. U. Lemieux, *Pure Appl. Chem.*, 1971, **27**, 527.
[717] K. S. Vijayalakshmi and V. S. R. Rao, *Carbohydrate Res.*, 1972, **22**, 413.
[718] A. Saran and G. Govil, *Indian J. Chem.*, 1971, **9**, 1095.
[719] J. C. Martin, *Ann. Chim. (France)*, 1971, **6**, 205.
[720] G. A. Jeffrey, J. A. Pople, and L. Radom, *Carbohydrate Res.*, 1972, **25**, 117.

enopyranosides to yield pairs of epimeric methyl 6-deoxyglycopyranoside 6-sulphites, it was found that proportionality exists between the ratios of the 5-epimers formed and the relative conformational stability of the respective hex-5-enopyranosides. Starting with five different methyl 6-deoxy-hex-5-enopyranosides, the ratios of the glycopyranoside 6-sulphites were determined by using ^{35}S-labelled compounds. By means of a trial and error method using known interaction energies and the measured differences in free energy between the pairs of diastereoisomers, new values for the absolute anomeric effect in methyl glycopyranosides have been calculated.[398a]

2-Alkoxy-dihydropyrans and -chromans have been prepared and their conformational properties, related to the anomeric effect, were examined by n.m.r. methods. The role of the solvent and the influence of solvent polarity were studied.[721]

N.m.r. studies of various derivatives of α- and β-D-idopyranose have shown, in agreement with the studies of Lemieux (Vol. 3, p. 171), that *syn*-diaxial interactions of acetoxy-groups are appreciably less than the corresponding interaction of two methoxy-groups.[722] The influence of solvent, hydrogen bonding, and dipolar effects (particularly the anomeric effect) on these factors was discussed in detail. From n.m.r. studies and rotational calculations, it was shown that the preferred conformation of methyl 4,6-*O*-benzylidene-α-D-idopyranoside changes from *C*1(D) in chloroform to a skew-boat in DMSO–water.[723]

The conformations of α- and β-D-galactopyranose derivatives (6-deoxy-, 6-CO_2H, 2-NHAc) in deuterium oxide have been studied by n.m.r. spectroscopy. It was concluded that the chair conformation is somewhat distorted from the ideal shape.[724] Although the conformation in the crystalline state of 2,3,4-tri-*O*-acetyl-β-D-arabinopyranosyl bromide has been found to be essentially the same as that in solution [*i.e.* the 1*C*(D) conformation], definitive quantitative discrepancies were found between measured dihedral angles from *X*-ray data and those calculated from coupling constants.[272a]

Detailed n.m.r. analyses of thirty-three fully acetylated glycopyranosylamine derivatives containing mainly the indole nucleus have been described.[353] A Fourier transform method has been used to measure the T_1-values of the H-1 protons of eight pairs of anomers. In every case, the axial proton had a shorter relaxation time than the corresponding equatorial proton. This procedure is potentially useful for assigning the anomeric configuration where $J_{1,2}$ values are not diagnostic as, for example, in mannopyranose derivatives.[725]

D-Fructose 1,6-diphosphate and stereochemical analogues of its α- and

[721] G. Descotes, J. C. Martin, and N. Mathicolonis, *Bull. Soc. chim. France*, 1972, 1077.
[722] H. Paulsen and M. Friedmann, *Chem. Ber.*, 1972, **105**, 705.
[723] H. Paulsen and M. Friedmann, *Chem. Ber.*, 1972, **105**, 718.
[724] K. Izumi, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1816.
[725] L. D. Hall and C. Preston, *J.C.S. Chem. Comm.*, 1972, 1319.

β-furanose forms have been examined by Fourier transform ^{31}P n.m.r. spectroscopy. New evidence has confirmed earlier work (Vol. 5, p. 9; Vol. 4, p. 8) which demonstrated that acyclic free and hydrated *keto*-forms of D-fructose 1,6-diphosphate are not present to any appreciable extent. The amounts of β-furanose and α-furanose forms observed were 90 and 10%, respectively.[249]

N.m.r. evidence for the existence of glycolaldehyde in solution as a monomer, a 1,4-dioxan dimer, and a 1,3-dioxolan dimer has been reported.[199]

N.m.r. evidence for the participation of a twist form in the conformational equilibrium of *r*-1,*t*-2-dibromo-*c*-3-(t-butyl)cyclohexanone has been provided.[726] The structures and energies of cyclic carbonyl compounds have been investigated by force-field calculations.[727]

^{14}N N.m.r. studies of azoles, triazoles, oxazoles, and related compounds showed the existence of a linear relationship between the chemical shift and the π-electron distribution.[728]

^{199}Hg–^{1}H N.m.r. studies of a number of mercuri-sugar derivatives have been reported, and it was suggested that an extension of such studies would be helpful in establishing relations between coupling constants and chemical shifts in mercury derivatives.[410]

The 2′,3′-unsaturated products from acid-catalysed treatment of acetylated glycals with benzotriazole and 5,6-dimethyl- and 5,6-dichlorobenzotriazole have been subjected to detailed and computerized n.m.r. analysis.[426, 426a]

Furanoid Systems

The conformations in solution of seven anomeric pairs of methyl *O*-methyl-D-xylofuranosides[729] and of dioxolans,[730] substituted at the 4- and 5-positions by either phenyl or methoxycarbonyl groups and at C-2 by methyl or phenyl groups, have been studied by n.m.r. spectroscopy.

A valuable discussion has appeared on the detailed conformations of furanoid rings of the 1,2:5,6-di-*O*-isopropylidene-D-hexofuranoses, which were shown to adopt rigid ring-forms rather than *pseudo*-rotating systems.[731] As well as the 3-hydroxy-compounds, 3-deoxy-compounds, 3-*O*-substituted compounds, and 3-uloses were examined.

Provided that the deuterium is coupled to a proton, the deuterium chemical shifts of specifically labelled carbohydrates can be measured by

[726] P. L. Barki, G. Bellucci, G. Ingrosso, F. Marioni, and I. Morelli, *Tetrahedron*, 1972, **28**, 4583.
[727] N. L. Allinger, M. T. Tribble, and M. A. Miller, *Tetrahedron*, 1972, **28**, 1173.
[728] M. Witanowski, L. Stefaniak, H. Januszewski, and Z. Grabowski, *Tetrahedron*, 1972, **28**, 637.
[729] J. Alfrödi, C. Peciar, R. Palovčík, and P. Kováč, *Carbohydrate Res.*, 1972, **25**, 249.
[730] M. Farines, J. Soulier, and R. Soulier, *Bull. Soc. chim. France*, 1972, 1066.
[731] L. D. Hall, S. A. Black, K. N. Slessor, and A. S. Tracey, *Canad. J. Chem.*, 1972, **50**, 1912.

the ^{1}H-^{2}H INDOR technique; the procedure was demonstrated by considering 1,2:5,6-di-*O*-isopropylidene-α-D-[3-^{2}H]allofuranose.[732] The torsional energies of different half-chair and envelope conformations of some methylated tetrahydrofurans have been calculated using Pitzer's method. For all the examples studied, the half-chair form was shown to be more stable than the envelope form.[733] N.m.r. studies of 1-deoxy-2,3:4,6-di-*O*-isopropylidene-1-phthalimido-α-L-sorbofuranose have revealed that the molecule contains the C-2—O bond of the isopropylidene ring trans-coplanar with the C-1—N bond.[734]

A new description of furanose-ring conformations in nucleosides is referred to in Chapter 21. Hückel calculations have been used to determine the most probable conformation of the D-ribose unit in nucleic acids.[735]

A number of papers [664, 665, 668, 671, 672] describing the assignment of *syn* and *anti* conformations to nucleosides are discussed in Chapter 21. The nuclear Overhauser effect has also been used in these studies. The conformation of 2′,3′-*O*-isopropylideneadenosine 5′-acetate in several organic solvents has been studied by quantitative application of the nuclear Overhauser effect, and it was shown that computer fitting of multiple, intramolecular Overhauser effects is a sensitive means of determining the conformation of small molecules in solution.[736] The glycosyl conformations of adenosine, inosine, guanosine, their isopropylidene derivatives, and xanthosine in DMSO have been examined by this technique. The 2′,3′-*O*-isopropylidenepurine nucleosides in DMSO strongly preferred a single glycosyl conformation in the *syn* range. There was more latitude in the torsion angle of the parent nucleosides, which in DMSO solution possessed a C-2′-*endo* conformation. The glycosyl conformation appeared to be more responsive to nucleobase substituents.[737] Two other papers [738, 739] have reported the use of the nuclear Overhauser effect for studies of nucleosides and other conformationally mobile molecules.

Disaccharides and Polysaccharides

δ-Values and coupling constants of the anomeric protons of thirty fully trimethylsilylated oligosaccharides have been reported and a method, based on these data, has been developed for determining the configuration of the glycosidic bond in oligosaccharides.[740] Chemical shifts of the

[732] J. R. Campbell, L. D. Hall, and P. R. Steiner, *Canad. J. Chem.*, 1972, **50**, 504.
[733] Y. Infarnet, J. Bogner, J. Huet, J.-C. Duplan, and J. Delmau, *Bull. Soc. chim. France*, 1972, 3616.
[734] R. S. Glass and T. Williams, *J. Org. Chem.*, 1972, **37**, 3366.
[735] D. Vasilescu, F. Camous, and J. N. Lespinasse, *F.E.B.S. Letters*, 1972, **21**, 179.
[736] J. P. Davis, *Tetrahedron*, 1972, **28**, 1155.
[737] J. P. Davis and P. A. Hart, *Tetrahedron*, 1972, **28**, 2883.
[738] R. E. Schirmer, J. P. Davis, J. H. Noggle, and P. A. Hart, *J. Amer. Chem. Soc.*, 1972, **94**, 2561.
[739] P. A. Hart and J. P. Davis, *J. Amer. Chem. Soc.*, 1972, **94**, 2572.
[740] J. P. Kamerling, M. J. A. DeBie, and J. F. G. Vliegenthart, *Tetrahedron*, 1972, **28**, 3037.

anomeric protons in a series of disaccharide methyl ethers have also been recorded.[741]

A ^{1}H n.m.r. kinetic approach to the stereochemistry of the enzymic hydrolysis of carbohydrates has been described.[742] The hydrolysis of amylopectin by barley β-amylase and that of benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside by boar epidermis *N*-acetyl-D-glucosaminidase were used to test the approach. The method was then applied to the hydrolysis of hyaluronic acid by testicular hyaluronidase, which was found to cleave the 4-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl bond of the substrate with retention of configuration at the anomeric carbon atom.

Detailed 220 MHz n.m.r. data have been used to provide evidence for a terminal α-D-galactopyranosyl residue in a galactosylgalactosylglucosylceramide isolated from human kidney.[140]

In kanamycin and related compounds, the chemical shift of H-1 was diagnostic of the absolute configuration of 4- or 6-linked derivatives.[555]

Acyclic Derivatives

The ^{1}H n.m.r. spectra of all the fully acetylated pentitols and several fully acetylated hexitols have been analysed; in some cases, recourse to 250 MHz spectra or to computation by iterative analysis was necessary. Useful notations for describing the conformations of alditols were put forward. The planar zigzag conformation was found to predominate only in those configurations (*arabino* and *manno*) that do not possess parallel 1,3-interactions between acetoxy-groups. The other compounds were found to be mixtures of two or more conformers, none of which had a planar zigzag conformation excepting allitol hexa-acetate.[743] Similar results were obtained by other workers.[744] Conformational studies of 3,4,5,6,7-penta-acetoxy-1-nitro-1-heptenes (with D-*galacto*, D-*gluco*, and D-*talo* configurations)[441] and of peracetylated aldopentose dimethyl acetals (with D-*ribo*, D-*xylo*, L-*arabino*, and D-*lyxo* configurations)[745] have provided results consistent with other studies of acyclic sugar derivatives.

3,4-Di-*O*-acetyl-1,2:5,6-diepithiohexitols with *manno*, *ido*, and *gluco* configurations adopted non-planar zigzag conformations with the C-3 and C-4 acetoxy-groups having a *trans*-arrangement.[746]

Conformational features of acyclic carbohydrates in general and of acetylated phenylosotriazole derivatives in particular have been discussed.[747]

[741] D. E. Minnikin, *Carbohydrate Res.*, 1972, **23**, 139.
[742] I. V. Vikha, V. G. Sakharovsky, V. F. Bystrov, and A. Ya. Khorlin, *Carbohydrate Res.*, 1972, **25**, 143.
[743] S. J. Angyal, R. Le Fur, and D. Gagnaire, *Carbohydrate Res.*, 1972, **23**, 121.
[744] L. Maijs, L. S. Bresler, I. Yu. Tsereteli, E. I. Karabanova, and O. Lukevics, *Lat. R.S.R. Zinat. Akad. Vestis, Kim. Ser.*, 1971, **5**, 545.
[745] J. Defaye, D. Gagnaire, D. Horton, and M. Muesser, *Carbohydrate Res.*, 1972, **21**, 407.
[746] P. Sohar and J. Kuszmann, *Org. Magn. Resonance*, 1971, **3**, 647.
[747] H. El Khadem, D. Horton, and J. D. Wander, *J. Org. Chem.*, 1972, **37**, 1630.

A general method for distinguishing between *threo* and *erythro* vicinal diols, amino-alcohols, *etc.* in acyclic compounds relied on their conversion into isopropylidene (or related) derivatives, which were examined by n.m.r. techniques. Nuclear Overhauser effects and long-range coupling constants were found to be particularly useful in the assignment of structures.[748]

N.M.R. Studies for Establishing the Positions of Substituents

^{1}H N.m.r. parameters for the di-[749] and tri-*O*-methyl ethers[750] of D-galacto-pyranose, methyl D-galactopyranosides, and galactitol have been obtained. The results of these and earlier studies showed that change of the anomeric configuration greatly affects the chemical shift of the 2-methoxy-signals (in derivatives of D-glucose, D-galactose, and D-mannose) and that a change of configuration at C-4 (D-galactose compared with D-glucose) affects the chemical shift of the neighbouring 3-methoxy-signal much more than that of the 4-methoxy-signal. The work was extended in another paper.[751]

The chemical shifts of the methylene protons of the iodomethyl group in (225) were separated by 0.64 p.p.m. and were more affected by the double bond than those of the corresponding protons in (226).[467]

The N*H* signal of acetamido-sugars having *J*(HCNH) in the range 8—9.5 Hz is indicative of a *trans* arrangement.[752]

Lanthanide Shift Reagents

It has been emphasized that reproducible data from studies of carbohydrates with lanthanide shift reagents are only possible if rigorous control of all experimentally variable parameters is maintained.[753] The conformation of adenosine 5′-phosphate in DMSO, determined using a lanthanide probe technique, was found to differ from that previously observed in water. The technique was able to define in precise terms the nature of solvent-dependent conformational changes for this molecule and could be extended to the study of other molecules.[754] It has been demonstrated, using D-ribose 5-phosphate as an example, that ^{13}C chemical shifts may be assigned unequivocally using europium(III) ion-induced pseudo-contact shifts and C—H heteronuclear spin-coupling techniques.[755]

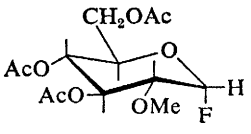
[748] K. Nakanishi, D. A. Schooley, M. Koreeda, and I. Miura, *J. Amer. Chem. Soc.*, 1972, **94**, 2865.
[749] E. B. Rathbone, A. M. Stephen, and K. G. R. Pachler, *Carbohydrate Res.*, 1972, **21**, 73.
[750] E. B. Rathbone, A. M. Stephen, and K. G. R. Pachler, *Carbohydrate Res.*, 1972, **21**, 83.
[751] E. B. Rathbone, A. M. Stephen, and K. G. R. Pachler, *Carbohydrate Res.*, 1972, **23**, 275.
[752] S. Hirano, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1071.
[753] I. Armitage and L. D. Hall, *Carbohydrate Res.*, 1972, **24**, 221.
[754] C. D. Barry, J. A. Glasel, A. C. T. North, R. J. P. Williams, and A. V. Xavier, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 166.
[755] B. Birdsall, J. Feeney, J. A. Glasel, R. J. P. Williams, and A. V. Xavier, *Chem. Comm.*, 1971, 1473.

^{19}F N.M.R. Spectroscopy

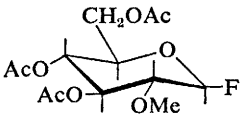
A general review of ^{19}F n.m.r. spectroscopy is of relevance for carbohydrate chemists.[756]

An n.m.r. study of the effects of fluorine substituents on the association between lysozyme and fluorinated derivatives of 2-amino-2-deoxy-D-glucose has been reported in preliminary form.[757] Rates of association and dissociation of the complex were studied with a highly selective pulse n.m.r. spectrometer. It was pointed out that, although a change in the chemical shift of the probe site of a small reversible inhibitor substance can be readily interpreted in terms of a molecular association, the absence of an observed shift does not necessarily indicate the absence of a molecular association.

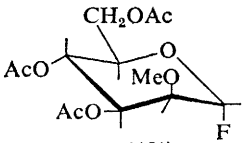
The binding of 2-deoxy-2-trifluoroacetamido-D-glucose and its chitobiose, -triose, and -tetraose analogues to hen egg-white lysozyme has been studied using ^{19}F n.m.r. spectroscopy. The *N*-trifluoroacetyl resonances underwent a change in shift upon binding to lysozyme.[758] ^{5}J(F, H) Coupling has been observed between F-1 and the 2-methoxy-group in each of the glycosyl fluorides (449) (1.0 Hz), (450) (1.4 Hz), and (451) (0.55 Hz) where the orientation of the two substituents is respectively *eq-ax*, *eq-eq*, and *ax-ax*.[759]



(449)



(450)



(451)

A solution of 3-deoxy-3-fluoro-L-idose in water or deuterium oxide at equilibrium showed four ^{19}F resonances, which were assigned to the $\alpha\beta$-pyranose and $\alpha\beta$-furanose forms; the proportions of the four tautomers corresponded closely to those observed for solutions of D-idose. It was possible to deduce the conformation of each pyranose form from the F—H splittings of the appropriate ^{19}F resonances, while the ^{19}F shifts also supported the conclusions reached.[43]

^{13}C N.M.R. Spectroscopy

The use of ^{13}C n.m.r. spectroscopy in determining the configurations of branched-chain sugars has been amply illustrated.[447, 457, 459]

^{13}C Signals in pulse Fourier transform spectra from the anomeric furanoses and pyranoses of D-ribose and 2-deoxy-D-*erythro*-pentose were assigned from reference spectra of methyl β-D-ribopyranoside, methyl

[756] R. Fields, *Ann. Report N.M.R. Spectroscopy*, 1972, **5A**, 99.
[757] L. D. Hall and C. W. M. Grant, *Carbohydrate Res.*, 1972, **24**, 218.
[758] F. Millett and M. A. Raftery, *Biochemistry*, 1972, **11**, 1639.
[759] A. B. Foster, R. Hems, and J. H. Westwood, *Carbohydrate Res.*, 1972, **23**, 316.

β-D-ribofuranoside, adenosine, 2′-deoxyadenosine, and 9-β-D-ribopyranosyladenine.[760]

The ^{13}C-5 signal in *trans*-fused, bicyclic hexopyranoside derivatives appeared noticeably upfield from its position in unsubstituted glycosides.[761]

It has been found for a variety of ^{13}C-labelled carbohydrates that vicinal $^{13}C—^{1}H$ couplings through both C—C and C—O bonds exhibit an orientational dependence analogous to that for protons: *i.e.* dihedral angles of 60—100° are associated with smaller couplings (0—3 Hz) than angles of 140—180° (4.5—5.5 Hz). Splittings of almost twice these values were observed with sp^2-carbon atoms. The spacing was observed to be smaller when an oxygen atom is in the plane of the coupling pathway than when it is out of plane for both vicinal and geminal $^{13}C—^{1}H$ interactions. There is evidence that the characteristics of coupling between ^{13}C and ^{1}H nuclei may be as valuable as $^{1}H—^{1}H$ coupling in the study of rotational isomerism, *etc.*[762]

Tentative assignments have been made for the ^{13}C resonances in the oligosaccharides 1-kestose and nystose.[763] The ^{13}C spectra of lactose and melibiose have been compared with those of D-glucose, methyl β-lactoside, D-galactose, and methyl α-D-galactopyranoside.[764] Using pulse Fourier transform techniques, the spectra were recorded before mutarotation ensued, thereby permitting assignment of the signals due to α- and β-anomers.

The disaccharide 2,5-anhydro-4-*O*-(α-L-idopyranosyluronic acid 2-sulphate)-D-mannose 6-sulphate, prepared as a model for the deaminative degradation of heparin, has been examined by ^{13}C Fourier transform n.m.r. spectroscopy.[765] The natural abundance ^{13}C n.m.r. spectra of aqueous solutions of D-fructose 1,6-diphosphate and D-fructose 6-phosphate gave the anomeric compositions in each case as α-D-furanose 20 ± 4% and β-D-furanose 80 ± 10% (see also p. 165).[248]

For α- and β-D-glucopyranoses, fair correlation was demonstrated between the ^{13}C chemical shifts and the electron densities at the respective carbon atoms calculated by iterative extended Hückel theory.[766]

Despite the use of differently bonded, vicinally related carbon atoms and protons in various cyclonucleosides of known geometry, a Karplus-type relation has been found for vicinal $^{13}C—^{1}H$ coupling constants and the relevant torsion angles;[767] key values are $\Phi = 30°$, $J = 3$ Hz; $\Phi = 90°$,

[760] E. Breitmaier, G. Jung, and W. Voelter, *Chimia*, 1972, **26**, 136.
[761] E. Conway, R. D. Guthrie, S. D. Gero, G. Lukacs, A. M. Sepulchre, E. W. Hagaman, and E. Wenkert, *Tetrahedron Letters*, 1972, 4879.
[762] J. A. Schwarz and A. S. Perlin, *Canad. J. Chem.*, 1972, **50**, 3667.
[763] W. W. Binkley, D. Horton, N. S. Bhacca, and J. D. Wander, *Carbohydrate Res.*, 1972. **23**, 301.
[764] E. Breitmaier, G. Jung, and W. Voelter, *Chimia*, 1971, **25**, 362.
[765] A. S. Perlin, N. M. K. Ng Ying Kin, S. S. Bhattacharjee, and L. F. Johnson, *Canad. J. Chem.*, 1972, **50**, 2437.
[766] N. Cyr, A. S. Perlin, and M. A. Whitehead, *Canad. J. Chem.*, 1972, **50**, 814.
[767] R. U. Lemieux, T. L. Nagabhushan, and B. Paul, *Canad. J. Chem.*, 1972, **50**, 773.

$J = 0$ Hz; and $\Phi = 180°$, $J = 8$ Hz. The establishment of this type of relation has important implications for the conformational analysis of nucleosides, peptides, and glycosides.

Borate complexes of carbohydrates have been studied by ^{13}C pulse Fourier transform n.m.r. spectroscopy.[768] For example, the ^{13}C signals of nucleosides at various pH's were recorded in the presence and in the absence of borate. In the presence of borate, C-2′ and C-3′ in adenosine were 6 p.p.m. lower than in the absence of borate, whereas C-5′ was little affected. The C-3′ signal for 2′-deoxyadenosine had the same chemical shift both in the absence and in the presence of borate.

[768] W. Voelter, C. Bürvenich, and E. Breitmaier, *Angew. Chem. Internat. Edn.*, 1972, **11**, 539.

24
Other Physical Methods

I.R. Spectroscopy

A theoretical study of the vibrational spectra of α-D-glucopyranose has been carried out by normal-co-ordinate analysis. The calculated vibrational frequencies were compared with the observed i.r. and Raman bands measured on the solid and in aqueous solution; satisfactory agreement was achieved. Although this represents a significant development – D-glucose being the most complex molecule examined by this method to date – it is unlikely that the method will lead to a major enhancement in the value of i.r. spectroscopy in structural analysis.[50]

Mass Spectroscopy

Several interesting developments have been described this year in this rapidly expanding aspect of carbohydrate chemistry.

The chemical ionization mass spectra of a number of sugars, measured with ammonia as the reagent gas, all gave major peaks corresponding to $(M + \overset{+}{N}H_4)$ and revealed the molecular weight readily. Corresponding spectra using methane gave prominent $(\overset{+}{M}H - H_2O)$, $(\overset{+}{M}H - 2H_2O)$, and $(\overset{+}{M}H - 3H_2O)$ ions. As expected, the spectrum of methyl α-D-glucopyranoside showed peaks corresponding to $(\overset{+}{M}H - MeOH)$ and fragment ions formed by loss of water. Examination of 3-*O*-methyl-D-glucose revealed significant differences, suggesting that the position of methylation of sugars could be examined by this method.[769]

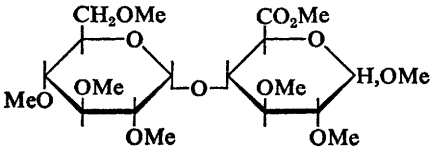
Although diastereoisomers are not normally considered to be distinguishable by mass spectrometry, Dutch workers have shown that detailed consideration of the ion ratios can lead to interpretable differences and have suggested that the method could be applied to the qualitative analysis of sugars on a μg scale. Using TMS ethers and from a consideration of eight ion pairs, it was possible to identify seven hexoses. However, among the seven were α- and β-D-glucopyranose, which reveals that the method is anomer dependent, and since μg quantities of sugars are unlikely to be obtainable in anomerically pure form, this hypothesis seems to contain a fundamental weakness. Nevertheless, this problem could be readily overcome and the paper reported a point of appreciable interest. Statistical

[769] A. M. Hogg and T. L. Nagabushan, *Tetrahedron Letters*, 1972, 4827.

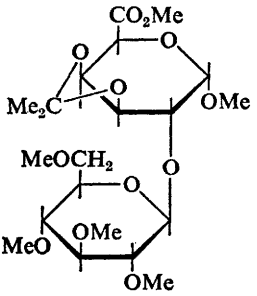
and computerized methods were used for matching the ion ratios of unknown compounds with those of reference sugars.[770] Other workers have also shown that the ion intensities of TMS ethers of pyranoses are stereochemically dependent.[771]

Several specific classes of compound have received attention. 6-Deoxy-6-halogeno-α-D-glucopyranose acetates have been examined with regard to the effect of the nature of the halogen on the fragmentations. The ease with which atomic halogen or hydrogen halide was eliminated from the molecular ion or fragment ions increased with decreasing electronegativity of the halogen.[772] The number and the location of methyl groups on methyl *O*-methylpentofuranosides can be determined with derivatization.[773] Phenylboronates with five-, six-, and seven-membered rings have been investigated with particular reference to alditol esters.[258] Partially methylated alditol acetates have continued to receive attention,[774] and related 2- and 3-deoxyalditol derivatives have also been studied.[775]

The structures of the methylated *pseudo*-aldobiouronic acids (452) and (453) have been investigated by mass spectrometry[776] and D-fructose-containing oligosaccharides can be distinguished as their TMS ethers.[777]



(452)



(453)

Other natural products to be examined were flavonoid oligosaccharides (as perdeuteriomethyl derivatives),[778] adenine nucleosides (as the trifluoroacetyl esters can be characterized according to ring-size and anomeric

[770] J. Vink, J. H. W. Bruins Slot, J. J. De Ridder, J. P. Kamerling, and J. F. G. Vliegenthart, *J. Amer. Chem. Soc.*, 1972, **94**, 2542.
[771] S. C. Havlicek, M. R. Brennan, and P. J. Scheuer, *Org. Mass Spectrometry*, 1971, **5**, 1273.
[772] O. S. Chizhov, A. B. Foster, M. Jarman, and J. H. Westwood, *Carbohydrate Res.*, 1972, **22**, 37.
[773] V. Kováčik and P. Kováč, *Carbohydrate Res.*, 1972, **24**, 23.
[774] K. Axberg, H. Björndal, Å. Pilotti, and S. Svensson, *Acta Chem. Scand.*, 1972, **26**, 1319.
[775] H. B. Borén, P. J. Garegg, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1971, **25**, 3299.
[776] C. K. Kuenzle, *Carbohydrate Res.*, 1972, **24**, 169.
[777] J. P. Kamerling, J. F. G. Vliegenthart, J. Vink, and J. J. De Ridder, *Tetrahedron*, 1972, **28**, 4375.
[778] R. D. Schmid, P. Varenne, and R. Paris, *Tetrahedron*, 1972, **28**, 5037.

configuration),[779] and anhydrouridines (as partially acetylated derivatives).[780] In the last investigation, intra- and inter-molecular acetyl migration was detected and this observation has led to a synthesis of the inaccessible 5′-*O*-acetylanhydrouridine. Studies of pyrimidine cyclonucleosides with 2,2′-, 2,3′-, 2′,6-, and 5′,6- anhydro-rings have shown that 2′- and 3′-bridged compounds could be readily distinguished from the 5′,6-isomers, but not from each other.[686]

X-Ray Crystallography

The rapid growth in the application of crystallography in carbohydrate chemistry has continued; the approximate numbers of structures that have been analysed in the years covered by this series are as follows: 1967, 5; 1968, 20; 1969, 20; 1970, 30; 1971, 30; 1972, 40. However, this year the change was apparently more than quantitative, since there was obviously an extension to the study of sugar derivatives holding unusual interest from a stereochemical viewpoint and into more complex systems such as trisaccharides.

Theoretical predictions of crystal structures by a method dependent on minimization of non-bonded repulsion energy gave good agreement with experimental findings for a number of sugars, but not for β-D-arabinose.[781]

Several anhydro-derivatives have been examined, namely 1,6-anhydro-β-D-mannofuranose,[782] 2,6-anhydro-β-D-fructofuranose,[179] methyl 3,6-anhydro-α-D-galactopyranoside,[184] methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside,[783] and 3,6-anhydro-α-D-glucopyranosyl 1,4:3,6-dianhydro-β-D-fructofuranoside.[190]

In a continuation of their work on alditols, Jeffrey and his co-workers have reported that allitol and D-iditol have the expected bent-chain conformations.[784]

The following salts and complexes have been examined: potassium D-gluconate,[485] D-mannose,$CaCl_2$,$4H_2O$,[508] sodium iodide complexes of α-cellobiose and α-isomaltose,[507] and hydrated calcium bromide complexes of D-galactose, lactose, and *myo*-inositol.[54a]

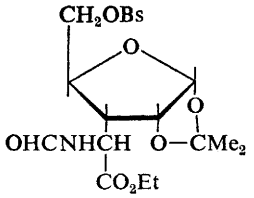
Stevens' work on septanoses has led to reports on the crystal structures of 3-*O*-acetyl-1,2:4,5-di-*O*-isopropylidene-α-D-glucoseptanose [206] and ethyl 2,3:4,5-di-*O*-isopropylidene-1-thio-β-D-glucoseptanoside.[785]

In the glycoside area, the crystal structure of methyl β-D-ribopyranoside has been examined as part of a study aimed at a comparison between the

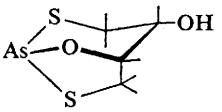
[779] W. A. König, K. Zech, R. Uhmann, and W. Voelter, *Chem. Ber.*, 1972, **105**, 262.
[780] K. K. Ogilvie, J. B. Westmore, G. Taylor, D. Lin, D. Iwacha, H. Wayborn, and G. E. Dunn, *Canad. J. Chem.*, 1972, **50**, 2365.
[781] P. Zugenmaier and A. Sarko, *Acta Cryst.*, 1972, **B28**, 3158.
[782] J. Lechat and G. A. Jeffrey, *Acta Cryst.*, 1972, **B28**, 3410.
[783] A.-M. Pilotti and B. Stensland, *Acta Cryst.*, 1972, **B28**, 2821.
[784] N. Azarnia, G. A. Jeffrey, and M. S. Shen, *Acta Cryst.*, 1972, **B28**, 1007.
[785] J. P. Beale, N. C. Stephenson, and J. D. Stevens, *Acta Cryst.*, 1972, **B28**, 3115.

results of *X*-ray and neutron diffraction.[786] The structure of a naturally occurring γ-lactone D-glucoside has also been reported.[787]

Other structures to have been determined are as follows: α-D-xylopyranose 1,2,4-orthobenzoate and 3-*O*-(4-bromobenzoyl)-α-D-xylopyranose 1,2,4-orthobenzoate,[788] 5-*O*-(bromobenzene-*p*-sulphonyl)-3-deoxy-3-*C*-(*R*)-(ethoxycarbonylformamido)methyl-1,2-*O*-isopropylidene-α-D-ribofuranose (454),[466] 2-amino-2-deoxy-β-D-galactopyranose hydrochloride,[319] 3,6-*N*-acetylepimino-3,6-dideoxy-1,2-*O*-isopropylidene-β-L-idofuranose,[375] 3-chloro-3-deoxy-1,2:5,6-di-*O*-isopropylidene-β-D-idofuranose,[276] ethyl 2-*S*-ethyl-1,2-dithio-α-D-mannofuranoside,[400] and the arsenite ester of 1,4-dithiothreitol (455).[789] The crystal structures of dehydro-L-ascorbic acid [790] and D-*arabino*-ascorbic acid have been determined.[791]



(454)



(455)

In the area of di- and oligo-saccharides, two papers have appeared on the crystal structure of αα-trehalose dihydrate,[792, 793] and the following trisaccharides have been reported: 1-kestose [*O*-α-D-glucopyranosyl-(1→2)-*O*-β-D-fructofuranosyl-(1→2)-β-D-fructofuranoside],[794] planteose [*O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside],[795] and *O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose.[796]

Interest in nucleosides and their derivatives remains high. Adenine derivatives to be examined included adenosine,[797] 8-bromo-2′-*O*-(triisopropylbenzenesulphonyl)adenosine,[798] and the phosphinyl analogues (456) and (413) of adenosine 3′,5′ cyclic-phosphate [799] and 3′-phosphate,[674] respectively.

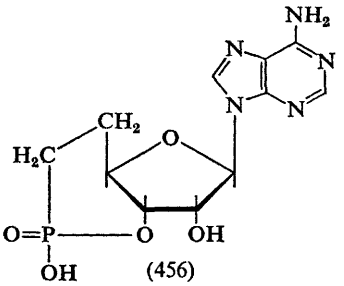
[786] V. J. James and J. D. Stevens, *Carbohydrate Res.*, 1972, **21**, 334.
[787] R. A. Mariezcurrena, S. E. Rasmussen, J. Lam, and E. Wollenweber, *Tetrahedron Letters*, 1972, 3091.
[788] L. G. Vorontsova, A. F. Bochkov, I. V. Obruchnikov, V. I. Andrianov, and B. L. Tarnopolsky, *Carbohydrate Res.*, 1972, **23**, 326.
[789] W. B. T. Cruse and M. N. G. James, *Acta Cryst.*, 1972, **B28**, 1325.
[790] J. Hvoslef, *Acta Cryst.*, 1972, **B28**, 916.
[791] N. Azarnia, H. M. Berman, and R. D. Rosenstein, *Acta Cryst.*, 1972, **B28**, 2157.
[792] G. M. Brown, D. C. Rohrer, B. Berking, C. A. Beevers, R. O. Gould, and R. Simpson, *Acta Cryst.*, 1972, **B28**, 3145.
[793] T. Taga, M. Senma, and K. Osaki, *Acta Cryst.*, 1972, **B28**, 3258.
[794] G. A. Jeffrey and Y. J. Park, *Acta Cryst.*, 1972, **B28**, 257.
[795] D. C. Rohrer, *Acta Cryst.*, 1972, **B28**, 425.
[796] R. A. Moran and G. F. Richards, *Carbohydrate Res.*, 1972, **25**, 270.
[797] T. F. Lai and R. E. Marsh, *Acta Cryst.*, 1972, **B28**, 1982.
[798] T. Fujihara, T. Fujiwara, K. Tomita, M. Kaneko, and M. Ikehara, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 708.
[799] M. Sundaralingam and J. Abola, *J. Amer. Chem. Soc.*, 1972, **94**, 5070.

Uracil derivatives to be examined included 2′-deoxyuridine,[800] dihydrouridine,[801] 6-methyluridine,[802, 803] uridine 3′-phosphate(disodium salt),[804] and 1-β-D-arabinofuranosyl-4-thiouracil.[805]

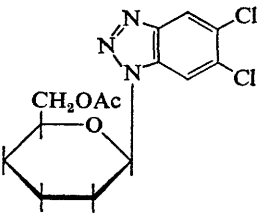
In the guanine series, 6-thioguanosine[806] and N^2-dimethylguanosine[807] have been examined.

The trideoxynucleoside analogue (457) has been characterized by crystallographic methods.[808]

The structure of a steroidal glycoside containing the unusual sugar 2-*O*-acetyl-6-deoxy-α-L-*ribo*-hexopyranos-3-ulose has been determined.[809]



(456)



(457)

[800] A. Rahman and H. R. Wilson, *Acta Cryst.*, 1972, **B28**, 2260.
[801] D. Suck, W. Saenger, and K. Zechmeister, *Acta Cryst.*, 1972, **B28**, 596.
[802] D. Suck, W. Saenger, and H. Vorbrüggen, *Nature*, 1972, **235**, 333.
[803] D. Suck and W. Saenger, *J. Amer. Chem. Soc.*, 1972, **94**, 6520.
[804] M. A. Viswamitra, B. Swaminatha Reddy, M. N. G. James, and G. J. B. Williams, *Acta Cryst.*, 1972, **B28**, 1108.
[805] W. Saenger, *J. Amer. Chem. Soc.*, 1972, **94**, 621.
[806] U. Thewalt and C. E. Bugg, *J. Amer. Chem. Soc.*, 1972, **94**, 8892.
[807] T. Brennan, C. Weeks, E. Shefter, S. T. Rao, and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1972, **94**, 8548.
[808] J. Fayos and S. García-Blanco, *Acta Cryst.*, 1972, **B28**, 2863.
[809] S. M. Kupchan, C. W. Sigel, L. J. Guttman, R. J. Restivo, and R. F. Bryan, *J. Amer. Chem. Soc.*, 1972, **94**, 1353.

25
Polarimetry

The polarimetric methods developed by Nakanishi and his group for determining the chirality of diol systems have been reviewed; their dibenzoate chirality rule has been renamed the 'exciton chirality method'.[810]

In a particularly interesting development, the c.d. spectra of D-glucose, D-galactose, and D-xylose have been measured down to 164 nm by the use of vacuum techniques. D-Glucose showed a positive dichroism maximum at 169.5 nm and D-xylose showed a similar curve centred at 167.5 nm; D-galactose, on the other hand, exhibited negative maxima at both 177 nm and below 164 nm; it appeared, as had been suspected previously, that the c.d. spectra of sugars depend very largely on the C-4 configuration and presumably on the rotamer state about the C-5—C-6 bond.[811] By way of comparison, it has been reported that aldopentoses exhibit a very weak c.d. band at 290 nm in aqueous solution, which has been attributed to the $n \rightarrow \pi^*$ transition of the *aldehydo*-sugar carbonyl group. D-Glucose and sucrose did not exhibit this band.[53]

The c.d. spectra of several hexopyranoside monoacetates have been correlated in terms of pair-wise interactions between the ester functions and vicinally related oxygenated functions.[812]

Satoh and his co-workers have continued their work on 1-deoxy-1-nitroalditol derivatives and have reported on the c.d. and o.r.d. spectra of 1-deoxy-1-nitroalditols acetates,[813] and 1,2-dideoxy-1-nitroalditols [364] and their acetates.[814] Their findings enabled the chirality at asymmetric centres nearest to the nitro-group to be determined.

Detailed conformations have been determined by crystallographic and polarimetric methods of both mono- and di-nitrates of a wide range of terpenes and various carbohydrates.[265]

O.r.d. studies of aldobiouronic acids have been reported.[815]

[810] N. Harada and K. Nakanishi, *Accounts Chem. Res.*, 1972, **5**, 257.
[811] R. G. Nelson and W. C. Johnson, *J. Amer. Chem. Soc.*, 1972, **94**, 3343.
[812] H. B. Borén, P. J. Garegg, L. Kenne, L. Maron, and S. Svensson, *Acta Chem. Scand.*, 1972, **26**, 644.
[813] C. Satoh and A. Kiyomoto, *Yakugaku Zasshi*, 1972, **92**, 176.
[814] C. Satoh, K. Shiba, and A. Kiyomoto, *J. Pharm. Soc. Japan*, 1972, **92**, 108.
[815] T. Sticzay, C. Peciar, J. Rosik, and J. Kubala, *Chem. Zvesti*, 1972, **26**, 160.

26
Separatory and Analytical Methods

Chromatographic Methods

The work of Michael Tswett, the founder of chromatography, has been reviewed on the centenary of his birth.[3]

Gas–Liquid Chromatography.—Trimethylsilyl (TMS) ethers have retained their pre-eminent position as volatile derivatives for use in g.l.c. and reports have been published of their applications to the study of hexosamines (simultaneous *N*-acetylation and *O*-trimethylsilylation was used),[816] pentoses and hexoses (using capillary columns),[817] partially methylated alditols,[818] pent-2- and -3-uloses and tetruloses,[819] borate complexes of hexoses,[820] glycosyl derivatives of amino-acids (*e.g.* 3-*O*-β-D-xylopyranosyl-L-serine),[821] and disaccharides occurring in human body fluids.[822] Another report has specified improved methods for the collection and measurement of TMS derivatives of [^{14}C]glycerol and D-[^{14}C]glucose, *etc.*[823]

Acetates and trifluoroacetates of reduced *O*-methyl-D-fructoses and -glucoses have been studied,[824] as have the trifluoroacetates of glycosides of pentoses, hexoses, and hexosamines.[825] Partially methylated hexoses have also been examined as their acetates.[826]

Column and Ion-exchange Chromatography.—A report has appeared on the separation of sugars and their methylated derivatives on Sephadex G-15.[827] The following sugars have been separated on ion-exchange resins: L-ascorbic, dehydro-L-ascorbic, and 2,3-diketo-L-*threo*-hexonic acid,[828] tetruloses and pentuloses,[819] the linkage isomers of trehalose,[829] and steroidal D-gluco-

[816] S. Hara and Y. Matsushima, *J. Biochem.* (*Japan*), 1972, **71**, 907.
[817] K. Tesařík, *J. Chromatog.*, 1972, **65**, 295.
[818] B. H. Freeman, A. M. Stephen, and P. van der Bijl, *J. Chromatog.*, 1972, **73**, 29.
[819] J. Havlicek, G. Petersson, and O. Samuelson, *Acta Chem. Scand.*, 1972, **26**, 2205.
[820] H. Jacin, *Stärke*, 1972, **24**, 19.
[821] G. W. Jourdian and J. J. Distler, *Carbohydrate Res.*, 1972, **22**, 369.
[822] H. Nakamura and Z. Tamura, *Chem. and Pharm. Bull.* (*Japan*), 1972, **20**, 1070.
[823] G. R. Watson and J. P. Williams, *J. Chromatog.*, 1972, **67**, 221.
[824] G. Kubberød, *Acta Chem. Scand.*, 1972, **26**, 2544.
[825] J. P. Zanetta, W. C. Breckenridge, and G. Vincendon, *J. Chromatog.*, 1972, **69**, 291.
[826] G. M. Bebault, G. G. S. Dutton, and R. H. Walker, *Carbohydrate Res.*, 1972, **23**, 430.
[827] L. F. Martin, N. R. Bertoniere, and S. P. Rowland, *J. Chromatog.*, 1972, **64**, 263.
[828] J. Hegenauer and P. Saltman, *J. Chromatog.*, 1972, **74**, 133.
[829] R. Onishi and I. Karasawa, *Doshisha Joshi Daigaku Gakujutsu Kenkyu Nempo*, 1970, **21**, 14 (*Chem. Abs.*, 1972, **76**, 127 294).

siduronic acids (using liquid ion-exchangers).[830] A further colorimetric procedure, based on the reaction with sulphuric acid, has been developed for detecting carbohydrates eluted from columns during chromatographic separations.[831] An automated separation of sugars as borate complexes on ion-exchangers has been mentioned earlier.[7]

Paper Chromatography and Electrophoresis.—Paper chromatographic and electrophoretic studies of glycosyl derivatives of amino-acids [821] and the paper chromatographic separations of phenyl- and 4-nitrophenyl-osazones [832] have been reported. L-Ascorbic acid and its oxidation products dehydro-L-ascorbic and 2,3-diketo-L-*threo*-hexonic acid have been separated on glass-fibre papers.[833] A modified *p*-anisidine reagent for the detection and permanent recording of D-fructose oligosaccharides on paper chromatograms has been developed.[834]

Thin-layer Chromatography.—A new modification designated 'coupled layer' chromatography, in which adjacent adsorbent layers of different resolving powers are used, has been applied to the separation of carbohydrates,[835] and another adaptation used silica gel and fused-glass powder soaked in aqueous sodium bisulphite.[836]

A relationship between the structure and t.l.c. behaviour of twenty substituted methyl α-D-glucopyranosides has been proposed.[837] Glycosides of cholesterol, phytosterol, and their esters have been examined under a variety of conditions.[838] The separation of all four ribonucleoside cyclic 3′,5′-phosphates can be effected on alumina using an aqueous, ethanolic ammonium acetate buffer as the developing solvent.[609] Separations of sugars on polyamide layers have been referred to earlier.[8]

Other Analytical Methods

A new autoanalyser operating in the 10^{-10} mol l^{-1} region, which has eliminated some of the deficiencies of commercial instruments, has been reported.[839] A further automated procedure has been used for the analysis of sugars, alditols, and their deoxy- and anhydro-derivatives.[840]

An interesting report has described an enzyme electrode for the determination of D-glucose.[841]

Specific analytical procedures have been advocated for 2-deoxy-D-

[830] V. R. Mattox, J. E. Goodrich, and R. D. Litwiller, *J. Chromatog.*, 1972, **66**, 337.
[831] S. Katz and L. H. Thacker, *J. Chromatog.*, 1972, **64**, 247.
[832] N. F. Komissarenko and I. G. Levashova, *Khim. prirod. Soedinenii*, 1971, **7**, 516.
[833] D. Hornig, *J. Chromatog.*, 1972, **71**, 169.
[834] D. H. Lewis, C. Chen, G. Woods, and L. A. Culpin, *J. Chromatog.*, 1972, **74**, 369.
[835] T. Mezzetti, M. Ghebregziabhier, S. Rufini, G. Ciuffini, and M. Lato, *J. Chromatog.*, 1972, **74**, 273.
[836] T. Okumura, T. Kadono, and M. Nakatani, *J. Chromatog.*, 1972, **74**, 73.
[837] H. B. Sinclair, *J. Chromatog.*, 1972, **64**, 117.
[838] M. L. Boisio, *J. Chromatog.*, 1972, **73**, 279.
[839] K. Mopper and E. T. Degens, *Analyt. Biochem.*, 1972, **45**, 147.
[840] H. Matsui, E. Päärt, and O. Samuelson, *Chim. Scripta*, 1971, **1**, 45.
[841] G. G. Guilbault and G. J. Lubrano, *Analyt. Chim. Acta*, 1972, **60**, 254.

erythro-pentose,[842] hexosamines,[843] glucuronic acid,[844] and muramic acid (by use of an automatic amino-acid analyser).[845] A technique dependent on measuring the remission spectrum was recommended as a new sub-micro method for the assay of L-ascorbic acid.[846]

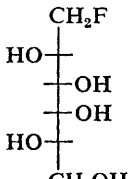
[842] R. Q. W. Pestell and H. R. Woodland, *Biochem. J.*, 1972, **127**, 589.
[843] A. R. Johnson, *Analyt. Biochem.*, 1971, **44**, 628.
[844] J. Tomašić and D. Keglević, *Analyt. Biochem.*, 1972, **45**, 164.
[845] B. Rosan, *Analyt. Biochem.*, 1972, **48**, 624.
[846] H. Fredholm, *Acta Chem. Scand.*, 1971, **25**, 3886.

27
Alditols

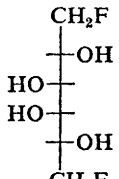
The presence of small quantities of D-mannitol and D-glucitol caused significant changes in the solvent and structural properties of water.[847]

A number of branched- and straight-chain alditols were identified (by combined g.l.c. and mass spectrometry) following reduction of the products obtained by condensation of formaldehyde under catalysis by calcium hydroxide (the formose reaction).[452] Alditols identified in the reduced formose reaction by g.l.c. of either their TMS ethers or trifluoroacetates included ethane-1,2-diol, glycerol, hydroxymethylglycerol, erythritol, threitol, 2-*C*-(hydroxymethyl)-tetritols and -pentitols, all of the straight-chain pentitols and hexitols, and *glycero-gulo-* and *glycero-galacto*-heptitols. G.l.c. of TMS derivatives has also been used in the separation of partially methylated alditols[818] and in the identification of a pentitol and mannitol in the sugar fraction of cocoa beans.[10]

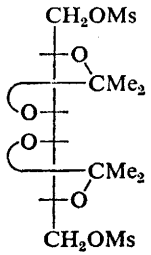
Reduction of 6-deoxy-6-fluoro-α-D-galactopyranose with sodium borohydride yielded 1-deoxy-1-fluoro-L-galactitol (458), which was also obtained



(458)



(459)



(460)

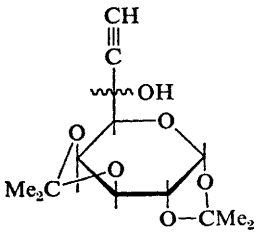
by the action of a polyol dehydrogenase (from rabbit-lens capsules) on the free sugar.[284] Biological reduction of 6-deoxy-6-fluoro-D-galactose proceeded more rapidly than that of D-galactose. 1,6-Disubstituted galactitol and mannitol derivatives, containing substituent chloro-, bromo-, and fluoro-groups, have been prepared by appropriate nucleophilic displacements; for example, 1,6-dideoxy-1,6-difluorogalactitol (459) was readily synthesized from 2,3:4,5-di-*O*-isopropylidene-5,6-di-*O*-methylsulphonylgalactitol (460) by direct displacement with tetraethylammonium fluoride

[847] J. H. Stern and M. E. O'Connor, *J. Phys. Chem.*, 1972, **76**, 3077.

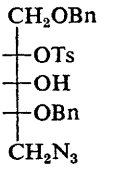
and subsequent removal of the protecting groups.[285] The halogenated derivatives were prepared as potential antitumour agents.

D-*glycero*-D-*galacto*-Heptitol (perseitol) and L-*glycero*-D-*galacto*-heptitol have been synthesized by a route involving ethynylation of 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose.[482] The epimeric acetylenic derivatives (461) were separated and characterized before each was converted into the corresponding heptitol by proven procedures.

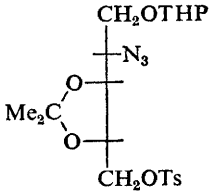
5-Azido-1,4-di-*O*-benzyl-5-deoxy-2-*O*-toluene-*p*-sulphonyl-D-ribitol (462) and 2-azido-2-deoxy-3,4-*O*-isopropylidene-1-*O*-(tetrahydropyran-2-yl)-5-*O*-toluene-*p*-sulphonyl-L-arabinitol (463) have been prepared from D-ribono-1,4-lactone.[490] The intramolecular cyclization of (462) and (463) to give N-analogues of 2,5-anhydro-D- and -L-arabinitol has been referred to earlier.[401]



(461)



(462)



(463)

THP = tetrahydropyranyl

1,3:2,4:5,6-Tri-*O*-benzylidenegalactitol was formed when 2,3:4,5-di-*O*-benzylidene- or -isopropylidene-galactitol was treated with benzaldehyde in the presence of zinc chloride. From a comparison of the benzylidenation of D-glucitol under conditions of kinetic and thermodynamic control, it was suggested that Baker and Bourne's rules (*J. Chem. Soc.*, 1952, 905) apply only when acetalation of alditols is performed under kinetic control.[211] Photolysis of 2,4-dinitrobenzylidene derivatives of hexitols afforded 2-nitroso-4-nitrobenzoate esters and, in the case of an acetal bridging primary and secondary diol groups, the ester formed was located at the primary position.[196]

The crystal structures of the arsenite ester of 1,4-dithiothreitol (455),[789] and of allitol and D-iditol [784] have been determined; both the hexitols were shown to assume the expected bent-chain conformation. A planar, zigzag conformation was found to predominate in solutions of only those alditol acetates (*i.e. arabino* and *manno*) that do not possess parallel 1,3-interactions between acetoxy-groups on alternate carbon atoms.[743] Other compounds were found to be mixtures of two or more conformers, none of which has the planar, zigzag conformation. The conformations of alditols in the crystalline state and of alditol acetates in solution were compared. N.m.r. studies have also demonstrated a non-planar, zigzag conformation for 3,4-di-*O*-acetyl-1,2:5,6-diepithiohexitols with the *manno*, *ido*, and *gluco* configurations.[746]

Part II

MACROMOLECULES

By

J. F. Kennedy
R. J. Sturgeon

1
Introduction

The primary objectives of Part II have followed the lines of previous Reports in the series. The coverage of journals is largely that noted in Volume 5, Chapter 1, but a number of journals appearing for the first time during 1972 have been added to the list.

As will be evident from a further increase in size of this Part, the number of papers published annually on carbohydrases and macromolecular species containing carbohydrates continues to increase at a substantial rate. The chapter and section headings introduced in Volume 4 have been followed along with the subsection headings added in Volume 5.

In the case of Chapter 5 (Glycoproteins), the increased diversity of material has necessitated the introduction of additional sections. Papers appearing on algal glycoproteins have been included in the section on plant glycoproteins and a new section has been introduced to accommodate viral glycoproteins. Because of the increasing number of references to haemagglutinins, these molecules are now reported in a separate section, which has been subdivided according to the source of the lectin. Where molecules have been shown to exhibit blood-group activity, the report has been included in the section on blood-group substances rather than in those dealing with materials from cells, tissues, fluids, *etc.* However, readers are advised to refer to other sections for reports on molecules that may be related to blood-group-active molecules, but for which no such activity was mentioned. The section dealing with the occurrence, isolation, measurement, and structure of glycosaminoglycuronans, glycosaminoglycans, and their protein and peptide derivatives has hitherto contained reports of two types: those dealing with macromolecules in a general way without specifying their sources and those dealing with macromolecules as obtained from specific sources. Whereas in Volumes 4 and 5 the two types were dealt with under one heading, they are now covered in separate subsections.

Chapter 6 (Enzymes) reports on a number of aspects of work on carbohydrases published during 1972. New sections have been introduced to cover enzymes not previously reported, *e.g.* iduronidases, dermatan sulphate hydrolases, and lyases; α-acetamidodeoxy-galactosidases and -glucosidases, α-D-fucosidases, and α-glucuronidases are also reported.

Developments in the use of insolubilized enzymes, affinity chromatography, and immunoadsorbents have accelerated very rapidly since 1970,

and the use of polysaccharides and their derivatives as insolubilizing matrices continues to be popular (Chapter 8, Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, and Glycolipids). Tables have been introduced to facilitate easy reference to both enzymes and carbohydrate-containing macromolecules derivatized by insolubilization and to the use of Sepharose cyclic imidocarbonate as a matrix for insolubilization.

The naming of organisms has continued to present problems, particularly in view of the constant re-naming and re-classification advocated in the interests of taxonomy and systematization. In this Report, the author's designation of the organism has been used, but readers are advised to look for references under all possible names for the organism.

2
General Methods

Analysis

A number of methods have been reported for the colorimetric determination of total carbohydrates. The reaction of acid hydrazides and reducing sugars in alkaline solutions with the production of yellow anions has been investigated.[1] Calcium ions and very high protein concentrations were the only interfering factors found when 4-hydroxybenzoic acid hydrazide was used in the detection of D-glucose. The determination of total carbohydrate by the phenol–sulphuric acid procedure has been carried out in the presence of DMSO and guanidine hydrochloride.[2] Automated estimation of total carbohydrate has been achieved by measuring the reducing sugars (from acid hydrolysates) potentiometrically with a redox electrode system.[3] A semi-automated method for the determination of carbohydrate material has used the orcinol–sulphuric acid method after takadiastase digestion of poultry feedstuffs.[4] The fluorescent properties of aldehydes heated with anthrone in sulphuric acid have been used in a microdetermination of glycogen and resulted in a 200-fold increase in sensitivity over colorimetric procedures.[5] Alkaline copper reagents, but not alkaline 3,5-dinitrosalicylate, gave a stoicheiometric relationship with the number of hemiacetal reducing groups of maltodextrins; the use of 3,5-dinitrosalicylate gave erroneously high estimates of α-amylase activity.[6] Three automated alkaline ferricyanide procedures gave reliable means of quantitatively determining the number of reducing groups for maltodextrins and α-(1 → 4)-linked polysaccharides.[7] Changes in the concentration of a non-fluorescent substrate such as 4-methylumbelliferone β-D-glucoside were measured directly by recording the increase in fluorescence as the aglycone was produced, thus establishing a procedure for the determination of β-glucosidase.[8] Gel electrophoresis of proteoglycans and glycosaminoglycans on large-pore composite polyacrylamide–agarose gels, having

[1] M. Lever, *Analyt. Biochem.*, 1972, **47**, 273.
[2] F. R. Dintzis, *Analyt. Biochem.*, 1972, **46**, 108.
[3] G. K. Buckee, *J. Inst. Brewing*, 1972, **78**, 222.
[4] D. W. F. Shannon, *Analyst*, 1972, **97**, 209.
[5] G. R. Morrison, *Analyt. Biochem.*, 1972, **47**, 1.
[6] J. F. Robyt and W. J. Whelan, *Analyt. Biochem.*, 1972, **45**, 510.
[7] J. F. Robyt, R. J. Ackerman, and J. G. King, *Analyt. Biochem.*, 1972, **45**, 517.
[8] R. M. Couture and D. G. Routley, *Phytochemistry*, 1972, **11**, 1947.

average pore diameters sufficient to allow penetration of all but the very largest polymers, has made it possible to distinguish 'aggregates' from disaggregated molecules.[9] The formation of precipitates with DEAE-dextrans and acidic polysaccharides has been used in the detection of acidic polysaccharides after electrophoresis in gels. The converse procedure of using dextran sulphate as a precipitant for basic polysaccharides was also effective.[10] A microcolorimetric method, based on the solubilization of the Toluidine Blue–polyanionic glycosaminoglycan complexes from cellulose acetate membranes, has been used for the quantitative determination of heparin, hyaluronic acid, keratan sulphate, and chondroitin sulphate.[11] By a combination of gel and paper electrophoreses, a simple micro-method was established for the identification of acidic mucopolysaccharides.[12] Cerebrosides have been fractionated by column chromatography of their benzoylated derivatives.[13] By suitable chemical derivatization (involving methanolysis, *N*-acetylation, and trimethylsilylation), a g.l.c. procedure has been reported to be suitable for the quantitation of small amounts of carbohydrate residues in gangliosides.[14] A g.l.c. estimation of carbohydrates in glycosphingolipids has been reported.[15] A modification of the cysteine–sulphuric acid procedure for determining heptose and hexose residues in lipopolysaccharides has given increased sensitivity.[16] An improved quantitative assay has allowed the rapid estimation of deoxy-sugars (characteristic of Gram-negative bacterial lipopolysaccharides) and diaminopimelic acid (characteristic of some bacterial peptidoglycans).[17] An improved method has been reported for the detection of muramic acid in an automated amino-acid analytical procedure.[18] Glycoproteins separated by polyacrylamide gel electrophoresis have been stained with Alcian Blue.[19] The inhibition of peptide hydrolysis by acid mucopolysaccharides and acid glycoproteins has been investigated, and the degree of protectivity afforded was assessed.[20] Products surviving after periodate oxidation and borohydride reduction of glycopeptides and related materials have been separated by g.l.c.[21] Aldoses and uronic acids obtained from the depolymerization of plant cell-wall polysaccharides were separated by ion-exchange chromatography after reduction to the corresponding alditols and aldonic acids. The recovered aldonic acids, after conversion into the alditols *via* the aldono-

[9] C. A. McDevitt and H. Muir, *Analyt. Biochem.*, 1971, **44**, 612.
[10] W. F. Dudman, *Analyt. Biochem.*, 1972, **46**, 668.
[11] D. Hsu, P. Hoffman, and T. A. Mashburn, *Analyt. Biochem.*, 1972, **46**, 156.
[12] C. P. Dietrich and S. M. C. Dietrich, *Analyt. Biochem.*, 1972, **46**, 209.
[13] A. J. Acher and J. N. Kanfer, *J. Lipid Res.*, 1972, **13**, 139.
[14] I. Dijong, P. T. Mora, and R. O. Brady, *Biochemistry*, 1971, **10**, 4039.
[15] C. C. Sweeley and R. V. P. Tao, *Methods Carbohydrate Chem.*, 1972, **6**, 6.
[16] B. G. Wright and P. A. Rebers, *Analyt. Biochem.*, 1972, **49**, 307.
[17] R. W. Hendler and A. H. Burgess, *Analyt. Biochem.*, 1972, **47**, 109.
[18] B. Rosan, *Analyt. Biochem.*, 1972, **48**, 624.
[19] A. H. Wardi and G. A. Michos, *Analyt. Biochem.*, 1972, **49**, 607.
[20] C. André, A. Berard, and R. Lambert, *Compt. rend. Soc. Biol.*, 1970, **164**, 853.
[21] K. W. Hughes and J. R. Clamp, *Biochim. Biophys. Acta*, 1972, **264**, 418.

lactones, were derivatized and examined by g.l.c.[22] The analysis of monosaccharides by g.l.c. of the methyl glycosides as their trifluoroacetate derivatives has been applied to the study of glycoproteins and glycolipids.[23] A rapid, highly sensitive and reproducible method for the separation of monosaccharide mixtures has been achieved using strong ion-exchange resins.[24] An improved method for the automated quantitative analysis of glycoproteins and polysaccharides is based on the ion-exchange separation of the sugar–borate complexes. Estimation of the sugars liberated during acidic hydrolysis was investigated and a variety of methods for neutralizing the hydrolysates was evaluated.[25] The stability of monosaccharides to methanolysis and other factors used in the analyses of glycopeptides and oligosaccharides have been investigated. Several reagents for trimethylsilylation were studied and conditions suitable for the derivatization of monosaccharides were recommended.[26] In a general method developed for the quantitative determination of monosaccharides released by enzymic hydrolysis of glycoproteins and glycopeptides, the sugars were separated from high molecular weight material by ultrafiltration before conversion into derivatives for examination by g.l.c.[27] Improvements in the analysis of carbohydrates by g.l.c. have been reported; these included modification of the conditions of hydrolysis, equilibration of the sugar anomers, and *N*-acetylation of hexosamines.[28] The separation of some pentoses and hexoses has been achieved using capillary-column g.l.c.[29] Lactose, maltose, and sucrose have been estimated in human blood and urine after oral administration.[30] The analyses by g.l.c. of mono- and oligo-saccharides in corn syrup [31] and of small amounts of D-glucose and glycerol in agar culture media [32] have been reported.

The use of an internal support of siliconized glass beads was found to improve the flow rates obtainable with dextran gels without significant alteration of other properties of the gels.[33] An investigation of the use of cross-linked polyacrylamide as a matrix for gel filtration of proteins and glycosaminoglycans has revealed the operation of adsorption phenomena, which could be overcome by using an ionic medium instead of water as eluant.[34] A review on the gel chromatography of carbohydrates has dealt with its application to the separation of monosaccharides, oligosaccharides, and polysaccharides.[35] Stereoselectivity in the elution of

[22] T. M. Jones and P. Albersheim, *Plant Physiol.*, 1972, **49**, 926.
[23] J. P. Zanetta, W. C. Breckenbridge, and G. Vincendon, *J. Chromatog.*, 1972, **69**, 291.
[24] K. Mopper and E. T. Degens, *Analyt. Biochem.*, 1972, **45**, 147.
[25] L. Hough, J. V. S. Jones, and P. Wusteman, *Carbohydrate Res.*, 1972, **21**, 9.
[26] R. E. Chambers and J. R. Clamp, *Biochem. J.*, 1971, **125**, 1009.
[27] R. B. Carlsen and J. G. Pierce, *J. Biol. Chem.*, 1972, **247**, 23.
[28] B. Salfner, *Z. klin. Chem. klin. Biochem.*, 1971, **9**, 486.
[29] K. Tesařík, *J. Chromatog.*, 1972, **65**, 295.
[30] H. Nakamura and Z. Tamura, *Clinica Chim. Acta*, 1972, **39**, 367.
[31] K. M. Brobst, *Methods Carbohydrate Chem.*, 1972, **6**, 3.
[32] F. Calhoun and H. B. Howe, *J. Chromatog. Sci.*, 1972, **10**, 639.
[33] D. H. Sachs and E. Painter, *Science*, 1972, **175**, 781.
[34] J. F. Kennedy, *J. Chromatog.*, 1972, **69**, 325.
[35] S. H. Churms, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 13.

sugars from dextran gels was observed by the decrease in R_g values in going from deoxy- to hydroxy- to methoxy-groups in monosaccharides.[36] The dynamic mixing of column effluent with sulphuric acid, followed by photometry, has been used as the basis of a new sensitive ultraviolet detection system for carbohydrates in which good discrimination from non-carbohydrate material was obtained by measurement at two wavelengths.[37] Corrected, tabulated data for the separation of xylodextrins by gel chromatography have been reported.[38] Mannose-containing oligosaccharides up to mannoheptaose have been separated by gel chromatography; their properties were compared with those of cellodextrins and xylodextrins on t.l.c. and gels.[39] The synthesis of tightly cross-linked cellulose gels in bead form has been described. Chemisorption was found with cellodextrins of chain lengths greater than three units with water as eluant. Weak adsorption was found for xylodextrins and mannodextrins in the comparable size interval. The results suggest that an exact structural correspondence between adsorbent and adsorbate is necessary for the adsorption of oligosaccharides on to cellulose.[40]

Free aldopentoses have been recovered in over 90% yield using two t.l.c. methods,[41] and a quantitative analysis of sugars by densitometric inspection of thin-layer chromatograms has been described.[42] Molybdic and phosphotungstic acid complexes of oligosaccharides were shown to have higher R_f values than the corresponding boric acid complexes. Up to fifteen oligosaccharides and monosaccharides were separated on a one-dimensional chromatogram.[43] Fructo-oligosaccharides up to DP21 could be separated by t.l.c. on Kieselguhr.[44] A system involving the use of continuous elution on pre-coated silica gel plates has been developed for the separation of kestoses.[45] The advantages of using butanol in the production of cellulose thin layers have been discussed.[46] The separation of sugars on polyamide layers has been shown to depend on the number of and the distance between the peptide groups in the macromolecular matrix. It was possible to compare and determine the kind of polyamide used from the R_f values of the sugars.[47] Detailed information on the qualitative,[48] quantitative,[49] and preparative [50] techniques of t.l.c. have been reported.

[36] H. R. Bertoniere, L. F. Martin, and S. P. Rowland, *Carbohydrate Res.*, 1971, **19**, 189.
[37] S. Katz and L. H. Thacker, *J. Chromatog.*, 1972, **64**, 247.
[38] W. Brown and Ö. Andersson, *J. Chromatog.*, 1972, **67**, 162.
[39] W. Brown and K. Chitumbo, *J. Chromatog.*, 1972, **66**, 370.
[40] K. Chitumbo and W. Brown, *J. Polymer Sci.*, 1971, 279.
[41] D. J. Bell and M. Q. K. Talukder, *Clinica Chim. Acta*, 1972, **40**, 13.
[42] B. L. Welch and N. E. Martin, *J. Chromatog.*, 1972, **72**, 359.
[43] T. Mezzetti, M. Lato, S. Rufini, and G. Ciuffini, *J. Chromatog.*, 1971, **63**, 329.
[44] F. W. Collins and K. R. Chandorkar, *J. Chromatog.*, 1971, **56**, 163.
[45] K. J. Schäffler and P. G. Morel du Boil, *J. Chromatog.*, 1972, **72**, 212.
[46] E. Marklová and I. M. Hais, *J. Chromatog.*, 1971, **63**, 446.
[47] I. Grafe and H. Englehardt, *Chromatographia*, 1972, **5**, 307.
[48] R. E. Wing and J. N. BeMiller, *Methods Carbohydrate Chem.*, 1972, **6**, 42.
[49] R. E. Wing and J. N. BeMiller, *Methods Carbohydrate Chem.*, 1972, **6**, 54.
[50] R. E. Wing and J. N. BeMiller, *Methods Carbohydrate Chem.*, 1972, **6**, 60.

Mono- and oligo-saccharides have been separated by partition chromatography on ion-exchange resins.[51]

A direct spectrophotometric assay of D-glucuronic acid in the presence of labile D-glucosiduronic acids has been based on a colorimetric reaction of D-glucuronic acid with benzidine. The method has been applied to the estimation of D-glucuronic acid in enzyme digests.[52] Direct measurement of 4-nitrophenyl D-glucuronide, rather than the disappearance of 4-nitrophenol, has been used in the study of glucuronidation reactions.[53] The transformation of D-glucaro-1,4-lactone into D-glucaric acid and D-glucaro-6,3-lactone under physiological conditions has been studied using g.l.c.[54] Thorin [1-(*O*-arsenophenylazo)-2-naphthol-3,6-disulphonic acid] has been used as a surface adsorption indicator for the barium salts of sugar sulphates. It does not interact chemically with the substrate and, once the sugar has been located, the indicator can be removed.[55]

The TMS derivatives of synthetic and naturally occurring D-glucosyl derivatives of amino-acids, 3-*O*-β-D-xylopyranosyl-L-serine, 3-*O*-α-L-arabinopyranosyl-L-serine, 5-*O*-β-D-galactopyranosyl-DL-lysine and 2-acetamido-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine have been separated by g.l.c. The paper chromatographic and electrophoretic mobilities of these glycosyl derivatives of amino-acids were also reported.[56] The ion-exchange chromatographic separation of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose has been reported using a scaled-down Elson–Morgan procedure and a scintillation counting system.[57] A procedure for the simultaneous *N*-acetylation and *O*-trimethylsilylation of these hexosamines,[58] and their separation as TMS derivatives,[59] has been described.

An automated fluorometric analysis for micromolar quantities of D-glucose has been based on its enzymic conversion into D-gluconate 6-phosphate by hexokinase, glucose 6-phosphate dehydrogenase, ATP, and NADP.[60] A similar procedure has been used for the determination of glycogen after its quantitative conversion into D-glucose with amylo-α-1,4-α-1,6-glucosidase.[61] An enzymic determination of D-glucose in haemolysed blood samples has been achieved, without interference by D-fructose, by elimination of phosphoglucose isomerase, an enzyme occurring in erythrocytes.[62] Three commercially available purified enzymes (hexo-

[51] O. Samuelson, *Methods Carbohydrate Chem.*, 1972, **6**, 65.
[52] J. Tomasic and D. Keglevic, *Analyt. Biochem.*, 1972, **45**, 164.
[53] A. R. Temple, A. K. Done, M. S. Clement, and A. J. Peart, *J. Lab. Clin. Medicine*, 1971, **77**, 1015.
[54] I. Matsunaga and Z. Tamura, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 284.
[55] W. M. Pasika and A. C. West, *J. Chromatog.*, 1971, **63**, 357.
[56] G. W. Jourdian and J. J. Distler, *Carbohydrate Res.*, 1972, **22**, 369.
[57] S. Lohmander, *Biochim. Biophys. Acta*, 1972, **264**, 411.
[58] S. Hara and Y. Matsushima, *J. Biochem.*, 1972, **71**, 907.
[59] W. H. Stimson, *F.E.B.S. Letters*, 1971, **13**, 17.
[60] H. J. Leese and J. R. Bronk, *Analyt. Biochem.*, 1972, **45**, 211.
[61] S. R. Nahorski and K. J. Rogers, *Analyt. Biochem.*, 1972, **49**, 492.
[62] F. Dafonseca-Wollheim, *Z. klin. Chem. klin. Biochem.*, 1971, **9**, 497.

kinase, glucose 6-phosphate dehydrogenase, and gluconate 6-phosphate dehydrogenase) have been used to convert D-glucose into D-ribulose 5-phosphate and carbon dioxide. A spectrophotometric method is based on the measurement of D-gluconate 6-phosphate for the non-radioactive determination of D-glucose, and on the measurement of $^{14}CO_2$ for the radioactive determination of D-[1-^{14}C]glucose.[63] The technique has been adapted for the measurement of D-[U-^{14}C]glucose, D-glucose 6-phosphate, D-gluconate 6-phosphate, and D-gluconate in plasma.[64] A critical appraisal of the specific estimation of D-glucose using glucose oxidase has examined the potential errors due to temperature dependence, pH behaviour of tris buffers, and the presence of high concentrations of buffer salts, glycerol, and related substances.[65] Two sulphonylureas, tolazamide and tolbutamide, interfered in estimation of blood glucose by the glucose oxidase–*o*-dianisidine and glucose oxidase–PERID methods. Tolazamide gave falsely low results unless the sample was deproteinized, whereas tolbutamide gave increased levels.[66] Improved colour reagents for the estimation of D-glucose by the glucose oxidase system have been proposed using either potassium hexacyanoferrate(II)[67] or 4-aminophenazone and sulphonated 2,4-dichlorophenol[68] in place of *o*-dianisidine. An assessment has been made of a commercially available analyser for the determination of D-glucose by the glucose oxidase system, which involves the measurement of oxygen by an oxygen electrode.[69] Polarographic measurements have been made of the rates of oxygen consumption, due to enzymic oxidation with β-D-glucose oxidase, of the β-D-glucose formed from α-D-glucose in the absence and presence of mutarotase.[70] L-Arabinose and D-galactose have been determined quantitatively using an NAD-dependent galactose dehydrogenase from *Pseudomonas fluorescens*. Since D-galactose is converted into D-galactose 1-phosphate by phosphorylation with ATP and galactokinase from *Saccharomyces fragilis*, and this product does not react with galactose dehydrogenase, the two aldoses could be determined together.[71] A simple ultramicro-method for the determination of serum xylose has been developed based on the *o*-toluidine reaction after removal of D-glucose from the serum with glucose oxidase.[72] Lactose has been determined, after hydrolysis with β-galactosidase, by measurement of the liberated D-glucose by the hexokinase method.[73, 74] Sugars in foodstuffs

[63] J. Costello, J. M. Scott, and E. Bourke, *Analyt. Biochem.*, 1972, **46**, 654.
[64] J. Costello, J. M. Scott, and E. Bourke, *Biochem. J.*, 1971, **125**, 114P.
[65] W. Banks and C. T. Greenwood, *Stärke*, 1971, **23**, 222.
[66] P. Sharp, C. Riley, J. G. H. Cook, and P. J. F. Pink, *Clinica Chim. Acta*, 1972, **36**, 93.
[67] H. Müller, *Stärke*, 1971, **23**, 314.
[68] D. Barham and P. Trinder, *Analyst*, 1972, **97**, 142.
[69] B. Morrison, C. J. Scotland, and A. Fleck, *Clinica Chim. Acta*, 1972, **39**, 301.
[70] I. Miwa, *Analyt. Biochem.*, 1972, **45**, 441.
[71] K. Möhler and W. Schmolck, *Z. Lebensm.-Untersuch.*, 1971, **146**, 319.
[72] M. B. Smith and J. L. Braidwood, *Clinical Biochem.*, 1971, **4**, 118.
[73] R. K. Bahl, *Analyst*, 1972, **97**, 559.
[74] R. K. Bahl, *Analyst*, 1972, **97**, 213.

have been determined with a continuous flow technique using a redox detector.[75]

The iodate produced on oxidation of carbohydrates with periodate ions has been converted into the tri-iodide and measured spectrophotometrically.[76] Methods for the quantitative removal of iodate and periodate ions by ion-exchange chromatography have been described in connection with the direct spectrophotometric determination of iodate.[77] Rapid and accurate determinations of periodate and formaldehyde have been obtained from polarographic measurements.[78] Spectrophotometric methods for the determination of formic acid arising from the periodate oxidation of carbohydrates have been based on reactions with 2-thiobarbituric acid and formate : tetrafolate ligase.[79]

Conditions for obtaining accurate and reproducible assays of *O*-ethyl and *O*-propyl substituents in polysaccharide derivatives have been developed.[80] Normal carbonyl compounds have been determined spectrophotometrically in the presence of carbohydrates, without prior separation, using 2,4-dinitrophenylhydrazine. The method exploited a technique that suppresses the formation of the carbohydrate 2,4-dinitrophenylhydrazone.[81] Sodium cyanide has been used in a method to determine the total carbonyl contents of starch and cellulose.[82] Oximation followed by oxime hydrolysis and chlorous acid–sodium borohydride treatments have been described for the determination of carbonyl groups in oxidized cellulose.[83]

Structural Methods

A new parameter has been presented for the conformational analysis of polysaccharide chains in solution, which involves a comparison of o.r.d. measurements in water with those made in urea, guanidine hydrochloride, and acidic and basic solutions.[84] An iterative procedure for fixing the minimum energy position of substituents in simple monosaccharides and polysaccharides converged to the same answer independently of the starting position of the side-group. The method proved useful in conformational studies of complex heterosaccharides and 2-acetamido-2-deoxy-sugars.[85]

Details have been reported of the chemical shifts of anomeric protons from ^{1}H n.m.r. spectra of methyl ethers of disaccharides.[86] Measurements of δ-values and coupling constants of the anomeric protons from the

[75] D. G. Porter and R. Sawyer, *Analyst*, 1972, **97**, 569.
[76] S. A. Barker, P. V. Peplow, and P. J. Somers, *Carbohydrate Res.*, 1972, **22**, 201.
[77] J. X. Khym, *Methods Carbohydrate Chem.*, 1972, **6**, 87.
[78] R. D. Corlett, W. G. Breck, and G. W. Hay, *Canad. J. Chem.*, 1970, **48**, 2474.
[79] J. F. Kennedy, *Methods Carbohydrate Chem.*, 1972, **6**, 93.
[80] A. N. de Belder, A. Persson, and S. Makstrom, *Stärke*, 1972, **24**, 361.
[81] E. B. Sanders and J. Schubert, *Analyt. Chem.*, 1971, **43**, 59.
[82] M. Lewin, *Methods Carbohydrate Chem.*, 1972, **6**, 76.
[83] M. Pašteka, *Methods Carbohydrate Chem.*, 1972, **6**, 80.
[84] S. Hirano, *Life Sci. II*, 1971, **10**, 151.
[85] P. R. Sundararajan and R. H. Marchessault, *Biopolymers*, 1972, **11**, 829.
[86] D. E. Minnikin, *Carbohydrate Res.*, 1972, **23**, 139.

^{1}H n.m.r. spectra of TMS derivatives of oligosaccharides have been used to determine the configuration of the glycosidic bonds.[87] A number of fully acetylated glycosides, oligosaccharide alditols, and polysaccharides have been treated with chromium trioxide in acetic acid. Pyranosidic pentose and hexose residues in which the aglyone occupied an equatorial position were readily oxidized, whereas the corresponding residues in which the aglycone occupied an axial position were resistant to oxidation. Similar results were obtained for carbohydrates containing pyranosidic 6-deoxy- and 2-acetamido-2-deoxy-sugar residues, thereby allowing the anomeric nature of sugar residues in oligosaccharides and polysaccharides to be determined.[88] Hydroxy-acids produced by Smith degradation and bromine oxidation of the aldonic acid termini of oligosaccharides have been separated by g.l.c. in a method used for the linkage analysis of oligosaccharides.[89] A method has been established for the structural analysis of polysaccharides which involves the application in sequence of periodate oxidation, hypoiodite oxidation, and acidic hydrolysis. The resulting products were separated by anion-exchange chromatography.[90] Investigations into the periodate oxidation of polysaccharides have led to the conclusions that inter-residue hemiacetal formation is a general phenomenon and that the relative abundance of inter-residue and intra-residue cyclic structures in any instance is fairly predictable from an already familiar hierarchy of stabilities based on ring-size and -type.[91]

The use of g.l.c.–mass spectrometry (m.s.) as an aid to structural studies has again been the subject of many reports. The biochemical application of mass spectrometry has been reviewed with regard to such monosaccharides as deoxy-sugars, amino-sugars, uronic acids, sugar phosphates, glycosides, and their derivatives.[92] The fragmentation patterns of carbohydrate derivatives have been described for partially methylated monosaccharides and oligosaccharides.[93] Methylated alditol acetates were readily identified by g.l.c.–m.s. when the gas chromatograph was physically separate from the mass spectrometer. Substances that were not separated by g.l.c. were collected from the effluent and resolved by t.l.c. prior to identification by m.s.[94] Computer-aided m.s. identification has been applied to stereoisomeric monosaccharides,[95] and to the TMS derivatives of sorbose, 2-keto-L-gulonic acid, and L-ascorbic acid.[96] M.s. of some partially

[87] J. P. Kamerling, M. J. A. de Bie, and J. F. G. Vliegenthart, *Tetrahedron*, 1972, **28**, 3037.
[88] J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1972, **26**, 661.
[89] H. Yamaguchi and M. Kuriyama, *J. Biochem.*, 1972, **71**, 239.
[90] P. V. Peplow and P. J. Somers, *Carbohydrate Res.*, 1972, **22**, 281.
[91] M. F. Ishak and T. Painter, *Acta Chem. Scand.*, 1971, **25**, 3875.
[92] T. Radford and D. C. De Jongh, in 'Biochemical Applications of Mass Spectroscopy', ed. G. R. Waller, Wiley–Interscience, New York, 1972, p. 313.
[93] N. K. Kochetkov and O. S. Chizhov, *Methods Carbohydrate Chem.*, 1972, **6**, 540.
[94] Y. M. Choy, G. G. S. Dutton, K. B. Gibney, S. Kabir, and J. N. C. Whyte, *J. Chromatog.*, 1972, **72**, 13.
[95] J. Vink, J. H. W. B. Slot, J. J. de Ridder, J. P. Kamerling, and J. F. G. Vliegenthart, *J. Amer. Chem. Soc.*, 1972, **94**, 2542.
[96] H. G. J. de Wilt, *J. Chromatog.*, 1971, **63**, 379.

methylated 2- and 3-deoxyalditol acetates were studied using deuterium-labelling techniques. As a result of these studies, the origins of the primary fragments have been established.[97] Further detailed mechanisms for the formation of secondary fragments obtained on m.s. of certain partially methylated alditol acetates were studied using the same techniques.[98] The fragmentation of methyl *O*-methyl-D-xylofuranosides under the conditions of electron-impact m.s. was studied and compared with that of fully methylated methyl pentofuranosides. Characteristic differences in the fragmentations of variously methylated methyl D-xylofuranosides permitted unambiguous assignment of both the number and location of the methyl groups.[99] The m.s. fragmentation patterns of methylated pseudoaldobiouronic acids have been reported.[100] The fragmentation patterns of the TMS derivatives of disaccharides of the type aldohexosyl-(1 → *x*)-fructose have been compared and could be divided into two main groups, *viz.* the (1 → 1)- and (1 → 2)-linked disaccharides and the (1 → 3)-, (1 → 4)-, (1 → 5)-, and (1 → 6)-linked disaccharides. Further differentiation was possible within both groups.[101] TMS derivatives of homogeneous cerebrosides were used for m.s. studies of long-chain bases and fatty acids and sugar components.[102] The technique of chemical-ionization m.s. using ammonia reagent gas has provided a ready means of determining the molecular weights of pentoses, hexoses, simple glycosides of monosaccharides, *O*-acetyl derivatives of monosaccharides, and disaccharides. The use of methane as reagent gas with *O*-acetylated sugars allowed the position of substitution on the sugar ring to be determined.[103]

The separation by g.l.c. of tetra-*O*-methyl ethers of D-glucose, D-galactose, and D-mannose, and other sugars as acetates has been reported.[104] Details of procedures for the preparation and g.l.c. separation of alditol acetates [105] and methylated sugars [106] have been published.

Lead tetra-acetate has been used for the oxidation of polysaccharides in DMSO on both analytical and preparative scales.[107]

The uses of concanavalin A for structural studies have been summarized.[108] The specificity of five anti-2-acetamido-2-deoxy-D-galactose lectins from seeds has been investigated. Dissimilarities with respect to the

[97] H. B. Borén, P. J. Garegg, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1971, **25**, 3299.
[98] K. Axberg, H. Björndal, Å. Pilotti, and S. Svensson, *Acta Chem. Scand.*, 1972, **26**, 1319.
[99] V. Kováčik and P. Kováč, *Carbohydrate Res.*, 1972, **24**, 23.
[100] G. C. Kuenzle, *Carbohydrate Res.*, 1972, **24**, 169.
[101] J. P. Kamerling, J. F. G. Vliegenthart, J. Vink, and J. J. de Ridder, *Tetrahedron*, 1972, **28**, 4375.
[102] K. A. Karlsson, I. Pascher, B. E. Samuelsson, and G. O. Steen, *Chem. and Phys. Lipids*, 1972, **9**, 230.
[103] A. M. Hogg and N. L. Nagabhushan, *Tetrahedron Letters*, 1972, 4827.
[104] G. G. S. Dutton and R. H. Walker, *Carbohydrate Res.*, 1972, **23**, 430.
[105] J. H. Sloneker, *Methods Carbohydrate Chem.*, 1972, **6**, 21.
[106] H. G. Jones, *Methods Carbohydrate Chem.*, 1972, **6**, 25.
[107] C. T. Bishop, *Methods Carbohydrate Chem.*, 1972, **6**, 350.
[108] I. J. Goldstein, *Methods Carbohydrate Chem.*, 1972, **6**, 106.

combining sites were elucidated and a comparison with other heterophilic 2-acetamido-2-deoxy-D-galactose specific agglutinins was presented.[109] Quantitative precipitin and inhibition studies using a protein in shark serum, which reacts specifically with fructosans, demonstrated that the combining site reacts specifically with β-D-fructofuranosyl-(2 → 1)-β-D-fructofuranosyl and β-D-fructofuranosyl-(2 → 6)-α-D-glucopyranosyl residues of fructosans, levans, and inulin.[110]

Alkaline sodium borohydride has been used to cleave the glycosidic linkage of oligosaccharide chains attached to L-serine and L-threonine residues, and resulted in reduction of the terminal 2-acetamido-2-deoxy-D-galactose residue with minimum degradation. The method proved useful in the isolation of complete oligosaccharides from blood-group substances.[111]

[109] G. Uhlenbruck and W. Dahr, *Vox Sanguinis*, 1971, **21**, 338.
[110] W. Harisdangkul, E. A. Kabat, R. J. McDonough, and M. M. Sigel, *J. Immunol.*, 1972, **108**, 1244.
[111] B. Anderson, L. Rovis, and E. A. Kabat, *Arch. Biochem. Biophys.*, 1972, **148**, 304.

3
Plant and Algal Polysaccharides

Starch

A number of specific methods have been developed for the analysis of starch. The amylose content in rice has been determined using an iodine method on the alkali-dispersed flour.[112] Very small amounts of starch in leaf tissue have been estimated by measuring the D-glucose liberated after digestion with amyloglucosidase; the results agreed closely with those obtained by the specific iodine precipitation method.[113] A polarimetric determination of starch solubilized in DMSO gave results in good agreement with those using extraction with calcium chloride.[114] The estimation of starch using glucamylase has been described.[115]

In the fractionation of amylomaize starch, a method has been developed for the isolation of anomalous polysaccharide material that did not conform to the classical definitions of either amylose or amylopectin.[116] The amylose and amylase contents of triticale and its parent species have been reported.[117] Amylose of high purity and high molecular weight has been isolated and characterized from starch granules dispersed with urea.[118] The self-hydrolysis of starch at temperatures below the starting point of irreversible swelling has been shown to lead to the formation of soluble, degraded products of low molecular weight containing bound phosphoric acid.[119]

From the yields of D-glucose 3-phosphate and D-glucose 6-phosphate isolated from partial acid hydrolysates of potato starch, the distribution of the phosphate ester was estimated to be 38% on C-3 and 61% on C-6 of the D-glucose residues.[120] Studies on the oxidation of starch by Fenton's reagent indicated that incomplete oxidation to carbon dioxide had occurred.[121] Small starch granules from barley were shown to have a higher amylose content and a higher gelatinization temperature than large

[112] C. M. Sowbhagya and K. R. Bhattacharya, *Stärke*, 1971, **23**, 53.
[113] J. C. MacRae, *Planta*, 1971, **96**, 101.
[114] W. J. Garcia and M. J. Wolf, *Cereal Chem.*, 1972, **41**, 298.
[115] P. Thivend, C. Mercier, and A. Guilbot, *Methods Carbohydrate Chem.*, 1972, **6**, 100.
[116] W. Banks, C. T. Greenwood, and D. D. Muir, *Stärke*, 1971, **23**, 199.
[117] A. J. Klassen and R. D. Hill, *Cereal Chem.*, 1971, **48**, 647.
[118] N. B. Patil and N. R. Kale, *Biochem. J.*, 1972, **128**, 61P.
[119] M. Palasinski and F. Schierbaum, *Stärke*, 1971, **23**, 383.
[120] S. Tabata and S. Hizukuri, *Stärke*, 1971, **23**, 267.
[121] D. L. Ingles, *Austral. J. Chem.*, 1972, **25**, 105.

granules and were usually associated with more protein. In contrast, large and small granules from wheat differ only slightly in their respective amylose contents. It was proposed that starch synthesis in small granules of barley is under different genetic control from that in large granules.[122]

A comparison of the distribution of linear α-(1 → 4)-linked unit chains of amylopectin and its β-limit dextrin has indicated that the 'B chains' in the original polysaccharide consist of two components, the longer chains having a DP 40—80 and the shorter chains having a DP 20—40.[123] Purified limit dextrinase had no effect on amylopectin, but readily hydrolysed α-limit dextrins.[124] It has been demonstrated that Q enzyme (α-1,4-glucan-α-1,4-glucan 6-glucosyltransferase) introduces additional branch points into amylopectin with the formation of significant proportions of malto-hexosyl side-chains.[125, 125a]

The properties of amylose and amylopectin from barley starches showing a wide variation in viscosity curves have been studied. Evidence has been provided that a low β-limit on one amylopectin is due to a block in the action of β-amylase.[126] The hydrolysis of soluble starch by rat and human α-amylases has been characterized as a biphasic reaction. Hydrolysis by rat pancreatic α-amylase was characterized by a first phase, corresponding to the rapid hydrolysis of starch to maltodextrins greater than maltodecaose, followed abruptly by a second phase during which slow hydrolysis of these oligosaccharides to the maltopentaose–maltose range occurred.[127]

Of two separable amylose components, one component was degraded to a higher dextrin together with an intermediate product during sodium persulphate oxidation of potato starch.[128] A colorimetric method for the determination of aldehyde groups by a formazan reaction has been found to characterize oxidized starches.[129] Monosaccharides, deoxy-compounds, sugar anhydrides, furan, and probably pyran compounds have been identified in a study of the low molecular weight degradation products isolated from irradiated starch.[130]

Light-scattering and ultracentrifugal experiments on amylopectin have shown that the large size of the macromolecule is real and not due to aggregation resulting from hydrogen bonding. The difference in hydrodynamic behaviour of amylopectin and glycogen was shown to be due to

[122] G. N. Bathgate and G. H. Palmer, *Stärke*, 1972, **24**, 336.
[123] H. Akai, K. Yokobayashi, A. Misaki, and T. Harada, *Biochim. Biophys. Acta*, 1971, **252**, 427.
[124] D. J. Manners and K. L. Rowe, *J. Inst. Brewing*, 1971, **77**, 358.
[125] G. S. Drummond, E. E. Smith, and W. J. Whelan, *European J. Biochem.*, 1972, **26**, 168.
[125a] G. S. Drummond, E. E. Smith, and W. J. Whelan, *European J. Biochem.*, 1972, **26**, 594.
[126] B. W. DeHaas and K. J. Goering, *Stärke*, 1972, **24**, 145.
[127] A. Vandermeers, M.-C. Vandermeers-Piket, and J. Christophe, *Biochemie*, 1971, **53**, 859.
[128] J. Seidemann, *Stärke*, 1972, **24**, 128.
[129] V. Prey and S. Siklossy, *Stärke*, 1972, **23**, 235.
[130] H. Scherz, *Stärke*, 1972, **23**, 259.

the much more flexible coil-like nature of amylopectin.[131] Experimental results have not supported the theoretical predictions that amylose has a pronounced helical character in aqueous solutions,[132, 133] thus differing from those nucleic acids and proteins possessing a helical structure in the crystalline state which is retained in solution.[134] A worm-like helical chain has been proposed as the most adequate model for amylose on the bases of o.r.d. and c.d. studies.[135] The relationship between the conformation of the D-glucopyranose units and the molecular configuration of amylose has been studied. Under conditions which are favourable to the twisting of the planes of two consecutive D-glucose rings, hydrogen bonds are stabilized and the homologues maltose and amylose achieve a helical molecular configuration.[136] The value of heat changes in the dehydration of starch was shown to be consistent with the rupture of two hydrogen bonds for the removal of each water molecule; it was suggested that each water molecule is hydrogen-bonded between adjacent grains or between two sites on the same coiled chain.[137] Changes were observed in the molecular volume of amylose as potassium chloride was added to a solution of the polysaccharide at pH 12, giving a minimum value of viscosity number identical with that observed for the addition of butan-1-ol and iodine to neutral, aqueous solutions of amylose; the results suggest that amylose undergoes a coil-to-helix transition in alkaline salt solution.[138] Binding parameters have been examined for the complexes formed between potato starch and alcohols, fatty acids, menthone, menthol, and β-pinene. The binding constants and stoicheiometry of the complexes were found to be related to the molecular structure of the ligands. Binding was by inclusion and, hence, governed by helix formation or by interactions with the surface of the helix.[139] The results of sedimentation and viscosity studies on amylose in ethylenediamine have shown that the polymer has a spherical shape in solution.[140]

Amylose–iodine complexes crystallized from dilute solutions in single, rod-like molecules of 40 Å diameter and had a fibrillar structure, provided the iodine concentration was sufficient for complete filling of the amylose helices. A model was proposed in which the molecules have folded chains oriented parallel to the fibril axes.[141] Amylose–iodine complexes in the presence of such complexing agents as DMSO, butan-1-ol, or cyclohexanol were shown to have acquired resistance to hydrolysis by α- and β-amylase,

[131] W. Banks, R. Geddes, C. T. Greenwood, and I. G. Jones, *Stärke*, 1972, **24**, 245.
[132] W. Banks and C. T. Greenwood, *Biopolymers*, 1972, **11**, 315.
[133] W. Banks and C. T. Greenwood, *Stärke*, 1971, **23**, 300.
[134] W. Banks and C. T. Greenwood, *Polymer*, 1971, **12**, 141.
[135] B. Pfannemuller, H. Mayerhofer, and R. C. Schulz, *Biopolymers*, 1971, **10**, 243.
[136] J. Szejtli, *Stärke*, 1971, **23**, 295.
[137] R. Collison and A. Dickson, *Stärke*, 1971, **23**, 45.
[138] W. Banks and C. T. Greenwood, *Carbohydrate Res.*, 1972, **21**, 229.
[139] F. Osman-Ismail and J. Solms, *Stärke*, 1972, **7**, 213.
[140] F. R. Dintzis, G. E. Babcock, and R. Tobin, *Carbohydrate Res.*, 1971, **17**, 216.
[141] H. Bittiger, E. Husemann, and B. Pfannemuller, *Stärke*, 1971, **23**, 113.

and to exhibit absorption differences, which appeared to be due to ligand-induced conformational changes.[142] The technique of semi-micro differential titration has been applied to starch and its components with the result that increased values for iodine-binding capacities were obtained.[143] Rate studies of the amylose–iodine reaction in unfractionated starch samples of diverse plant origins have shown variations that have been explained in terms of differences in the average molecular weights of the amyloses.[144] The rate of absorption of iodine vapour by aqueous potassium iodide was found to be faster when starch granules were present.[145]

By combination of α-amylase digestion and mild periodate oxidation, it was shown that the organization of the peripheral and mid areas and the central part of the potato starch granule are different.[146] Anisotropic light-scattering studies on tapioca starch indicated that, if layering is present at all, the layer thickness is comparable to the wavelength of incident radiation. Potato starch morphology was characterized by relatively coarse layering, with few layers and considerable differences in the anisotropy between successive layers.[147] The results from similar studies on transverse sections of potato starch indicated the presence of a coarsely layered morphology, including an isotropic region near the centre of the granule.[148] Crystalline regions of native starch granules, retrograded amylose, and amylodextrins were resistant to acidic hydrolysis at room temperature.[149] No retrogradation was observed from light-scattering experiments on amylopectins from various starches.[150] Water bound to starch has been determined from dielectric permeability measurements.[151]

Association of gelatinized wheat starch and wheat protein occurred at acidic and neutral pH, but diminished in alkaline media.[152] Starch isolated from a barley mutant was apparently anomalous with respect to chain length, β-amylolysis limit, and iodine uptake. This was apparently due to heterogeneity in the polysaccharide rather than to an increase in length of the external chains.[153] Starches of the gametophytic and sporophytic generations of pollen amylomaizes, which are homozygous for the amylose extender gene, were found to be of similar type.[154] Sedimentation and counting methods have been used to analyse the particle size of starches.[155] Detailed studies of the polysaccharide content of tea plants immediately

[142] S. V. Bhide and N. R. Kale, *Biochem., J.* 1972, **128**, 60P.
[143] W. Banks, C. T. Greenwood, and D. D. Muir, *Stärke*, 1971, **23**, 119.
[144] E. Hamori and M. C. Kallay, *Biopolymers*, 1972, **11**, 475.
[145] R. S. Freeman, G. T. Tsao, and E. B. Lancaster, *Stärke*, 1971, **23**, 3.
[146] D. Gallant, C. Mercier, and A. Guilbot, *Cereal Chem.*, 1972, **49**, 354.
[147] R. S. Finkelstein and A. Sarko, *Biopolymers*, 1972, **11**, 881.
[148] R. S. Finkelstein and A. Sarko, *Carbohydrate Res.*, 1972, **23**, 31.
[149] K. Kainuma and D. French, *Biopolymers*, 1971, **10**, 1673.
[150] C. T. Greenwood and D. I. Hourston, *Stärke*, 1971, **23**, 344.
[151] P. K. Kobushkin, Y. N. Yudin, and A. M. Krasnitskaya, *Biofizika*, 1972, **17**, 92.
[152] L. K. Dahle, *Cereal Chem.*, 1971, **48**, 706.
[153] W. Banks, C. T. Greenwood, and J. T. Walker, *Stärke*, 1971, **23**, 12.
[154] W. Banks, C. T. Greenwood, D. D. Muir, and J. T. Walker, *Stärke*, 1971, **23**, 380.
[155] V. Rasper, *J. Sci. Food Agric.*, 1971, **22**, 572.

after pruning and during recovery suggested that starch is the main reserve polysaccharide.[156]

In non-aqueous environments, such as olive oil and liquid paraffin, starch synthesis was reported to occur in the absence of any external sugar supply in non-growing lemon fruit cells. The non-aqueous media provided a physiological situation that induced the quiescent cells to utilize their endogenous sugar for starch production.[157] Incorporation of radioactivity from 2-amino-2-deoxy-D-[1-^{14}C]glucose into the starch of *Lemna minor* has been reported, suggesting that the amino-sugar could be deaminated and converted into hexose phosphates under certain conditions.[158] The amylose content and the activities of starch-synthesizing enzymes decreased in cultures of the alga *Polytoma uvella*, but remained unchanged in potatoes maintained at different temperatures.[159] Red light has been reported to stimulate the accumulation of sucrose and starch in the leaves of *Vicia faba* plants owing to extra ATP production in cyclic phosphorylation.[160] The biosynthesis of starch from sugar nucleotides has been reviewed.[161]

Cellulose

Cellulose and cellulose derivatives have been the subject of an authoritative work, which has concentrated on studies of cellulose structure, including chapters on spectroscopic methods, enzymic synthesis, and formation of the microfibril.[162] Information on the degradation of cellulose by acid hydrolysis, alcoholysis, thermolysis, photolysis, and enzymolysis, together with technicological developments in cellulose production, has also been compiled.[163] The morphology and biosynthesis of cellulose and plant cell walls have been reviewed.[164]

The fine structure of highly crystalline cellulose microfibrils from the cell walls of the marine alga *Valonia ventricosa* has been investigated by observation of deformed and undeformed specimens in the electron microscope. On treatment with ultrasonic vibrations, the cell wall broke down into long microfibrils.[165] The laser-induced fluorescence of cellulosic materials has been attributed to trace amounts of transition-metal ions remaining after normal preparative procedures.[166] Evidence has been produced for the occurrence of flexible sugar-ring conformers in cellulosic

[156] R. R. Selvendran and S. Selvendran, *Phytochemistry*, 1972, **11**, 3167.
[157] H. A. Kordan, *Phytochemistry*, 1972, **11**, 2743.
[158] R. M. Roberts, A. B. Connor, and J. J. Cetorelli, *Biochem. J.*, 1971, **125**, 999.
[159] B. S. Mangat and N. P. Badenhuizen, *Canad. J. Botany*, 1971, **49**, 1787.
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[161] H. N. Nikaido and W. Z. Hassid, *Adv. Carbohydrate Chem. Biochem.*, 1971, **26**, 351.
[162] N. M. Bikales and L. Segal, 'Cellulose and Cellulose Derivatives', Wiley, New York, 1971, Part IV.
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[164] F. Shafizadeh and G. D. McGinnis, *Adv. Carbohydrate Chem. Biochem.*, 1971, **26**, 297.
[165] K. H. Gardner and J. Blackwell, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 327.
[166] R. H. Atalla and S. C. Nagel, *J.C.S. Chem. Comm.*, 1972, 1049.

chains.[167] The state of sorbed water on celluloses derived from cotton and woodpulp sources has been studied using pulsed n.m.r. techniques; the molecular motion of sorbed water molecules was found to depend on the physical state of the cellulose, particularly on the degree of crystallinity.[168] Values of the ratio of unperturbed to free rotation root-mean-square end-to-end distance (σ) have been calculated for chains composed of the unsolvated disaccharide repeating units of cellulose. Bulky side-groups attached near the glycosidic carbon atom could increase or decrease the unperturbed dimensions, and σ was increased with both the assumed atomic radii and θ, the supplement of the glycosidic bond angle.[169] A hypothesis has been developed for the formation of elementary fibrils of native cellulose with metastable structures.[170] The morphological transition of cellulose during mercerization has been examined with reference to a folding chain model. It was envisaged that the transition is a result of a partially restricted annealing of the cellulose crystals under the action of a swelling agent. Thus, low-temperature annealing of cellulose shortened the fold length of the molecule, whereas high-temperature annealing generated at least a partial reversal of mercerization.[171] Information has been presented to show that the total volume of micropores in cellulose fibres swollen in acetic acid correlated closely with acetylation reactivity. The relationship between cellulose structure and reactivity could be extended to chemical reactions other than acetylation.[172] The conformational terminology for crystalline cellulose has been discussed in relation to the 'bent chain' model of Hermans. For a given geometry of the D-glucose residues, the 'straight chain' corresponded to a unique value for the glycosidic angle (τ). When the value of τ exceeds this unique value, two 'bent chains' are possible and it was suggested that only the one allowing an intramolecular hydrogen bond (O-3$\cdots$O-5′) should be referred to as the Hermans conformation.[173] Eastern spruce cellulose has been examined by a solid-state light-scattering method that allowed a study of the scattering due to fluctuations in density and in anisotropy. This method has given information on the overall distribution of microfibrillar orientation and the void content in the cells.[174] Theoretical expressions of the intensity of light scattered by a continuous helix have been derived and found to be in fair agreement with the results obtained from solid-state light-scattering for a section of eastern spruce wood. A method was outlined for estimating the 'pitch' and 'tilt' of the helical segments.[175] Solid-state light-scattering

[167] D. A. Brant and K. D. Goebel, *Macromolecules*, 1972, **5**, 536.
[168] T. F. Child, *Polymer*, 1972, **13**, 259.
[169] R. L. Cleland, *Biopolymers*, 1971, **10**, 1925.
[170] V. E. Stockman, *Biopolymers*, 1972, **11**, 251.
[171] M. Chang, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 343.
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[173] P. R. Sundararajan and R. H. Marchessault, *Canad. J. Chem.*, 1972, **50**, 792.
[174] J. Borch, P. R. Sundararajan, and R. H. Marchessault, *J. Polymer Sci., Part A-2, Polymer Phys.*, 1971, **9**, 313.
[175] P. R. Sundararajan and R. H. Marchessault, *J. Mol. Biol.*, 1972, **63**, 305.

patterns from single wood fibres have been recorded. Comparison of relative intensities led to the conclusion that cellulose of the wood fibre behaves optically as a biaxial crystal in that the cellulose crystallites are biaxially orientated in an axial-tangentoplanar fashion in the cell wall.[176] Unperturbed dimensions have been calculated for several (1 → 3)- and (1 → 4)-linked homopolysaccharides. The characteristic ratio depended strongly on the bonding geometry and on the degree of rotational freedom about the glycosidic bonds. The unperturbed dimensions could be predicted qualitatively from a knowledge of the regular conformation of the polysaccharide.[177]

The thermal analysis of hardwood reflected the pyrolysis of its main components, including cellulose and xylan. From kinetic data, including the rates and energies of activation of the pyrolytic processes, it was concluded that cleavage of the glycosidic bond is directly influenced by variation of its electron density and that it constitutes the rate-determining step in pyrolysis of the carbohydrate compounds.[178] The exposure of cotton cellulose to hydrochloric acid, chlorine, and u.v. radiation in carbon tetrachloride led to the formation of metastable free macroradicals, which underwent recombination in the presence of water.[179] A postulation of the energetic non-equivalence of the glycosidic bonds of cellulose has led to the development of a linear equation for the decreased rate of depolymerization during acid hydrolysis. Hydrolysis proceeded by protonation of the glycosidic cellulose bond and this was followed by the rate-controlling hydrolysis of the protonated species.[180] The equivalent weights of celluloses have been determined by addition of hydrogen cyanide to the terminal carbonyl group, followed by hydrolysis of the cyanohydrin and estimation of the liberated ammonia.[181]

The kinetics of oxidative alkaline degradation of the end-group stabilized cellulose models cellobiitol, cellobionic acid, and methyl β-D-glucoside have been reported. The order of alkaline stability was shown to be α-hydroxy-acetals > α-hydroxy-primary alcohols > hydroxy-carboxylic acids ≫ α-hydroxy-hemiacetals (methyl β-D-glucoside) > cellobiitol > cellobionic acid ≫ cellobiose.[182] A study of the soluble monocarboxylic acids formed after oxygen–alkali treatment of hydrocellulose, which was carried out under such conditions that no appreciable drop in viscosity occurred, revealed that the major part of the added alkali was consumed in formation of glucoisosaccharinic, 3-deoxypentonic, 3,4-dihydroxybutyric, glycolic,

[176] R. Muggli, R. Marton, and A. Sarko, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 121.
[177] S. G. Whittington and R. M. Glover, *Macromolecules*, 1972, **5**, 55.
[178] F. Shafizadeh, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 21.
[179] V. I. Ivanov, N. Ya. Kuznetsova-Lenshina, V. I. Kogan, Z. A. Maslinkovskaia, and A. I. Bosova, *Doklady Akad. Nauk S.S.S.R.*, 1971, **196**, 1352.
[180] V. I. Ivanov, V. A. Afanasev, and R. I. Sarybaeva, *Doklady Akad. Nauk S.S.S.R.*, 1971, **196**, 380.
[181] V. L. Frampton, *Analyt. Chem.*, 1972, **44**, 866.
[182] R. M. Rowell, *Tappi*, 1972, **55**, 1326.

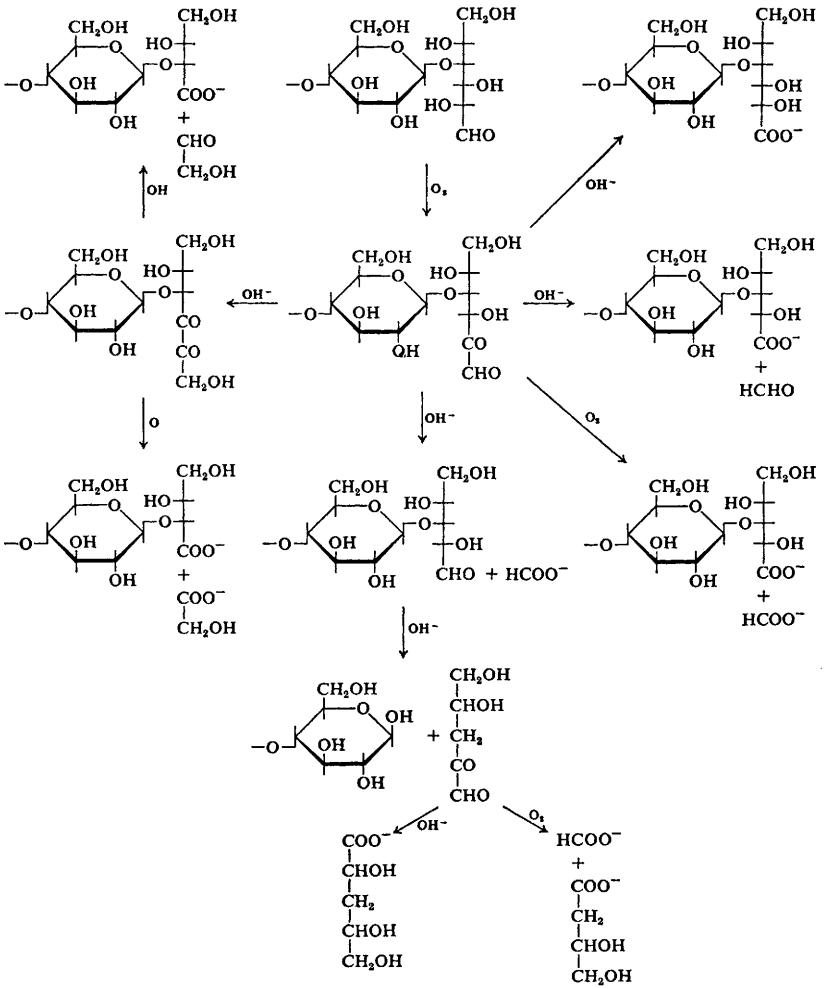
and formic acids. Appreciable amounts of anhydrosaccharinic, glyceric, lactic, and acetic acids, and minor amounts of glucometasaccharinic, erythronic, and threonic acids, were formed [183] (Scheme 1).

The very small amount of hydrogen peroxide generated during alkaline oxidation of cellulose in the presence of oxygen has been reported to induce further accelerated oxidation. Potassium iodide was found to be effective in stabilizing cellulose towards alkaline oxidation and depolymerization by removal of hydrogen peroxide and, possibly, hydroperoxides.[184] Oxidative reactions of cellulose initiated by free radicals have been investigated by means of e.s.r. spectroscopy. Dehydrogenative and oxidative depolymerization reactions were indicated, and the physical, chemical, and morphological effects of these reactions were summarized.[185] The main changes occurring in the distribution of the degree of polymerization of cellulose in wood pulp during refining have been examined by gel-permeation chromatography. Differences have been revealed that were not evident from viscometric measurements.[186] The effects of various treatments of rayon on the molecular-weight distribution of cellulose have been reported.[187]

Cellulose has been found to absorb cellulase strongly under conditions which are optimum for enzyme action. Sugars could be separated from the enzyme–cellulose complex by centrifugation on a column from which sugar solution was eluted.[188]

Changes in the molecular weight of cellulose in the pea epicotyl during growth have been studied. The degree of polymerization (DP) distribution patterns showed that a low molecular weight (< 500) component of cellulose is prominent in young tissues, whereas a high molecular weight (> 7000) component is predominant in mature tissues. Since the yield of soluble cellulase activity is known to decrease from high values in the meristem to barely detectable amounts in mature tissues, it was suggested that the relative levels and proportions of the hydrolytic and synthetic enzyme activities control the amount and DP of the cellulose formed during cell expansion in pea epicotyl.[189] Peroxyacetyl nitrate has been shown to cause a decrease in the incorporation of D-glucose into *Avena* coleoptile sections and it apparently inactivated cellulose synthetase and decreased cell growth by inhibiting the production of cell-wall constituents.[190] *X*-Ray diffraction analysis of the glucan formed in mung bean roots showed that cellulose I (natural cellulose) is not synthesized, but only

[183] H. Kolmodin and O. Samuelson, *Svensk Papperstidn.*, 1972, **75**, 369.
[184] J. L. Minor and N. Sanyer, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 73.
[185] J. C. Arthur and O. Hinojosa, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 53.
[186] W. J. Alexander and T. E. Muller, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 87.
[187] J. Dyer and L. H. Phifer, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 103.
[188] M. Mandels, J. Kostick, and R. Parizek, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 445.
[189] F. S. Spencer and G. A. Maclachlan, *Plant Physiol.*, 1972, **49**, 58.
[190] W. C. Gordon and L. Ordin, *Plant Physiol.*, 1972, **49**, 542.



Scheme 1

low molecular weight polysaccharides.[191] Protoplasts isolated from *Convolvulus* tissue regenerated a wall-like structure that could be degraded with cellulase, but which was resistant to protease, pectinase, and exo-β-(1 → 3)-glucanase, as was the original wall.[192] A particulate enzyme preparation from *Phaseolus aureus* catalysed the formation of an alkali-insoluble β-(1 → 4)-linked [^{14}C]glucan when UDP-α-D-[^{14}C]glucose was used as a substrate.[193] An increase in the levels of β-glucan synthetase [UDP-glucose-β-(1 → 4)-glucan-glucosyl transferase] has been reported when pea-stem tissue was treated with indolylacetic acid (IAA). Assays of the metabolic pools have supported the conclusions that the stimulation of polysaccharide biosynthesis by IAA is partly due to changes in hexokinase reaction rate, resulting from an increase in the metabolic D-glucose pool size caused by increased D-glucose uptake, and partly to increased activity at the polysaccharide synthetase level.[194] Glucose synthetase activity associated with the plasma membrane of onion stems was increased on addition of the auxin herbicide 2,4-dichlorophenoxyacetic acid.[195]

Hemicelluloses

Little difference was observed in either the extent or the rate of hydrolysis when either rumen hemicellulase or *Trichoderma viride* cellulase and hemicellulase were incubated with cell-wall preparations from oven-dried and freeze-dried samples of rye grass, cocksfoot, or white clover.[196] Particulate enzyme preparations from mung bean seedlings were able to catalyse the incorporation of radioactivity from UDP-D-[^{14}C]glucose, GDP-D-[^{14}C]-glucose, GDP-D-[^{14}C]mannose, UDP-D-[^{14}C]galactose, UDP-D-[^{14}C]xylose, UDP-D-[^{14}C]glucuronic acid, and UDP-L-[^{14}C]arabinose into both alkali-insoluble and alkali-soluble polysaccharides. It was shown that UDP-D-[^{14}C]glucose is a better D-glucosyl donor than GDP-D-[^{14}C]glucose with advancement of the age of the seedlings.[197] The results of the uptake of D-[U-^{14}C]glucose by subcellular particles of wheat seedling roots indicated that a hemicellulose-type of polysaccharide is widely distributed throughout the subcellular particles of the seedling roots.[198] D-Glucose from UDP-D-[^{14}C]glucose has been incorporated into glucans by cell-free extracts of *Lolium multiflorum*; the nature of the radioactive products depended on the concentration of UDPGlc in the incubation mixture. One of the products was a water-soluble glucan having structural features similar to those of a glucan containing both β-(1 → 3)- and β-(1 → 4)-linkages isolated from the cell walls.[199] A water-soluble galactan has been

[191] D. G. Robinson and R. D. Preston, *Biochim. Biophys. Acta*, 1972, **273**, 336.
[192] R. H. Horine and A. W. Ruesink, *Plant Physiol.*, 1972, **50**, 438.
[193] A. F. Clark and C. L. Villemez, *Plant Physiol.*, 1972, **50**, 371.
[194] A. A. Abdul-Baki and P. M. Ray, *Plant Physiol.*, 1971, **47**, 537.
[195] W. J. Van Der Woude, C. A. Lembi, and D. J. Morré, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 245.
[196] D. I. H. Jones and R. W. Bailey, *J. Sci. Food Agric.*, 1972, **23**, 609.
[197] S. S. Brar and A. D. Elbein, *Indian J. Biochem. Biophys.*, 1972, **9**, 131.
[198] R. Jilka, O. Brown, and P. Nordin, *Arch. Biochem. Biophys.*, 1972, **152**, 702.
[199] M. M. Smith and B. A. Stone, *Proc. Austral. Biochem. Soc.*, 1971, **4**, 103.

isolated from potato tubers. Methylation and periodate oxidation indicated that the polymer (molecular weight 1×10^4) is composed of about sixty β-(1 → 4)-linked D-galactopyranose residues containing two or three β-(1 → 4)- or β-(1 → 6)-linkages.[200] The structures of one trisaccharide and two tetrasaccharides have been obtained following graded hydrolysis of cashew nut-shell galactan. Smith degradation and methylation analysis supported their structures as being β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 3)-D-Gal*p*, β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 6)-D-Gal*p*, and a branched tetrasaccharide, β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 3)-[D-Gal*p*-(1 → 6)]-D-Gal*p*, respectively.[201] Complete hydrolysis of the hemicellulose A fraction, synthesized from GDP-D-[^{14}C]mannose by a particulate enzyme system from mung bean seedlings, released all the radioactivity as D-mannose. Enzymic hydrolysis with a hemicellulase released three neutral [^{14}C]oligosaccharides, one of which was tentatively identified as β-D-mannosyl-D-glucose. On Smith degradation, the larger [^{14}C]oligosaccharides yielded D-mannose and glycerol, but only a small amount of [^{14}C]erythritol, suggesting either a β-(1 → 3)-linkage or a branched structure.[202] The role of a mannosyl-lipid as an intermediate in the biosynthesis of polysaccharide in *Phaseolus aureus* seedlings has been studied. When the partially purified D-[^{14}C]mannosyl-lipid was treated with particulate enzymes under a variety of conditions, negligible amounts of material behaving as a polysaccharide were produced, implying that the lipid complex is not an obligatory precursor of polysaccharide.[203] Two decades of research on the biosynthesis of oligo- and poly-saccharides have been reviewed.[204] The occurrence, constitution, biosynthesis, and physiological action of polysaccharides of the cell walls of land and aquatic plants have been the subjects of reviews.[205, 206]

On the basis of the results of periodate oxidation, formic acid release, and i.r. spectroscopy, a polysaccharide isolated from deteriorated sugar cane syrup has been shown to be an α-glucan containing 70% (1 → 6)- and 20% (1 → 4)-linkages.[207] The acid hydrolysis of inulin has been shown to occur by initial hydrolysis of the furanose linkage, involving protonation of the oxygen of a furanose ring, followed by heterolysis of the linkage and formation of a tertiary cation. The value of the entropy of activation showed that the reaction is of the A-1 type and that the mechanism is similar to that of the hydrolysis of sucrose and raffinose.[208] Methyl β-D-fructofuranoside has been prepared by methanolysis of inulin. From

[200] P. J. Wood and I. R. Siddiqui, *Carbohydrate Res.*, 1972, **22**, 212.
[201] S. Bose and P. L. Soni, *J. Indian Chem. Soc.*, 1972, **49**, 593.
[202] S. S. Brar and A. D. Elbein, *Phytochemistry*, 1972, **11**, 651.
[203] D. L. Storm and W. Z. Hassid, *Plant Physiol.*, 1972, **50**, 473.
[204] L. F. Leloir, *Angew. Chem.*, 1971, **83**, 813.
[205] H. Grisebach and R. Schmid, *Angew. Chem.*, 1972, **84**, 192.
[206] H. Grisebach and R. Schmid, *Angew. Chem. Internat. Edn.*, 1972, **11**, 159.
[207] L. Brossard, J. Marinello, and C. Milian, 'Proceedings of the 8th International Symposium on the Chemistry of Natural Products', 1972, p. 359.
[208] J. Szejtli, R. D. Henriques, and M. Castineira, *Acta Chim. Acad. Sci. Hung.*, 1971, **70**, 379.

measurements of the activation energy and entropy, it was concluded that the mechanism of alcoholysis differs from that of hydrolysis.[209] It has been reported that alkaline degradation of inulin is due to depolymerization taking place during the isolation procedure. An alternative method for aqueous extraction of the polysaccharide has been described.[210] The results of analysis of fructosan formation during incubation of leaf discs of certain Asteraceae with sucrose have indicated a close correlation between transfructosylation mechanisms observed *in vitro* and the *de novo* synthesis of fructosans *in vivo*. Fructosan formation began with a logarithmic biphasic relationship between the amount of polymer synthesized and the chain length.[211] Sucrose, raffinose, mannotriose, stachyose, and verbascose have been isolated from water-soluble constituents of the roots of *Rehmannia glutinosa*.[212] Fructo-oligosaccharides of high DP have been separated using t.l.c. techniques.[44] A protein in normal shark serum has been shown to react specifically with fructosans.[110] Crude particulate enzyme preparations from mung bean shoots, which had been freed from sugar transferases, were able to synthesize pure araban from UDP-L-[^{14}C]arabinose and pure xylan from UDP-D-[^{14}C]xylose.[213]

The relationship between extensibility, growth rate, and carbohydrate composition in different sections of lupin hypcotyls has shown that the carbohydrate compositions of elongating and non-elongating regions are similar, although differences in extensibility are found. The nature of a possible arabinogalactan in the walls was studied with regard to its insolubility in pectic solvents.[214] Xylans and arabans present in tropical pasture herbage have been reported to be resistant to digestion by bovine rumen owing to physical protection by lignification in heavily lignified parts of the plant tissue. In less heavily lignified tissues, other factors such as cellulose crystallinity probably limit digestibility.[215] Galactinol, raffinose, and *myo*-inositol have been identified in samples of spear grass (*Heteropogon contortus*).[216] Relatively large amounts of hemicelluloses have been fractionated by continuous electrophoresis using a modified instrument.[217]

A study of the organization of the wheat cell wall has indicated the presence of an arabinoxylan, a glucomannan, and a glucan.[218] Oligosaccharides isolated from the leaves of *Phaseolus atropurpureus* have been shown to have the structures (1)—(3).[219]

[209] J. Szejtli, R. D. Henriques, and M. Castineira, *Acta Chim. Acad. Sci. Hung.*, 1972, **72**, 459.
[210] J. N. BeMiller, *Carbohydrate Res.*, 1972, **21**, 154.
[211] K. R. Chandorakar and F. W. Collins, *Canad. J. Botany*, 1972, **50**, 295.
[212] M. Tomoda, M. Tanaka, and N. Kondo, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 2411.
[213] W. Odzuck and H. Kauss, *Phytochemistry*, 1972, **11**, 2489.
[214] J. A. Munro, R. W. Bailey, and D. Penny, *Phytochemistry*, 1972, **11**, 1597.
[215] R. F. H. Dekker, G. N. Richards, and M. J. Playne, *Carbohydrate Res.*, 1972, **22**, 173.
[216] R. J. Beveridge, R. F. H. Dekker, G. N. Richards, and M. Towsey, *Austral. J. Chem.*, 1972, **25**, 677.
[217] N. W. H. Cheetham, R. J. McIlroy, and D. J. Winzor, *Carbohydrate Res.*, 1972, **21**, 479.
[218] D. J. Mares and B. A. Stone, *Proc. Austral. Biochem. Soc.*, 1971, **4**, 103.
[19] C. W. Ford, *Austral. J. Chem.*, 1972, **25**, 889.

D-Gal*p*-(1 → 6)-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 6)-D-Glc*p*
or D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 6)-D-Gal*p*-(1 → 6)-D-Glc*p*
(1)

β-D-Gal*p*-(1 → 6)-D-Glc*p* (2)

L-Ara*p*-(1 → 6)-D-Glc*p* (3)

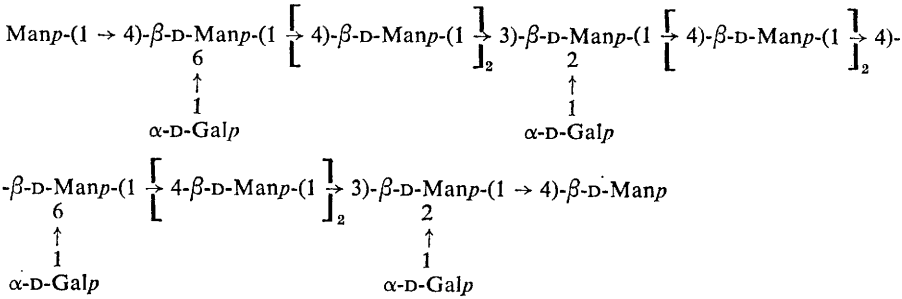
The preparation and properties of pure konjac mannan have been described.[220] From the rate constants for the acid hydrolysis of β-D-mannosidic and β-D-glucosidic linkages of konjac mannan, a statistical analysis of the hydrolysis of the main chain of the mannan was made whereby the yields of the fragmented disaccharides could be calculated for any degree of hydrolysis.[221] Extensive degradation of konjac mannan was observed after acetylation of the polysaccharide, and differences in rheological properties of rigid gels from different fractions were interpreted in terms of light-scattering measurements.[222] An analytical study of the carbohydrates during the growth of konjac plants has shown that the glucomannan content in growing bulbs gradually increases with maturation of the bulbs. The mannan in leaf blades was reported to have a different chemical composition from that of the bulbs.[223] A solubilized enzyme preparation from *Phaseolus aureus* has been shown to contain two types of glycosyltransferase activity, *viz.* a mannosyltransferase utilizing GDP-D-mannose and a glucosyltransferase utilizing GDP-D-glucose. A β-(1 → 4)-linked homopolysaccharide was formed when either nucleotide was the only substrate present, but a β-(1 → 4)-linked glucomannan was formed when both nucleotides were present. The glucosyltransferase required the continual production of mannose-containing acceptor molecules for maintenance of enzymic activity, but it was severely inhibited by GDP-D-glucose. These properties promoted the synthesis of β-(1 → 4)-linked glucomannan, rather than β-(1 → 4)-linked glucan, and β-(1 → 4)-linked mannan when both sugar nucleotides were present.[224] The glucomannan from the reserve polysaccharide of *Eremurus fuscus* has been subjected to partial acid hydrolysis. From the identification of four different disaccharides, it was concluded that the polysaccharide is composed of a linear chain of β-(1 → 4)-linked hexopyranose residues, with at least some parts of the molecule containing both contiguous D-glucose and D-mannose residues.[225] Water-soluble polysaccharides have been isolated from young tubers of *E. comosus* during fruit formation of the plant. Purification by means of copper complexing yielded a glucomannan and a galactan.[226]

[220] N. Sugiyama, H. Shimahara, and T. Andoh, *Bull. Chem. Soc. Japan*, 1972, **45**, 561.
[221] K. Kato and K. Matsuda, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 639.
[222] N. Sugiyama, H. Shimahara, T. Andoh, M. Takemoto, and T. Kamata, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1381.
[223] T. Murata, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 1.
[224] J. S. Heller and C. L. Villemez, *Biochem. J.*, 1972, **129**, 645.
[225] V. D. Shcherbukhin, N. K. Shcherbukhina, and V. L. Kirillina, *Doklady Akad. Nauk S.S.S.R.*, 1971, **200**, 1473.
[226] N. K. Shcherbukhina, V. L. Kirillina, and V. D. Shcherbukhin, *Doklady Akad. Nauk S.S.S.R.*, 1972, **202**, 1451.

When a glucomannan from Western hemlock was stabilized toward alkaline degradation, the rate of delignification on pulping was significantly retarded.[227] The polysaccharide from white willow (*Salix alba*) bark has been shown to be a glucomannan containing a linear chain of approximately thirty-two β-D-glucose and β-D-mannose residues in (1 → 4)-linkage.[228] Steam and water extracts of Southern pinewood have been fractionated to give a series of glucomannans and galactoglucomannans, which were characterized by gel-permeation chromatography.[229] A polysaccharide from Townsville lucerne (*Stylothanthes humilis*) has been purified by ion-exchange chromatography to apparent homogeneity. Structural studies indicated that the polysaccharide contains a backbone consisting of (1 → 4)-linked D-glucose and D-mannose units. D-Glucose and D-galactose residues were shown to be present as non-reducing end-groups, with the D-galactose units present as single-unit substituents attached to D-mannose units.[230]

The seeds of *Cassia fistula* have been found to contain a galactomannan. Periodate oxidation and methylation analysis indicated the existence of a continuous backbone of twenty-five (1 → 4)-β-D-mannose units with eight D-galactopyranose and two D-mannopyranose units attached to it by α-(1 → 6)-linkages.[231] A similar structure for a glucomannan, but lacking the mannopyranose side-chains, has also been reported.[232] The oligosaccharides liberated from partial acid hydrolysis of *C. absus* seed galactomannan corroborated previous reports that this polysaccharide is composed of a main chain of β-(1 → 4)-linked D-mannopyranose units. In addition, the identification of α-D-galactopyranosyl-(1 → 2)-D-mannose and α-D-galactopyranosyl-(1 → 6)-D-mannose provided evidence for the existence of both (1 → 2)- and (1 → 6)-linked D-galactose units.[233] When the polysaccharide was degraded by acid hydrolysis to a polymer of DP 16, periodate oxidation and methylation analysis of the degraded galactomannan indicated that it was a branched polysaccharide having the structure (4).[233a] The polysaccharides isolated by aqueous extraction of *C. occidentalis* and *Delonix regia* seeds contained D-galactose and D-mannose (1 : 3) and (1 : 2), respectively. They were shown to have structures similar to those of many other seed galactomannans in being composed of a main chain of β-(1 → 4)-linked D-mannose residues with side-chains of single α-(1 → 6)-linked D-galactose residues.[234, 235]

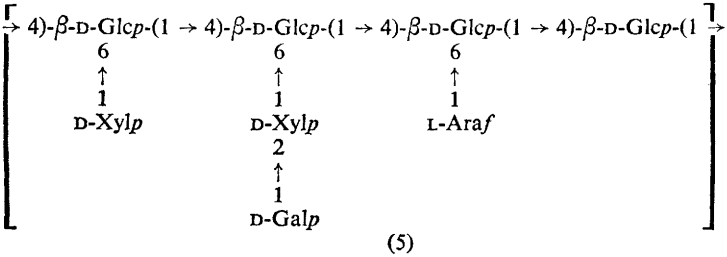
[227] A. R. Procter, *Tappi*, 1972, **55**, 424.
[228] R. Toman and Š. Karačsonyi, *Coll. Czech. Chem. Comm.*, 1972, **37**, 3640.
[229] K. D. Sears, A. Beélik, R. L. Casebier, R. J. Engen, J. K. Hamilton, and H. L. Hergert, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 425.
[230] M. Alam and G. N. Richards, *Austral. J. Chem.*, 1971, **24**, 2411.
[231] J. Lal and P. C. Gupta, *Planta Medica*, 1972, **22**, 71.
[232] P. S. Kelkar and S. Mukherjee, *Indian J. Chem.*, 1971, **9**, 1085.
[233] V. P. Kapoor and S. Mukherjee, *Phytochemistry*, 1971, **10**, 655.
[233a] V. P. Kapoor and S. Mukherjee, *Indian J. Chem.*, 1972, **10**, 155.
[234] D. S. Gupta and S. Mukherjee, ref. 207, p. 374.
[235] V. P. Kapoor, *Phytochemistry*, 1972, **11**, 1129.



(4)

The galactomannans isolated from black spruce (*Picea mariana*) and Sitka spruce (*P. sitchensis*) have been found to have similar structures, namely a (1 → 4)-linked mannan backbone with non-reducing end-groups consisting of D-mannose and a few single-unit side-chains of D-galactose.[236]

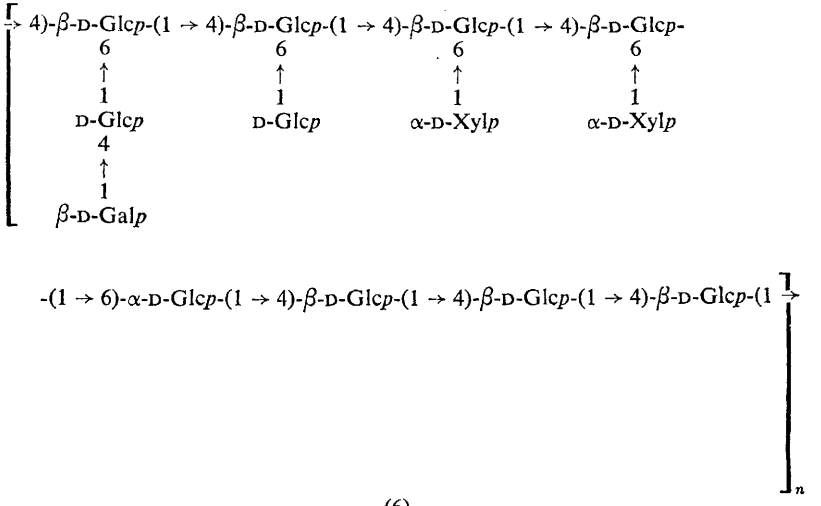
Aqueous extracts of Tamarind seed kernels have been fractionated by copper complexing to yield three polysaccharides, one of which (fraction III) was found to contain D-glucose, D-xylose, D-galactose, and L-arabinose (4 : 1 : 1 : 2, respectively). The results of methylation analysis suggested that the polysaccharide contains a main chain of (1 → 4)-D-glucosidic linkages with branches composed of D-xylose, L-arabinose, and D-galactose residues. From the strong resemblance in the structure to that of fraction II (which had been studied previously), it was considered likely that fraction III also contains branches composed of β-D-galactopyranosyl-(1 → 2)-D-xylose residues; these features can be accommodated in the proposed structure (5).[237, 238] In the presence of acid and heat, the polysaccharide was shown to undergo structural changes mainly by debranching and hydrolysis, rather than by transglycosidation as in the dextrinization of starch. The L-arabinofuranose residues present in the original polysaccharide were absent from this dextrin, which was composed



(5)

[236] G. G. S. Dutton and R. H. Walker, *Cellulose Chem. Technol.*, 1972, **6**, 295.
[237] H. C. Srivastava and T. N. Krishnamurthy, ref. 207, p. 371.
[238] H. C. Srivastava and T. N. Krishnamurthy, *Stärke*, 1972, **24**, 369.

of D-glucose, D-xylose, and D-galactose (10 : 2 : 1, respectively). From the results of periodate oxidation, Smith degradation, and methylation studies, the structure (6) has been proposed.[239] A study of the polysaccharides in compression wood of tamarack (*Larix laricina*) and in normal wood



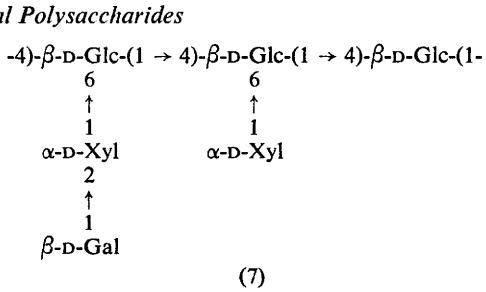
(6)

indicated that they contain similar quantities of hemicelluloses. In normal wood, however, the hexosans consist of various galactoglucomannans, whereas galactans and glucans are also present in compression wood. An alkali-soluble galactoglucomannan from compression wood has been shown to have the same composition and structure as that of the corresponding polysaccharide in normal wood.[240] A galactoglucomannan isolated from the stem tissues of the aquatic moss *Fontinalis antipyretica* has been reported to have a main chain of β-(1 → 4)-linked D-mannose and D-glucose residues with D-galactose and D-mannose residues attached to C-6 of the D-mannosyl, and to a lesser extent to the D-glucosyl, residues of the main chain.[241]

A re-examination of nasturtium 'amyloid', using methylation and partial acid hydrolysis techniques, has provided evidence supporting the revised structure (7).[242]

A g.l.c. method for determining the aldose and uronic acid constituents of plant cell-wall polysaccharides has been described.[22] The dehydration (using concentrated sulphuric acid) of total carbohydrate in wood or pulp to furans has been adapted to the measurement of glucans and xylans.

[239] H. C. Srivastava and T. N. Krishnamurthy, *Stärke*, 1972, **24**, 405.
[240] G. C. Hoffmann and T. E. Timell, *Svensk Papperstidn.*, 1972, **75**, 297.
[241] D. S. Geddes and K. C. B. Wilkie, *Carbohydrate Res.*, 1972, **23**, 349.
[242] P. Le Dizet, *Carbohydrate Res.*, 1972, **24**, 505.



(7)

Losses were usually smaller and less time was required than by the use of reducing-sugar methods.[243]

A water-soluble arabinogalactan containing D-galactose (53%), L-arabinose (33%), and D-glucuronic acid (14%) has been isolated from the leaves of *Phaseolus atropurpureus*. As a result of structural studies, the principal inter-glycosidic linkages were tentatively identified to involve (1 → 3)- and (1 → 6)-linked D-galactopyranose and (1 → 3)-linked L-arabinofuranose residues. In synthesizing a polysaccharide with these structural features, *P. atropurpureus* differs from other legumes such as soybean, lucerne, and *Centrosema*.[244] A flavonoid polysaccharide has been isolated from *Monoclea forsteri*; it had a molecular weight of 3200, based on the presence of one flavonoid group, and a structure in which 8-methoxy-5,7,3′,4′-tetrahydroxyflavone is glycosidically linked to a galacturonic acid residue of a water-soluble polysaccharide, which also contains glucose, galactose, and mannose residues.[245]

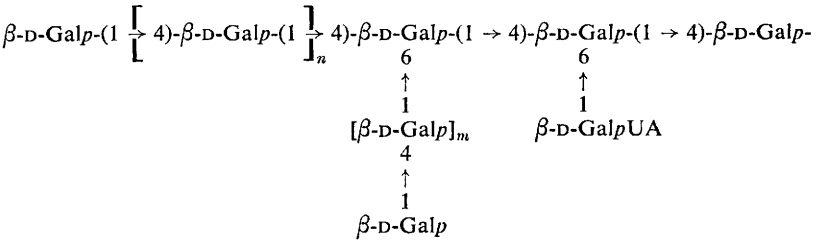
Gel-permeation chromatography and g.l.c. have been used to characterize the carbohydrates occurring in sulphite hydrolysates from wood pulp.[246] The polysaccharides occurring in the ray cells of normal wood of red pine (*Pinus resinosa*) on extraction and fractionation were found to contain cellulose (35%), β-(1 → 3)-glucan (2%), galactoglucomannan (9%), arabino-4-*O*-methylglucuronoxylan (11%), and pectin (2%). Compared with the tracheid cells, which contain no glucan, the ray cells contain less cellulose, much less galactoglucomannan, and more xylan. The structures of a xylan and a galactoglucomannan in ray cells were shown to be the same as those of the corresponding hemicelluloses in tracheid cells.[247] Although ray cells in normal and compression wood of *P. resinosa* have almost identical polysaccharide composition and contain hemicellulose of similar structure, they contain more cellulose and lack the high galactan content of the tracheid cells.[248] A quantitative method has been devised for studying the carbohydrate content of wood and the changes incurred in pulping and other types of chemical processing. After partial delignification of the

[243] R. W. Scott and J. Green, *Tappi*, 1972, **55**, 1061.
[244] C. W. Ford, *Phytochemistry*, 1972, **11**, 2559.
[245] K. R. Markham, *Phytochemistry*, 1972, **11**, 2047.
[246] J. W. Collins, *Tappi*, 1972, **55**, 1335.
[247] G. C. Hoffmann and T. E. Timell, *Tappi*, 1972, **55**, 733.
[248] G. C. Hoffmann and T. E. Timell, *Tappi*, 1972, **55**, 871.

tissue, the residue was sequentially extracted to yield an arabino-4-*O*-methylglucuronoxylan, a water-soluble galactoglucomannan, an alkali-soluble galactoglucomannan, and a residue of essentially pure cellulose. When this technique was applied to white fir wood, the analysis revealed the existence of four distinctive types of galactoglucomannan and two distinctive types of xylan.[249] The delignification of black spruce wood by acid chlorite has been followed by u.v. microscopy. It was shown that topochemical preference for the dissolution of lignin from the secondary wall correlated with the quantity of hemicellulose removed earlier in the extraction procedure.[250] The alkali-soluble xylan isolated from alder wood, *Alnus glutinosa*, was shown to have a structure similar to that of xylans from other woods in having a main chain composed of (1 → 4)-linked D-xylopyranose residues carrying (1 → 2)-linked residues of 4-*O*-methyl-D-glucuronic acid.[251] Chromatographic studies on the oligosaccharides produced from birch xylan on acid hydrolysis allowed the isolation of oligosaccharides containing from two to eighteen D-xylose residues. The results indicated that the 4-*O*-methyl-D-glucuronic acid groups are randomly distributed along the chain. Gel-permeation chromatography on ion-exchange resins could be applied to the separations of lower homologues of the β-(1 → 4)-linked D-xylose series.[252] The cell-wall polysaccharide of *Pinus radiata*, which was isolated from the chlorite holocellulose, had a DP of 78 and contained residues of 4-*O*-methyl-D-glucuronic acid, D-xylose, and L-arabinose (1 : 5.8 : 1.1, respectively). It appeared from methylation analysis that the uronic acid is attached to C-2, and the L-arabinose is linked to C-3, of individual D-xylose units.[253] A 4-*O*-methylglucuronoxylan, accounting for 86% of the alkali-extractable xylan from basswood (*Tilia americana* L), is composed of a backbone of one hundred and eighty β-(1 → 4)-linked D-xylopyranose units, with every ninth D-xylose unit (on average) carrying terminal side-chains of 4-*O*-methyl-α-D-glucuronic acid linked to C-2.[254] Further studies on laricinan, a glucan from the compression wood of tamarack (*Larix laricina*), have shown that it is an acidic glucan consisting of two hundred β-D-glucopyranose units, 6% of which are (1 → 4)-linked and the remainder (1 → 3)-linked. The average molecule contains eight branch points with D-glucuronic acid, and to a lesser extent D-galacturonic acid, residues attached to the glucan.[255] The compression wood contains an acidic galactan, which accounted for 90% of the galactan present in the wood. Methylation and other supporting evidence indicated the presence of a main chain of two- to three-hundred

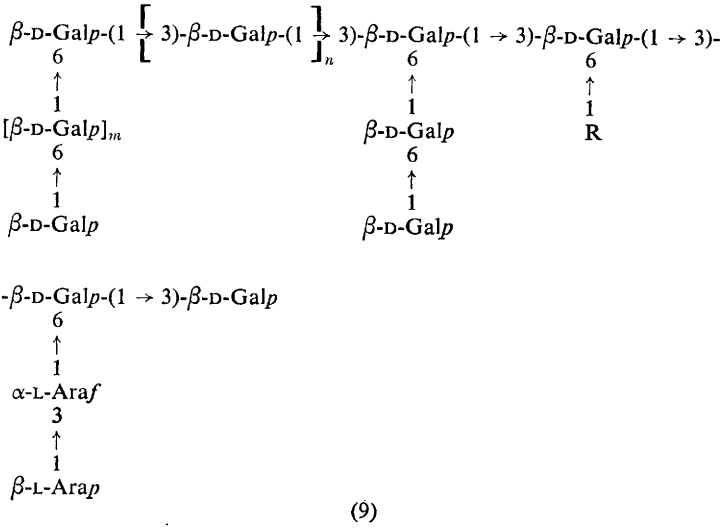
[249] D. L. Brink and A. A. Pohlman, *Tappi*, 1972, **55**, 380.
[250] J. R. Wood, P. A. Ahlgren, and D. A. I. Goring, *Svensk Papperstidn.*, 1972, **75**, 15.
[251] M. S. Dudkin, A. T. Bezusov, and N. G. Shkantova, *Zhur. obshchei. Khim.*, 1972, **42**, 1126.
[252] J. Havlicek and O. Samuelson, *Carbohydrate Res.*, 1972, **22**, 307.
[253] V. D. Hardwood, *Svensk Papperstidn.*, 1972, **75**, 207.
[254] M. J. Song and T. E. Timell, *Cellulose Chem. Technol.*, 1972, **6**, 67.
[255] G. C. Hoffmann and T. E. Timell, *Svensk Papperstidn.*, 1972, **75**, 135.

β-(1 → 4)-linked D-galactopyranose residues slightly branched at C-6. An average of one D-galacturonic acid residue per twenty D-galactose units is directly attached to C-6 of the latter. These features were accommodated in the proposed structure (8).[256] Methylation analysis of an arabino-4-*O*-methylglucuronoxylan from the compression wood of tamarack showed



(8)

it to be similar to the xylan present in normal larch wood, with the exception that it contains only half as many L-arabinose side-chains.[257] An acidic arabinogalactan, also present in tamarack compression wood, was subjected to structural analysis using methylation, partial acid hydrolysis, and Smith degradation. It was shown to have the same structure as the arabinogalactan present in the normal wood of the same species, but, contrary to present concepts, some of the D-galactose side-chains are thought to be considerably longer than two units [see (9)].[258]

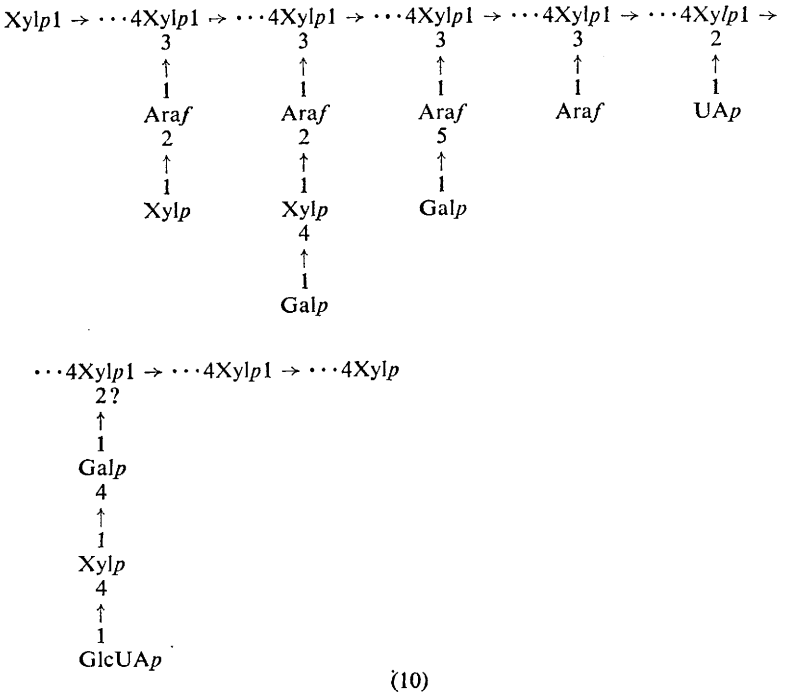


(9)

[256] K. S. Jiang and T. E. Timell, *Svensk Papperstidn.*, 1972, **75**, 592.
[257] G. C. Hoffmann and T. E. Timell, *Svensk Papperstidn.*, 1972, **75**, 241.
[258] Y. Fu and T. E. Timell, *Svensk Papperstidn.*, 1972, **75**, 680.

The carbohydrates of the leaves of the common barberry (*Berberis vulgaris*) have been extracted and fractionated to give an α-glucan [containing (1 → 4)-linkages with some residues branched through C-3 or C-6], a β-xylan, and three neutral and two uronic acid-containing glucoxylans.[259] An arabinoglucuronoxylan isolated from the leaf and stem of giant spear grass, *Cynodon plectostachyus*, was shown to contain D-xylose, L-arabinose, and D-glucose (17 : 22 : 1, respectively), together with 4-*O*-methyl-D-glucuronic acid. Methylation analysis indicated that the polysaccharide has similar structural features to those isolated from temperate grass species, namely a main chain of (1 → 4)-linked D-xylose units with L-arabinose being found exclusively as non-reducing, terminal units.[260] The solution properties of the hemicellulose B isolated from the tropical grass species *Setaria sphacelata* have been studied by equilibrium sedimentation and moving-boundary electrophoresis; the polysaccharide was shown to consist entirely of molecules having a molecular weight in the vicinity of 3.8×10^4.[261] The solutes in liquors after delignification of the coleoptile, leaf, stem, and root tissues of oat plants, *Avena sativa*, have been studied. Under the conditions used to isolate total hemicelluloses, which were required for studies of polysaccharide composition in relation to plant maturity, no significant losses of arabinose, xylose, or galactose residues occurred, but there was a small loss of glucan.[262] An acidic galactoarabino xylan from the stem of *A. sativa* has a DP 80 with a main xylan chain-carrying substituents of D-glucuronic acid and 4-*O*-methyl-D-glucuronic acid on the C-2 positions, D-glucopyranosyluronic acid-(1 → 4)-D-xylopyranosyl-(1 → 4)-D-galactose on the C-2 or C-3 positions, and α-L-arabinofuranosyl, D-galactopyranosyl-(1 → 5)-L-arabinofuranosyl, D-galactopyranosyl-(1 → 4 ?)-D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl, and D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl residues or side-chains, as illustrated in (10).[263] A water-soluble acidic arabinogalactan from rape seed was apparently homogeneous by electrophoretic and sedimentation criteria. Both methylation and periodate oxidation confirmed the complexity of this highly branched polysaccharide.[264] An arabinogalactan and two acidic polysaccharides, one of which was considered to be a high-molecular-weight galacturonan–arabinogalactan complex, were isolated from soybeans after cooking. The acidic polysaccharides were assumed to be the main components of the matrix and middle lamella of the soybean cell wall, respectively.[265] The values reported for the cation-exchange properties and uronic acid composition of pollen from a number of angiosperm and gymosperm species were of the same order as those of leaves,

[259] G. A. Henderson and G. W. Hay, *Carbohydrate Res.*, 1972, **23**, 379.
[260] N. W. H. Cheetham and R. J. McIlroy, *Carbohydrate Res.*, 1972, **21**, 201.
[261] N. W. H. Cheetham, R. J. McIlroy, and D. J. Winzor, *Carbohydrate Res.*, 1972, **23**, 57.
[262] A. J. Buchala, C. G. Fraser, and K. C. B. Wilkie, *Phytochemistry*, 1972, **11**, 1249.
[263] A. J. Buchala, C. G. Fraser, and K. C. B. Wilkie, *Phytochemistry*, 1972, **11**, 2803.
[264] I. R. Siddiqui and P. J. Wood, *Carbohydrrate Res.*, 1972, **24**, 1.
[265] T. Kikuchi, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 405.



(10)

roots, and seeds of the same species.[266] Pollen grain hemicellulose of *Phoenix dactylifera* is composed of L-arabinose (46%), D-galactose (25%), D-xylose (18%), L-rhamnose (9%), and uronic acid (2%). Periodate oxidation and methylation analysis indicated that the polysaccharide contains thirty-six residues in a structure which includes (1 → 4)-linked D-glucose, (1 → 2)-linked L-rhamnose, and (1 → 5)-linked L-arabinose residues.[267]

X-Ray crystallographic studies have shown that the unit-cell symmetry of oat-hull xylan appears to depend only on the xylan backbone, since the empty columnar space could accommodate the other more numerous side-chains of white birch xylan.[268] The characteristic ratios (C_N) of β-(1 → 4)-linked xylan and mannan, which were computed as a function of the angle τ at the bridge-oxygen atom and the degree of polymerization N, were found to converge to the asymptotic value at low DP values and high τ values. The low values of calculated C_N for xylan compared with those of cellulose and mannan for the same τ value indicated that the xylan is more flexible and assumes a compact configuration.[269] A comparison of the structures of xylan and cellulose showed that water is required to

[266] A. H. Knight, W. M. Crooke, and H. Shepherd, *J. Sci. Food Agric.*, 1972, **23**, 263.
[267] E. Samin and K. M. Kandeel, *Carbohydrate Res.*, 1972, **25**, 261.
[268] F. A. Bettelheim, *Carbohydrate Res.*, 1972, **21**, 427.
[269] N. Yathindra and V. S. R. Rao, *Biopolymers*, 1971, **10**, 1891.

stabilize the lattice structure of the xylan and, although conformational analysis indicated a strong preference for a two-fold screw axis as in cellulose, the conformation adopted by the xylan is presumably determined by the more favourable three-fold screw axis.[270] A study of the effect of the linkage on the possible conformations of di- and poly-saccharides of α-D-glucans has indicated that differences in the nature of the linkages affect the energetically preferred conformations. Thus, (1 → 2)-linkages would lead only to right-handed helical conformations, whereas (1 → 3)-linkages would lead to extended, left- or right-handed helical conformations. The extent of hydrogen-bonding between adjacent residues was also considered to be dependent on the nature of the linkage.[271] From steric considerations and energy contour diagrams plotted for disaccharide-like and helical structures of linear α-D-glucans having (1 → 2)-, (1 → 3)-, and (1 → 4)-linkages, the allowed conformation was shown to constitute only about 4% of the total conformations, indicating that the freedom of rotation of D-glucose residues is highly restricted in all three polysaccharides. In addition to the information already described, it was concluded that (1 → 4)-linkages lead to both right- and left-handed extended helical conformations.[272] The conformational energies for both α- and β-(1 → 4)-linked D-galactans have been computed; from the values of the characteristic ratios, which were higher for the α-D-galactan than for the β-D-galactan, it was concluded that the former assumes a highly extended conformation compared with the latter.[273] Conformational studies on linear β-(1 → 4)-linked galactan indicated that the freedom of rotation of the monomer units is highly restricted, as in cellulose and β-(1 → 4)-linked mannan, and that the galactan prefers a helical conformation similar to that of amylose.[274] Computation of electronic charge-distribution for twelve different types of molecular fragment, including di- and tri-saccharides having (1 → 2)-, (1 → 3)-, and (1 → 4)-linkages, has been reported.[275]

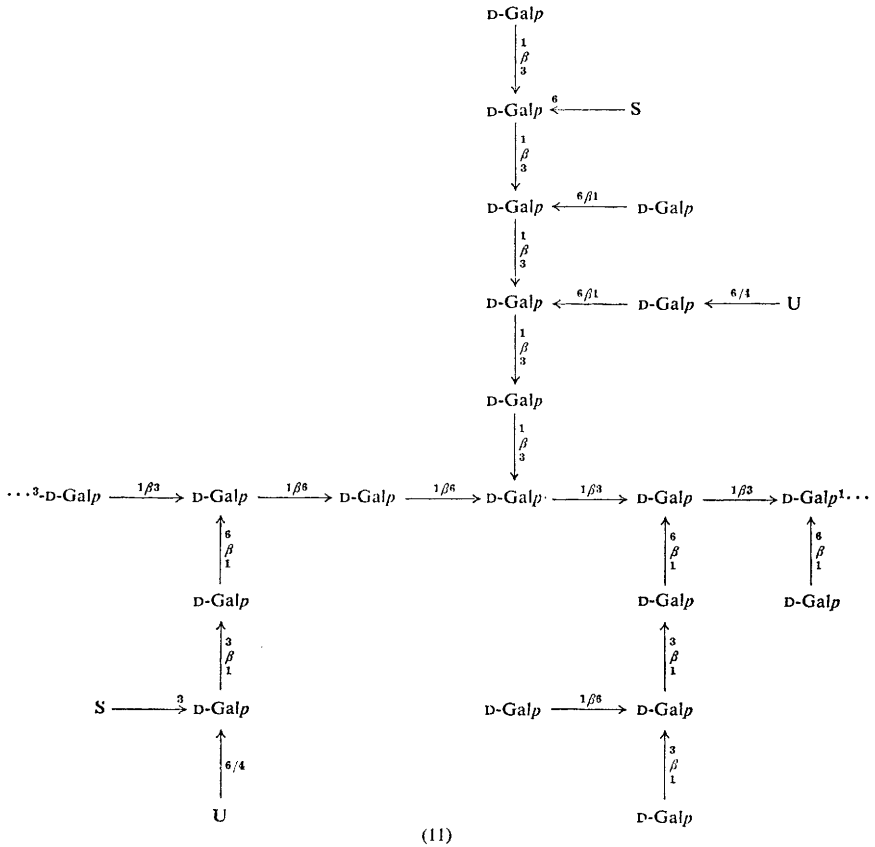
Gums and Pectic Substances

The physical chemistry of acidic polysaccharides has been the subject of a treatise that includes chapters on the chemical structure of gums, pectic substances, alginic acids, and hemicelluloses, as well as detailed information on the molecular parameters (molecular weight, size, and shape) of single molecules, the thermodynamics of dilute solutions, transport phenomena, and structural properties in the solid state.[276]

[270] I. A. Nieduszynski and R. H. Marchessault, *Biopolymers*, 1972, **11**, 1335.
[271] B. K. Sathyanarayana and V. S. R. Rao, *Biopolymers*, 1972, **11**, 1379.
[272] B. K. Sathyanarayana and V. S. R. Rao, *Biopolymers*, 1971, **10**, 1605.
[273] N. Yathindra and V. S. R. Rao, *J. Polymer Sci., Part A-2, Polymer Phys.*, 1972, **10**, 1369.
[274] P. R. Sundararajan and V. S. R. Rao, *Biopolymers*, 1970, **9**, 1239.
[275] N. Yathindra and V. S. R. Rao, *Carbohydrate Res.*, 1972, **25**, 256.
[276] F. A. Bettelheim, 'Physical Chemistry of Acidic Polysaccharides', ed. A. Veis, Marcel Dekker, New York, 1970, p. 131.

An analysis of the light-scattering data for aqueous salt solutions of gum arabic has suggested a rod-like shape for the aggregated arabate particles.[277] Approximately 35% of the branches of gum arabic have been shown to differ from the rest of the branches in the molecule in that they are made more susceptible to hydrolysis by a physical change (possibly dehydration). Electrophoretic and light-scattering data indicated that the molecular weight of the hydrolysis product is approximately 20% less than that of gum arabic.[278] A number of *Acacia* gum species have been analysed for their rhamnose content, acidity, intrinsic viscosity, and galactose to arabinose ratio.[279] When studied by gel chromatography, the molecular weights of several components in the elution pattern of hydrolysates approximated to values predicted for the products obtained on complete hydrolysis of peripheral arabinofuranoside and rhamnopyranoside linkages, and subsequent preferential removal of galactopyranose end-groups, from various components of the polymolecular gum.[280] Present evidence on the solubilization of gum exudates by sodium borohydride has indicated that minimal degradation occurs, provided that dilute solutions are used for short periods of time.[281] A water-soluble gum isolated from *Lannea humilis* trees was shown to contain galactose (75%), arabinose (11%), rhamnose (2%), and uronic acids (12%). Three aldobiouronic acids were isolated from partial acid hydrolysates, namely 4-*O*-(α-D-galacto-pyranosyluronic acid)-D-galactose, 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose. By characterization of neutral oligosaccharides isolated from the degraded gum and from methylation analysis, evidence was obtained to indicate that the gum molecules are very highly branched. The galactan framework consists of short chains of β-(1 → 3)-linked D-galactose residues, which are branched and interspersed with β-(1 → 6)-linkages. To positions 3 and 6 of this framework are attached either single D-galactose end-groups or short chains of D-galactose or L-arabinose residues, and three aldobiouronic acids [see (11)].[282] The amino-acid and 2-amino-2-deoxy-D-glucose composition of some plant gums has been reported.[283] Structural studies on Hualtaco gum from *Laxopterygium huansango* indicated that the polysaccharide has a main chain of (1 → 3)-linked D-galactopyranose residues with branches at C-6 of some of the hexose units. In some places, branches consist of single units of D-galactose and L-rhamnose, and in others aldobiouronic acid residues are present. Some of the acidic sugars are linked at C-4 either to an L-arabinofuranose residue or to the disaccharide β-D-galactose-(1 → 3)-L-arabinose.[284] A gum from *Odina woder* has been

[277] A. R. Sloniewsky and P. Ander, *Carbohydrate Res.*, 1971, **18**, 103.
[278] R. E. Nelson and P. Ander, *Carbohydrate Res.*, 1972, **25**, 81.
[279] D. M. W. Anderson, P. C. Bell, and C. G. A. McNab, *Phytochemistry*, 1972, **11**, 1751.
[280] S. C. Churms and A. M. Stephen, *Carbohydrate Res.*, 1972, **21**, 91.
[281] D. M. W. Anderson, P. C. Bell, and H. A. R. King, *Carbohydrate Res.*, 1972, **22**, 453.
[282] D. M. W. Anderson and A. Hendrie, *Carbohydrate Res.*, 1972, **22**, 265.
[283] D. M. W. Anderson, A. Hendrie, and A. C. Munro, *Phytochemistry*, 1972, **11**, 733.
[284] C. V. N. Rao and S. Samajpati, ref. 207, p. 369.

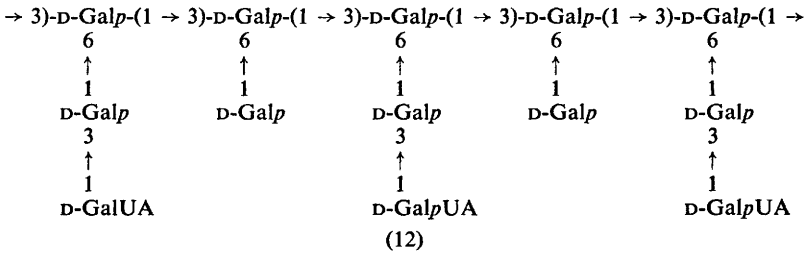


(11)

extracted with alkali and purified by ion-exchange chromatography; it was shown to contain L-arabinose, D-galactose, L-rhamnose (38 : 20 : 1, respectively), and D-galacturonic acid. On the basis of the identification of an aldobiouronic acid fragment and methylation and periodate oxidation evidence from the degraded gum, a possible partial structure (12) has been assigned.[285] A natural plant exudate, 'Gaz' gum, isolated from *Tamerix manifera*, has been found to contain glucose, fructose, mannose, xylose, and arabinose.[286] An attempted interpretation of i.r. spectra in the region of 1600—1800 cm^{-1} has been reported for peach gum polysaccharides.[287]

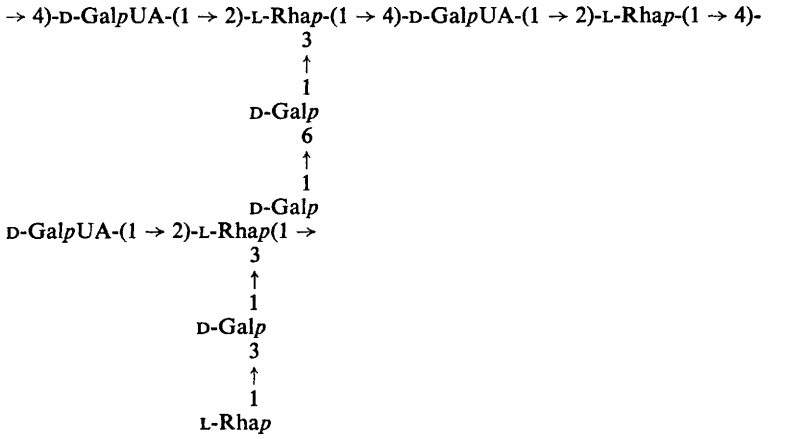
Complete methylation and hydrolysis of the mucilage from *Hibiscus ficuleneus* yielded 2,3,4,6-tetra-*O*-methyl-D-galactose (2), 2,3,4-tri-*O*-methyl-D-galactose (3), 2,4,6-tri-*O*-methyl-D-galactose (1), 2,3,4-tri-*O*-methyl-L-

[285] A. K. Gupta and S. Mukherjee, ref. 207, p. 360.
[286] M. Maleki and M. Djazayeri, *Stärke*, 1972, **24**, 296.
[287] J. Rosík, A. Kardošová, and J. Kubala, *Carbohydrate Res.*, 1971, **18**, 151.



(12)

rhamnose (1), 3,4-di-*O*-methyl-L-rhamnose (2), 4-*O*-methyl-L-rhamnose (3), and 2,3-di-*O*-methyl-D-glucuronic acid (6 parts). From these results and those from periodate oxidation, a tentative structure (13) has been postulated.[288] The polysaccharides from the seed mucilage of *Ocimum*



(13)

basilicum have been shown to be heterogeneous and probably highly branched and to contain bound and free lipids. They are composed of a Cetavlon-precipitable uronic acid–pentosan-rich fraction, as well as two other hexosan-rich fractions.[289] 'Odoratan', a mucilage isolated from the rhizome of *Polygonatum odoratum*, was shown to be homogeneous by gel chromatographic and electrophoretic techniques and to contain D-fructose, D-mannose, D-glucose, and D-glucuronic acid (6 : 3 : 1 : 1.5, respectively). Digestion with β-fructofuranosidase liberated 94% of the D-fructose, leaving a polymer of molecular weight 2.5×10^5.[290] An acidic polysaccharide having a molecular weight $28—40 \times 10^3$ and containing D-galacturonic acid, L-rhamnose, and D-galactose is probably present in the

[288] K. S. Bajpai and S. Mukherjee, *Indian J. Chem.*, 1971, **9**, 33.
[289] R. N. Tharanathan and Y. V. Anjaneyalu, *Current Sci.*, 1972, **41**, 214.
[290] M. Tomoda, Y. Yoshida, H. Tanaka, and M. Uno, *Chem. and Pharm. Bull.* (*Japan*), 1971, **19**, 2173.

mucilage of pea seeds.[291] The mucilage isolated from the tubers of *Dioscorea batatas* is composed of mannan associated with protein. A water-insoluble mannan ($[\alpha]_D$ − 31°), isolated by formation of the copper complex, was found to be a β-(1 → 4)-linked D-mannan of DP 110. From the results of methylation and periodate oxidation, it appeared to have a small degree of branching at C-3 in one out of every ten or eleven D-mannose units. Two oligosaccharides isolated after enzymic degradation were characterized as mannobiose and mannotriose.[292] The structure of slippery elm mucilage (*Ulmus fulva*) was investigated by means of methylation, periodate oxidation, and partial acid hydrolysis. It was concluded that this polysaccharide is composed of a main chain of alternating D-galacturonic acid and L-rhamnose residues, joined by α-linkages through position 4 of the uronic acid and position 2 of the L-rhamnose residues, with side-chains containing one or two residues of 3-*O*-methyl-D-galactopyranose or D-galactopyranose attached to some L-rhamnose residues at C-4 and C-3, respectively.[293]

Pectins

Pectic substances extracted from pasture legumes were hydrolysed with polygalacturonase and the galacturonic acid produced was determined by a modified carbazole reaction. Inclusion of the enzymic hydrolysis step gave a seven-fold increase in sensitivity compared with the acid-carbazole determination.[294] After pectolytic attack, a series of oligogalacturonic acids (up to DP 9) were separated on columns of DEAE–Sephadex.[295] A ^{14}C-labelled D-galacturonan has been isolated from *Chara globate* grown in the presence of $^{14}CO_2$, thereby providing a convenient source for the preparation of uniformly ^{14}C-labelled oligo(D-galacturonic acids).[296] The pectic acid isolated from garlic skins contains neither (1 → 3)-linked residues nor branch points; periodate oxidation indicated the existence of a linear (1 → 4)-linked galacturonan.[297] Mild acid extraction of cocoa-pod husks liberated a polysaccharide with a composition similar to that of apple pectin.[298] Two water-soluble, acidic polysaccharides (molecular weights 3.2×10^5 and 1.6×10^4, respectively) were isolated and purified from soy sauce; since the sugar composition of both polymers was close to that of the soybean cell-wall preparation, they were presumed to originate from the cell wall.[299] The affinity of some cations for the carboxy-groups of pectin was shown to increase slightly in the sequence calcium < strontium

[291] T. Shinohara, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 1075.
[292] A. Misaki, T. Ito, and T. Hadada, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 761.
[293] R. J. Beveridge, J. K. N. Jones, R. W. Lowe, and W. A. Szarek, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, **23**, 461.
[294] R. F. H. Dekker and G. N. Richards, *J. Sci. Food Agric.*, 1972, **23**, 475.
[295] C. Hatanaka and J. Ozawa, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 417.
[296] Y. Milner and G. Avigad, *Carbohydrate Res.*, 1972, **25**, 153.
[279] A. F. Abdel-Fattah and M. Edrees, *J. Sci. Food Agric.*, 1972, **23**, 871.
[298] D. Adomako, *Phytochemistry*, 1972, **11**, 1145.
[299] T. Kikuchi and T. Yokotsuka, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 544.

< barium.[300] Variation of polygalacturonase concentration was found to produce different patterns in the release of oligosaccharides from flax pectin. It was suggested that the enzyme has more affinity towards the polymer molecules than towards the lower oligosaccharides.[301] The relative energies of various D-galacturonic acid monomer conformations have been calculated by employing an iterative procedure to fix the positions of the rotating groups of the sugar ring.[302] A fibrillar, u.v.-absorbing substance has been isolated from the outer wall surface of suspension-cultured cells of *Daucus* and *Ipomeoa*.[303] Chemical analysis of the fibrils demonstrated that the major component is a linear polygalacturonic acid to which an u.v.-absorbing compound is attached.[304]

Algal Polysaccharides

The significance of cell-wall chemistry to the phylogeny in algae [305] and the histological localization of structural polysaccharides of seaweeds [306] have been the subjects of reviews.

Calcium-ion activities have been determined in solutions of calcium oligo- and poly-guluronates and calcium oligo- and poly-mannuronates. The activity coefficients in solutions of the calcium monouronates were close to that of a corresponding solution of calcium chloride and decreased with increasing DP, reaching constant values for polymers having a DP higher than thirty.[307] A study of oriented specimens of salts of polymannuronic acid have indicated a fibre repeat value of 15 Å in a helical chain, each turn containing three monosaccharide units in the *C*1 chair conformation. In contrast, salts of polyguluronic acid showed a two-fold repeat value of 8.7 Å.[308]

The time course for incorporation of photosynthesized materials into alginate in *Laminaria digitata* has indicated the presence of relatively large pools of alginate precursors. A large proportion of the total material incorporated was found in mannuronic acid and alternating mannuronic and guluronic acid block components.[309] Amino-acids, particularly L-aspartic acid and L-alanine, were considered the most likely sources of carbon for alginic acid synthesis, mainly by incorporation into mannuronic acid residues. Considerable amounts of the polysaccharide were laid down in the stripes of the seaweed during dark periods, but very little synthesis was found in the blades.[310] An investigation into the efficiency of reagents

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[301] A. F. Abdel-Fattah, S. S. Mabrouk, and M. Edrees, *J. Gen. Appl. Microbiol.*, 1971, **17**, 421.
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[303] G. G. Leppard, J. R. Colvin, D. Rose, and S. M. Martin, *J. Cell Biol.*, 1971, **50**, 63.
[304] G. G. Leppard and J. R. Colvin, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, 321.
[305] B. C. Parker, *Ann. New York Acad. Sci.*, 1970, **175**, 417.
[306] M. E. McCulley, *Ann. New York Acad. Sci.*, 1970, **175**, 702.
[307] R. Kohn and B. Larsen, *Acta Chem. Scand.*, 1972, **26**, 2455.
[308] W. Mackie, *Biochem. J.*, 1971, **125**, 89P.
[309] J. A. Hellebust and A. Haug, *Canad. J. Botany*, 1972, **50**, 169.
[310] J. A. Hellebust and A. Haug, *Canad. J. Botany*, 1972, **50**, 177.

used for extracting alginic acid from *Cystoseira barbara* has shown that aqueous sodium carbonate gave alginic acid in the highest yield and of high viscosity.[311] The results of a study on criteria for the crystallization of alginic acid and hyaluronic acid have indicated that the processes may be regarded in the same general terms as annealing phenomena, which is well recognized in the synthetic polymer and metallurgy fields.[312] When alginic and pectic acids were sulphated to a degree of substitution of 1.8, very little degradation of the polysaccharides was observed.[313] A method has been evaluated for the determination of the stiffness of poly-(1 → 4)-hexuronates in the gel state.[314] Although calcium polymannuronate did not form gels with a measurable stiffness, the modulus of stiffness was found to increase with increasing L-guluronic acid content of alginic acids. The properties of calcium pectate gels resembled those of alginates rich in L-guluronate.[315] A study of conformational energy maps calculated for β-D-mannuronic acid-(1 → 4)-α-L-guluronic acid and for α-L-guluronic acid-(1 → 4)-β-D-mannuronic acid has indicated that the alternating copolymer is less extended than either homopolymer.[316] Electrical cells, generating free radicals at a platinum electrode surface, have been used to degrade alginic acid. Depolymerization was normally followed by the generation of reducing properties.[317]

The agar isolated from *Gracilaria verrucosa* has been separated into a number of fractions of differing carbohydrate composition by ion-exchange chromatography. The findings suggested that agar is a family of polydisperse polysaccharides consisting of various intermediate molecular forms between two extreme types of macromolecules, one being a non-sulphated, highly methylated galactan, possibly made of repeating agarobiose residues, and the other being a non-methylated, highly sulphated galactan with a lesser proportion of anhydrogalactose residues.[318] An agar producing a firm gel with specific rheological properties has been isolated from *G. foliifera* by pre-treatment of the seaweed with alkali before extraction.[319] Steady-state and pulsed ^{1}H n.m.r. techniques have been used to study the synergistic increase in gel strength obtained on adding locust-bean gum to agarose. The results indicated that the gum did not form double-helix junctions and could decrease the rates of gelation by steric effects. At high agarose concentrations, the gum remained mainly in solution in the interstitial water, but investigations at low agarose concentrations suggested that the gum could link gel aggregates into a self-supporting structure, producing a synergistic increase in gel strength.[320] A molecular

[311] A. F. Abdel-Fattah, M. M. Hussein, and H. M. Salem, *J. Chem. U.A.R.*, 1971, **14**, 185.
[312] E. D. T. Atkins and W. Mackie, *Biopolymers*, 1972, **11**, 1685.
[313] R. G. Schweiger and T. R. Andrew, *Carbohydrate Res.*, 1972, **21**, 275.
[314] O. Smidsrød, A. Haug, and B. Lian, *Acta Chem. Scand.*, 1972, **26**, 71.
[315] O. Smidsrød and A. Haug, *Acta Chem. Scand.*, 1972, **26**, 79.
[316] S. G. Whittington, *Biopolymers*, 1971, **10**, 1617.
[317] M. J. Harris, A. Herp, and W. Pigman, *Arch. Biochem. Biophys.*, 1971, **142**, 615.
[318] K. Izumi, *J. Biochem.*, 1972, **72**, 135.
[319] T. Matsuhashi and K. Hayashi, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 1543.
[320] T. F. Child and N. G. Pryce, *Biopolymers*, 1972, **11**, 409.

view of agarose, furcellan, κ-carrageenan, and pectic gels has traced the characteristic differences between one gel and another, the variations in framework flexibility, and the number and nature of cross-linkages.[321] 'Shapely Polysaccharides' was the title of the Eighth Colworth Medal Lecture, which dealt with *in vivo* investigations of gel systems and the polysaccharide conformations of agarose and κ-carrageenan.[322] Differences in the fine structure of agaroids, carrageenans, and furcellans have been shown to alter the conformations of these polysaccharides and, consequently, the physical properties of the individual polymers. The evidence that gel formation occurs through association of chain segments into a three-dimensional framework, which is held together by hydrogen bonds, has been reviewed.[323] It has been shown that ordered binding could occur between agarose or carrageenan helices and parts of galactomannan backbones containing contiguous, unsubstituted D-mannose residues. This association of unlike polysaccharide entities was regarded as 'polysaccharide quaternary structure' and its influence was considered to be illustrative of ligand induction of polysaccharide tertiary structure.[324]

Some applications of computer model-building in polysaccharides have been described with reference to carrageenan.[325] Relatively undegraded cellulose sulphate showed properties similar to those of carrageenan in its reactivity with proteins and in the gelation of its aqueous solutions in the presence of potassium, rubidium, or caesium ions.[326] Carrageenan has been reported to cause an increase in the Michaelis constant for trypsin, suggesting that the polysaccharide acts as a competitive inhibitor towards the enzyme.[327] Studies on ligand-induced conformational transitions and selective cation binding have suggested that the double-helical carrageenans may have a biological function in maintaining a suitable intracellular ionic environment and that they may act as allosteric ion-exchange agents involved in the uptake and transport of specific ions.[328] Alkali treatment of κ-carrageenan from *Gigartina tenella* resulted in a change in the proportions of D-galactose, 3,6-anhydro-D-galactose, and sulphate. From identification of fragments liberated on hydrolysis of the methylated polysaccharide, it was concluded that the polysaccharide is composed of residues of β-(1 → 3)-linked D-galactose 4-sulphate, α-(1 → 4)-linked D-galactose 6-sulphate, and α-(1 → 4)-linked 3,6-anhydro-D-galactose and its 2-sulphate.[329]

The incorporation of $^{14}CO_2$ into the polysaccharides of *Fucus vesiculosus*, which were sequentially extracted and fractionated, yielded laminarin,

[321] D. A. Rees, *Chem. and Ind.*, 1972, 630.
[322] D. A. Rees, *Biochem. J.*, 1972, **126**, 257.
[323] E. Percival, *J. Sci. Food Agric.*, 1972, **23**, 933.
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[325] D. A. Rees, *Biochem. J.*, 1971, **125**, 86P.
[326] R. G. Schweiger, *Carbohydrate Res.*, 1972, **21**, 219.
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[328] F. B. Williamson, *Biochem. J.*, 1972, **129**, 28P.
[329] S. Hirase and K. Watanabe, *Bull. Chem. Soc. Japan*, 1972, **45**, 1839.

a xylofucogalactoglucuronan (*A*), a xyloglucuronogalactofucan (*B*), fucoidan (*C*), alginic acid, and residual polysaccharide material. Highly active low molecular weight carbohydrates present in acid extracts were suggested to be precursors of the polysaccharides. The fucose-containing polysaccharides are considered to represent the extremes in a family of polymers in which polysaccharide *A* is transformed into fucoidan by way of polysaccharide *B*.[330]

In the logarithmic growth phase, the cells of the marine diatom *Chaetoceros affinis* contained very little glucan, which was synthesized in large amounts, together with considerable amounts of an extracellular polysaccharide, in the stationary phase.[331] The extracellular polysaccharide, which behaved as a homogeneous, polyionic compound, contained rhamnose, fucose, arabinose, galactose, and sulphate.[332] The accumulation of [^{35}S]sulphate by *Porphyridium aerugineum* cells and the subsequent appearance of a solubilized, [^{35}S]sulphated capsular polysaccharide has been examined.[333] Two polysaccharides have been isolated after gel-permeation chromatography of aqueous extracts of *Chlorella* cells. One of the polymers was shown to contain mainly rhamnose and galactose, whereas the other is a glucan–protein complex. Proteolytic digestion of the D-glucose-containing polymer released a polyglucoside (molecular weight 3500), which had a stimulative activity on phagocytosis in the reticulo-endothelial system of the rat.[334] A kinetic expression has been presented for the rate of alkaline degradation of such linear β-(1 $\rightarrow$ 3)-glucans as laminarin and pachyman in terms of the mono- and di-anionic species formed from the reducing end-groups.[335] The principal products of photosynthesis of *Phaeosaccion collinsii* were identified as mannitol and a water-soluble glucan similar to laminarin.[336] A glucan from the blue-green alga *Nostoc muscorum* has been reported to have similar properties to glycogen in that it formed an iodine complex and was degraded to maltose by β-amylase.[337] By a combination of *X*-ray intensity data and chain-packing analysis, it was concluded that the overall number of permissible structures for the β-(1 $\rightarrow$ 4)-linked mannan of *Cymopolia barbata* is very limited, so that van der Waals forces play a major role in packing.[338] The carbohydrates of *Acetabularia crenulata* have been shown to include inulin-type oligosaccharides, a water-soluble inulin-type polysaccharide, and a complex

[330] R. G. S. Bidwell, E. Percival, and B. Smestad, *Canad. J. Botany*, 1972, **50**, 191.
[331] S. Myklestad and A. Haug, *J. Exptl. Marine Biol. Ecol.*, 1972, **9**, 125.
[332] S. Myklestad, A. Haug, and B. Larsen, *J. Exptl. Marine Biol. Ecol.*, 1972, **9**, 137.
[333] J. Ramus and S. T. Groves, *J. Cell Biol.*, 1972, **54**, 399.
[334] M. Kojima, S. Ino, N. Takase, K. Shishido, S. Kobayashi, M. Dobashi, and K. Wanatabe, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 373.
[335] R. A. Young, K. V. Sarkanan, P. G. Johnson, and G. G. Allan, *Carbohydrate Res.*, 1972, **21**, 111.
[336] J. S. Craigie, C. Leigh, L. C.-M. Chen, and J. MacLachlan, *Canad. J. Botany*, 1971, **49**, 1067.
[337] L. Chao and C. C. Bowen, *J. Bacteriol.*, 1971, **105**, 331.
[338] I. Nieduszynski and R. H. Marchessault, *Canad. J. Chem.*, 1972, **50**, 2130.

acid-containing polymer. Alkali solubilized a polydisperse β-(1 → 4)-linked mannan.[339] An acidic polysaccharide, which had been solubilized from the cell wall of *Chlorella pyrenoidosa* by treatment with alkaline sodium borohydride, was apparently homogeneous after purification by ion-exchange chromatography; it is composed of L-rhamnose, L-arabinose, D-xylose, D-mannose, DL-galactose, and D-glucuronic acid.[340] Studies of the sequence and configuration of the (1 → 4)- and (1 → 3)-linkages of a xylan isolated from *Chaetangium fastigiatum* have indicated that some of the (1 → 3)-linked units are grouped in zones containing several residues.[341] The purified cell walls of *Pleurotaenium trabecula* have been shown to contain mainly polymers of D-glucose and D-galactose.[342] Most of the hydroxyproline residues in *Chlamydomnas reinhardtii* have been found to be linked glycosidically to oligosaccharides.[343] A study of the pattern of incorporation of $^{14}CO_2$ into the thallus of an alga has suggested that an unknown compound, probably a sugar phosphate, and D-glucose are intermediates in the transfer of carbon to the lichen *Peltigera polydactyla*. The results are consistent with the hypothesis that the alga released a glucan that the lichen hydrolysed extracellularly to D-glucose, which was then incorporated into the lichen and converted into mannitol.[344] A water-soluble polysaccharide (DP 140) from *Stereocaulon paschale* was found to be of the α-glucan type. The results indicated that the polysaccharide is built up mainly of (1 → 4)-D-glucosidic linkages, with (1 → 3)-linkages as branch points.[345] Some members of the Entomophthorales have been isolated and analysed for the carbohydrate content of the cell walls. The genera were found to differ qualitatively and the glucose: mannose ratio emerged as the most useful taxonomic criterion.[346] A glycogen-type polysaccharide has been found to accumulate in heat-synchronized cells of *Tetrahymena pyriformis* and was shown to be capable of complexing with ribonucleic acid.[347] The molecular structures of polysaccharides from *Haematococcus pluvialis* and *Tetraselmis carteriiformis*, on examination by chemical and enzymic methods, were characterized as having starch-type structures similar in composition and structure to those of higher plants.[348]

[339] E. J. Bourne, E. Percival, and B. Smestad, *Carbohydrate Res.*, 1972, **22**, 75.
[340] R. C. White and G. A. Barber, *Biochim. Biophys. Acta*, 1972, **264**, 117.
[341] A. S. Cerezo, *Carbohydrate Res.*, 1972, **22**, 209.
[342] D. W. Lorch and A. Weber, *Akriv. Mikrobiol.*, 1972, **83**, 129.
[343] D. H. Miller, D. T. A. Lamport, and M. Miller, *Science*, 1972, **176**, 918.
[344] D. J. Hill, *New Phytol.*, 1972, **71**, 31.
[345] E. Hauan and O. Kjølberg, *Acta Chem. Scand.*, 1971, **25**, 2622.
[346] J. Hoddinott and O. A. Olsen, *Canad. J. Botany*, 1972, **50**, 1675.
[347] E. G. Christensson, *Comp. Biochem. Physiol.*, 1971, **40B**, 381.
[348] Sir E. L. Hirst, D. J. Manners, and I. R. Pennie, *Carbohydrate Res.*, 1972, **22**, 5.

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Microbial Polysaccharides

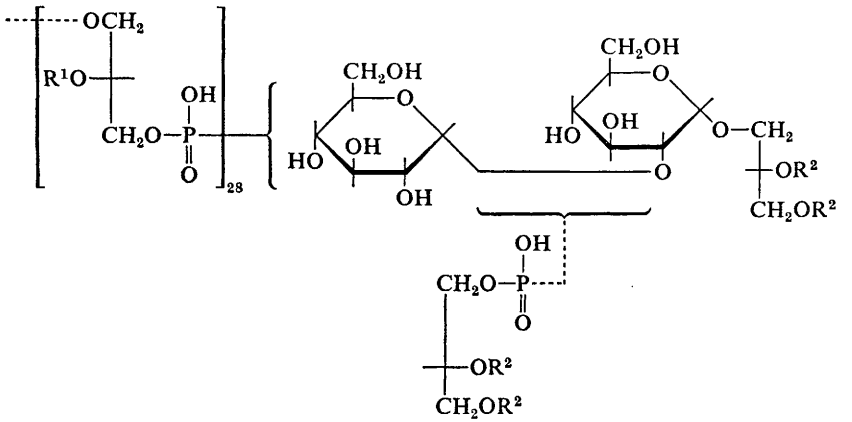
Bacterial Cell Walls and Membranes

Teichoic Acids.—A review of teichoic acids has included sections on the structure of glycerol phosphate polymers and ribitol phosphate polymers, their biosynthesis *via* nucleotide and lipid intermediates, and their function.[349] The occurrence, location, structure, biosynthesis, and function of teichoic acids in walls and membranes has also been reviewed.[350] Detailed procedures for the isolation and characterization of teichoic acids have been reported.[351] The role of membranes in the synthesis of teichoic acids, lipopolysaccharides, and peptidoglycans has been the subject of a treatise.[352]

The qualitative and quantitative chemical composition of five strains of streptococci have been studied. Presumptive evidence was presented for the existence of glycerol teichoic in *Streptococcus mutans*.[353] The lipid–teichoic acid complex in the cytoplasmic membrane of *S. faecalis* exhibited macromolecular properties in solution, which were shown to be due to micelle formation. From chemical studies it was concluded that the teichoic acid is a polyglycerol phosphate in which some of the glycerol hydroxy-groups possess kojibiosyl substituents as well as D-alanine ester residues. The lipid is a previously identified membrane component 1-kojibiosyl diglyceride and is attached to the teichoic acid through a phosphodiester linkage (14).[354]

Synthesis of teichoic acids was examined in two types of phage-resistant mutants having altered cell-wall composition. In Type I, a lack of 2-acetamido-2-deoxy-D-glucose was observed in the wall teichoic acid, although the level of CDP-ribitol:acceptor phosphoribitol transferase was normal. The Type II mutant lacked ribitol teichoic acid, although normal phosphoribitol transferase and 2-acetamido-2-deoxy-D-glucose transferase activities were found.[355] The effects of various salts on the autolysis of the

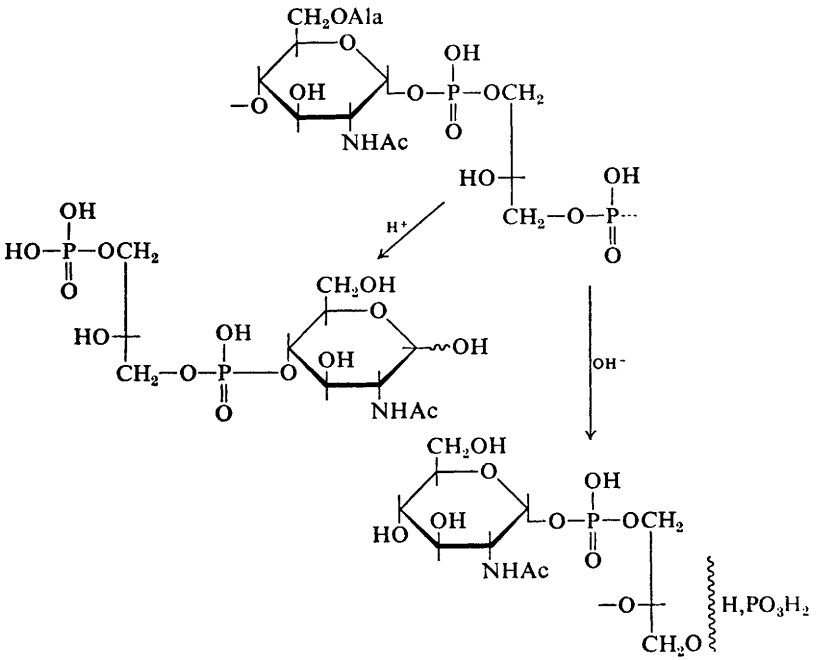
[349] J. Baddiley, *Accounts Chem. Res.*, 1970, **3**, 98.
[350] J. Baddiley, *Essays Biochem.*, 1972, **8**, 35.
[351] A. R. Archibald, *Methods Carbohydrate Chem.*, 1972, **6**, 162.
[352] M. J. Osborn, in 'Structure and Function of Biological Membranes', ed. L. I. Rothfield, Academic Press, 1971, p. 345.
[353] A. S. Bleiwis, R. A. Craig, D. D. Zinner, and J. M. Jablon, *Infection and Immunity*, 1971, **3**, 189.
[354] P. Toon, P. E. Brown, and J. Baddiley, *Biochem. J.*, 1972, **127**, 399.
[355] D. R. D. Shaw, D. Mirleman, A. N. Chatterjee, and J. T. Park, *J. Biol. Chem.*, 1970, **245**, 5101.



(14) R^1 = kojibiosyl or H; R^2 = fatty acid

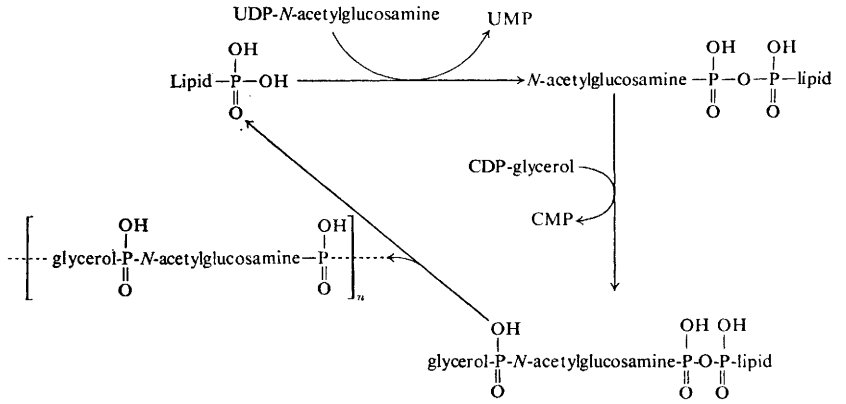
cell walls of a ribitol teichoic acid-deficient mutant of *S. aureus* have been compared with those on the parent strain. Lysis was stimulated by sodium chloride and was related to the activation of *N*-acylmuramyl-L-alanine amidase.[356] Antigenic analysis has been performed on forty-eight strains of *S. epidermidis*, and 67% of the strains were found to contain glycerol α-glucosyl teichoic acid.[357] Immunization of mammary glands of pregnant guinea-pigs with *S. aureus* or isolated mucopeptide induced a local immune response that persisted throughout lactation. When antiserum to guinea-pig immunoglobulin was used, a reaction with teichoic acid was demonstrated by immunoelectrophoresis.[358] Undigested cell walls of two strains of *S. epidermidis* and of two micrococcus strains have been isolated and subjected to chemical and serological examination. Glycerol and hexoses were present in all preparations.[359] The teichoic acid from the walls of *S. lactis* I3 was degraded in alkali, the degradation proceeding by selective hydrolysis of a phosphodiester group attached to an hydroxy-group of 2-acetamido-2-deoxy-D-glucose. Further studies on a different repeating unit, isolated by partial acidic hydrolysis, have shown that the glycerol diphosphate is attached to the 4-hydroxy-group of 2-acetamido-2-deoxy-D-glucose (15) and not to the 3-hydroxy-group as postulated earlier.[360] Particulate enzyme systems, which effect the synthesis of the wall teichoic acid of *S. lactis* I3, have been studied using CDP-glycerol and UDP-2-acetamido-2-deoxy-D-glucose labelled with ^{32}P and ^{14}C. It was shown that the synthesis proceeds by way of lipid intermediates which are derivatives of undecaprenol phosphate. Of two intermediates isolated, one contained

[356] R. W. Gilpin, A. N. Chatterjee, and F. E. Young, *J. Bacteriol.*, 1972, **111**, 272.
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[360] A. R. Archibald, J. Baddiley, J. E. Heckels, and S. Heptinstall, *Biochem. J.*, 1971, **125**, 353.



(15)

2-acetamido-2-deoxy-D-glucose 1-phosphate, whereas the repeating unit (16) of the teichoic acid occurred in the other.[361] The walls of *Micrococcus* sp. 24 have been shown to contain a polyribitol phosphate component, but the ribitol is not present as part of the repeating unit in a simple (1 → 3)-linked glycerol teichoic acid. A third chemically distinct teichoic



(16)

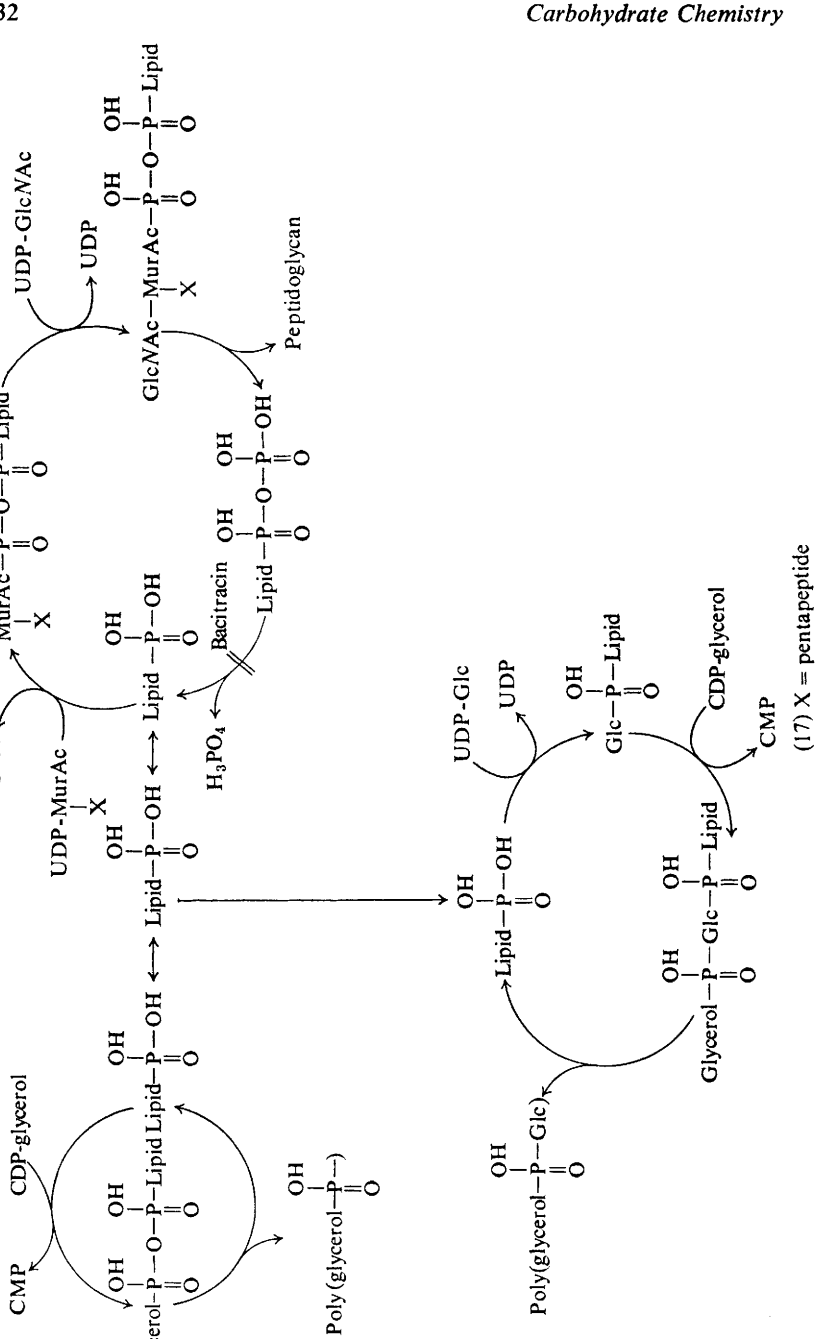
[361] H. Hussey and J. Baddiley, *Biochem. J.*, 1972, **127**, 39.

acid gave degradation fragments identical with those obtained from the teichoic acid of *S. lactis* I3 and is composed of repeating units of 2-acetamido-2-deoxy-D-glucosyl-4-*O*-D-glycerol 1-phosphate, which are connected by phosphodiester linkages between position 1 of the amino-sugar and position 3 of the glycerol residue.[362] Fifty-six *Micrococcus* strains representing eight biochemical types were found to share agglutinogens to a wide extent.[363] When tested against *Micrococcus* immune sera and antigen–antibody systems, it was shown that a large number of the strains contain a wall ribitol teichoic acid to which β-linked 2-acetamido-2-deoxy-D-glucose is attached.[364] Localization of teichoic acids in the cell walls of Gram-negative bacteria has been achieved by specific binding with concanavalin A labelled with mercury or colloidal gold. The teichoic acid localized by this technique showed areas of different density of labelling on electron microscopy, indicating an uneven distribution of the polymer in the cell wall.[365]

Specific antibodies to the wall ribitol teichoic acid of *Lactobacillus plantarum* have been shown to depend primarily on the α-D-glucosyl substituent, since the antibodies cross-reacted with α-D-glycosyl-substituted teichoic acids, but not with unsubstituted ribitol teichoic acid. The specificity of the antibodies to the membrane glycerol teichoic acid is directed against either the D-glucose or glycerol components, depending on the preparation injected.[366] Following a preliminary report,[367] it has been demonstrated that the enzymic incorporation of D-alanine into the membranes of *L. casei* is dependent on ATP, a supernatant fraction containing D-alanine:membrane acceptor ligase, and membrane fragments. The product of the reaction was characterized as a D-alanine ester, which showed a lability towards hydroxylamine similar to that observed for the D-alanine ester residues in glycerol teichoic acid.[368]

A purified enzyme from *Bacillus subtilis* was shown to be totally dependent on the addition of a heat-stable factor for activity. The factor, which was isolated from cell membranes, acted as an acceptor of glycerol phosphate units and contained glycerol phosphate, 2-acetamido-2-deoxy-D-glucose, and fatty acids.[369] Labelling experiments have shown that both teichoic and teichuronic acids of *B. subtilis*, which are synthesized during logarithmic growth and phosphate-limiting conditions, respectively, are attached only to glycopeptide chains synthesized at the same time as these polymers are formed. Examination of the teichoic acid–glycopeptide complex obtained by lysozyme digestion of cells labelled with 2-acetamido-2-deoxy-D-[^{3}H]glucose during phosphate-limiting conditions, and with

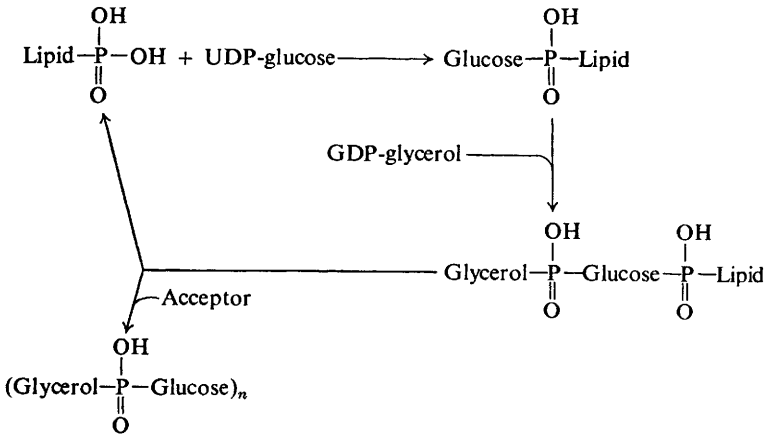
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[369] J. Mauck and L. Glaser, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 6238.


CMP
CDP-glycerol
Glycerol—P—O—P—Lipid
Lipid—P—OH
Poly (glycerol—P—)
UMP
UDP-MurAc
MurAc—P—O—P—Lipid
UDP-GlcNAc
UDP
GlcNAc—MurAc—P—O—P—Lipid
Peptidoglycan
Bacitracin
H_3PO_4
Lipid—P—O—P—OH
UDP-Glc
Glc—P—Lipid
CDP-glycerol
Glycerol—P—Glc—P—Lipid
Poly(glycerol—P—Glc)
(17) X = pentapeptide

2-amino-2-deoxy-D-[^{14}C]glucose after resumption of logarithmic growth, showed that the glycan chains adjacent to those linked covalently to teichoic acid represent a random selection of new and old chains. This indicated that during growth of the cell wall there was a random intercalation of new and old chains.[370] A decrease in teichoic acid synthesis, coupled with a rapid turnover of this polymer, has been observed in a temperature-sensitive mutant of *B. subtilis*, whose cells were shown to contain the enzymes involved in the biosynthesis and D-glucosylation of teichoic acids, but which were deficient in the autolytic enzyme *N*-acylmuramyl-L-alanine amidase.[371]

The synthesis of peptidoglycan and teichoic acid by cell-free preparations from *B. subtilis* and *B. licheniformis* has indicated that polyglycerol phosphate is synthesized by way of a lipid intermediate, as are all major bacterial wall polymers. Close correlation between the synthesis of peptidoglycan and teichoic acid was demonstrated; inhibition studies confirmed that the polyprenol phosphate molecules participating in the synthesis of peptidoglycan are shared with systems synthesizing teichoic acids. Nucleotides involved in the synthesis of one polymer were inhibitory towards the synthesis of the other, and these effects could be enhanced or diminished by pre-incubation of the enzyme systems with the appropriate nucleotide precursors. It was concluded that the return of undecaprenol phosphate to a common pool occurs only after completion of the polymer chains and not after each cycle in the attachment of individual repeating units. This and other observations supported a model (17) for bacterial-wall synthesis in which multi-enzyme systems for each polymer are closely aligned in the membranes with a molecule of undecaprenol phosphate located between them in a manner enabling it to be shared.[372] In the biosynthesis of the wall teichoic acid in *B. licheniformis*, it was shown that the first intermediate is the phosphodiester D-glucose polyprenol monophosphate, which is converted into a second intermediate by the donation of glycerol phosphate from CDP-glycerol. The D-glucose residue in the lipid intermediate has the β-configuration, indicating that chain extension in the synthesis of the polymer occurs by transglycosylation with inversion of configuration at two stages [see (18)].[373] However, it has been reported that in the D-glucosylation of the teichoic acid of *B. subtilis*, D-glucosyl transfer is mediated by both membrane-bound and soluble forms of UDP:polyglycerolteichoic acid glucosyl transferase in a mechanism not mediated by lipids.[374] α-Amylase formation by washed cell suspensions of *B. subtilis* was found to be accompanied by the excretion of 2-*O*-α-D-glucopyranosylglycerol 3-phosphate and a polymerized form (through phosphodiester

[370] J. Mauck and L. Glaser, *J. Biol. Chem.*, 1972, **247**, 1180.
[371] R. J. Boylan, N. H. Mendelson, D. Brooks, and F. E. Young, *J. Bacteriol.*, 1972, **110**, 281.
[372] R. G. Anderson, H. Hussey, and J. Baddiley, *Biochem. J.*, 1972, **127**, 11.
[373] I. C. Hancock and J. Baddiley, *Biochem. J.*, 1972, **127**, 27.
[374] D. Brooks, L. L. Mays, Y. Hatefi, and F. E. Young, *J. Bacteriol.*, 1971, **107**, 223.



(18)

linkages) involving C-3 of glycerol. This polymer, a kind of teichoic acid, was the dominant material excreted when the cells were incubated under conditions suitable for α-amylase formation, whereas the low molecular weight material prevailed when the cells were incubated under conditions unfavourable for enzyme formation.[375]

Concanavalin A has been used as a probe to study structural features of bacterial cell walls. The interaction between concanavalin A and cell-wall digests of *B. subtilis* resulted in the formation of insoluble complexes in which glucosyl teichoic acid was identified as the macromolecular constituent of the walls being complexed. The data suggested that, for complex formation to occur, a rigid rod conformation of the glucosylated teichoic acid is probably necessary.[376] Extracts from two *Bacillus* species have been purified and identified as glycerol teichoic acids. Although neither alanine nor other amino-acids were present, spontaneous adsorption to erythrocytes was demonstrated.[377]

Bacterial phosphodiesterases capable of cleaving the backbone of teichoic acid have been isolated after autoradiography of soil suspensions grown on a medium containing ^{32}P-labelled *B. subtilis* cell walls as the sole source of phosphate. Broken cell preparations were capable of depolymerizing teichoic acids in media of low ionic strength.[378]

A mutant of *Staphylococcus aureus* has been isolated by virtue of its inability to agglutinate with antibodies against the teichoic acid of *S. aureus*. Immunological studies revealed that the mutant possesses a new

[375] N. Hamada, J. Fukumoto, and T. Yamamoto, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1052.
[376] R. J. Doyle and D. C. Birdsell, *J. Bacteriol.*, 1972, **109**, 652.
[377] G. P. Decker, F. W. C. Horpenning, and G. T. Frederick, *J. Immunol.*, 1972, **108**, 214.
[378] E. M. Wise, R. S. Glickman, and E. Teimer, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 233.

surface antigen which, when separated from teichoic acid, was found to be composed of 2-acetamido-2-deoxy-D-fucose and 2-acetamido-2-deoxy-D-mannopyranosyluronic acid.[379] A teichoic acid composed of glycerol, 2-acetamido-2-deoxy-D-glucose, and D-alanine has been extracted from Group A streptococcal polysaccharide antigens; the antigenic specificity was shown to be due to the amino-sugar and D-alanine methyl ester.[380]

Peptidoglycans.—The chemical composition and the structure of peptidoglycans present in bacterial cell walls have been described.[381] Views on the biosynthesis of these polymers have been presented in a review in which the biosynthesis of intracellular precursors and the role of lipid intermediates were summarized.[382]

Muramic acid derived from bacterial cell walls has been determined in an automated amino-acid analyser.[18]

X-Ray diffraction studies of cell walls and peptidoglycans from Gram-positive bacteria have indicated that they have few stereochemically determined structures in common. Observations indicated that the structural order in the walls and peptidoglycans is short range and analogous to the order found in randomly cross-linked polymers.[383]

Degradation of the mucopeptide-lipoprotein complex of *Escherichia coli* by *Dictyostelium* involved cleavage of the bond between *N*-acetylmuramic acid and L-alanine, and resulted in the release of L-alanine from the peptide side-chain.[384] The lipid component of the complex was found to be attached to *N*-terminal serine, while the mucopeptide is bound to the 6-amino-group of the *C*-terminal lysine residue[385] and to the carboxy-group of the chiral L-centre of *meso*-diaminopimelic acid (DAP).[386, 387]

Studies on *E. coli* enzymes involved in the synthesis of UDP-*N*-acetylmuramyl pentapeptide have been reported.[388] A number of the properties of temperature-sensitive mutants of *E. coli* involved in mucopeptide synthesis have been described. One mutant, which had an impaired rate of mucopeptide synthesis *in vivo*, possessed a temperature-sensitive D-alanyl-D-alanine-adding enzyme and contained elevated levels of UDP-Mur*N*Ac-L-Ala-D-Glu-*meso*-DAP.[389] Mutants of *E. coli* possessing very low DAP-adding enzyme activity had impaired rates of mucopeptide synthesis and were shown to accumulate UDP-Mur*N*Ac-L-Ala-D-Glu.[390] A group of temperature-sensitive lysis mutants impaired in the synthesis

[379] T. C. M. Wu and J. T. Park, *J. Bacteriol.*, 1971, **108**, 874.
[380] T. Matsuno and H. D. Slade, *Infection and Immunity*, 1971, **3**, 385.
[381] A. Klein and A. Rais, *Postepy Biochem.*, 1972, **18**, 225.
[382] A. Rais and A. Klein, *Postepy Biochem.*, 1972, **18**, 241.
[383] H. H. M. Balyuzi, D. A. Reaveley, and R. E. Burge, *Nature New Biol.*, 1972, **235**, 252.
[384] V. Braun, K. Hantke, H. Wolff, and G. Gerisch, *European J. Biochem.*, 1972, **27**, 116.
[385] V. Braun and V. Bosch, *European J. Biochem.*, 1972, **28**, 51.
[386] V. Braun and V. Bosch, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 970.
[387] V. Braun and V. Bosch, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2354.
[388] E. J. J. Lugtenberg, *J. Bacteriol.*, 1972, **110**, 26.
[389] E. J. J. Lugtenberg and A. van Schijndel-Van Dam, *J. Bacteriol.*, 1972, **110**, 35.
[390] E. J. J. Lugtenberg and A. van Schijndel-Van Dam, *J. Bacteriol.*, 1972, **110**, 41.

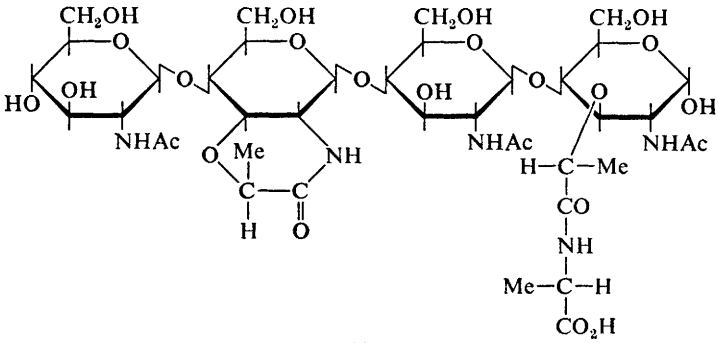
of UDP-Mur*N*Ac-pentapeptide and in the synthesis of mucopeptide amino-acids has been reported. The amount of mucopeptide precursor was considered to be regulated by feedback inhibition.[391] In another study, temperature-sensitive mutants of *E. coli*, which could not form cell-wall peptidoglycan, were isolated. The thermosensitive steps were characterized biochemically and the genes coding the enzymes of peptidoglycan synthesis were mapped. No simple relations were found between the gene location and the order or localization of enzymes involved in the sequence of reactions or cell-wall peptidoglycan biosynthesis.[392]

Proteolytic and lysozymic digestion of *Mycobacterium smegmatis* produced a water-soluble product (molecular weight 2×10^4) having a typical cell-wall composition of 2-acetamido-2-deoxy-D-glucose and muramic acid (in equimolar amounts), arabinose, galactose, alanine, glutamic acid, and diaminopimelic acid. The peptidoglycan is linked to an arabinogalactan characteristic of mycobacterial cell walls; this soluble fraction was strongly adjuvant active in increasing the amount of precipitating antibodies and of inducing hypersensitivity to ovalbumin in guinea-pigs.[393] A product having similar biological properties was prepared by a simple extraction technique from human mycobacterial cells.[394] A water-soluble peptidoglycan-arabinogalactan from mycobacterial cell walls was shown to be effective in increasing the immune response to viruses.[395] An arabinogalactan from the walls of *M. tuberculosis* has been degraded enzymically by *endo*-attack of arabinosidic linkages, producing high and low molecular weight fractions rich in galactose and arabinose, respectively.[396] A polysaccharide from *M. phlei* was found to contain ten (1 → 4)-linked 3-*O*-methyl-D-mannose residues in a homologous chain, which probably forms a macrocyclic ring. In addition, two D-mannose residues are linked to each other in (1 → 2)-linkage and occur either as a 'non-reducing' end of the polysaccharide or as a side-chain on the postulated macrocyclic ring. Inhibition of precipitin reactions and other evidence confirmed the presence of at least two α-(1 → 2)-linked D-mannose residues at a terminal position on the methylated mannose polysaccharide.[397]

Various methods for extraction and purification of the peptidoglycan from *Proteus vulgaris* have been investigated. A procedure was recommended in which the bacteria were neither disintegrated nor washed, but were treated with sodium dodecyl sulphate before proteolytic digestion.[398]

[391] E. J. J. Lugtenberg, L. De Haas-Menger, and W. H. M. Ruyters, *J. Bacteriol.*, 1972, **109**, 326.
[392] T. Miyakawa, H. Matsuzawa, M. Matsuhashi, and Y. Sugino, *J. Bacteriol.*, 1972, **112**, 950.
[393] A. Adam, R. Ciorbaru, J. F. Petit, and E. Lederer, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 851.
[394] D. Migliore-Samour and P. Jollès, *F.E.B.S. Letters*, 1972, **25**, 301.
[395] L. Chedid, M. Parant, F. Parant, R. H. Gustafson, and F. M. Berger, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 855.
[396] S. Kotani, T. Kato, T. Matsubara, M. Sakagoshi, and Y. Hirachi, *Biken J.*, 1972, **15**, 1.
[397] G. R. Grey and C. E. Ballou, *J. Biol. Chem.*, 1971, **246**, 6835.
[398] J. Fleck and M. Mock, *Compt. rend.*, 1971, **272**, *D*, 1560.

Autolysis of *Bacillus subtilis* vegetative cell walls cleaved the muramyl–L-alanine bond of the peptidoglycan to yield peptides, a glycan composed of 2-acetamido-2-deoxy-D-glucose and *N*-acetylmuramic acid, and a teichoic acid–polysaccharide complex. The glycan was shown to contain twenty-three hexosamine residues terminating at the reducing end with 2-acetamido-2-deoxy-D-glucose. The cell-wall peptides differ from peptides in *Escherichia coli* and *B. megaterium* cell walls and from those of *B. subtilis* and other spores in having diaminopimelyl amides and in lacking carboxy-terminal D-alanine.[399] Heat-inactivated spores of *B. subtilis* yielded a peptidoglycan after treatment with lysozyme. The glycan component consisted of long chains of alternating 2-acetamido-2-deoxy-D-glucose and muramic acid, probably all β-(1 → 4)-linked, with half of the muramic acid residues present as the 5-lactam. The remainder of the residues are *N*-acetylated and the side-chain carboxy-groups are substituted with either a peptide or L-alanine residue. Muramolactam glycosidic bonds were found to be resistant to hydrolysis by lysozyme; the principal products of digestion were tetrasaccharides containing an internal muramolactam and a reducing, terminal *N*-acetylmuramic acid residue (19).[400] The release of



(19)

mucopeptide from the spore coats of *B. megaterium* by lysozyme was inhibited by bubbling simple hydrocarbons into the system. The hydrocarbon did not inhibit directly the hydrolysis of β-(1 → 4)-glucosaminidic linkages in the polysaccharide, but inhibited the binding of the enzymes to the bacterial cells or spores by changing the states of these regions.[401]

Penicillin-sensitive transpeptidation during peptidoglycan biosynthesis in cell-free preparations from *B. megaterium* has been described. A particulate enzyme preparation utilized UDP-*N*-acetylmuramyl-pentapeptide (UDP-Mur*N*Ac-L-Ala-D-Glu-*meso*-DAP-D-Ala-D-Ala) and UDP-2-acetamido-2-deoxy-D-glucose. Unlike systems previously studied in Gram-

[399] A. D. Warth and J. L. Strominger, *Biochemistry*, 1971, **10**, 4349.
[400] A. D. Warth and J. L. Strominger, *Biochemistry*, 1972, **11**, 1389.
[401] K. Watanabe, *Enzymolgia*, 1971, **41**, 99.

positive micro-organisms, the preparation catalysed the terminal cross-linking reaction in cell-wall biosynthesis as well as incorporating free DAP. Incorporation of DAP was inhibited by penicillins and the DAP appeared to be incorporated on to a terminal position of a disaccharide-pentapeptide peptidoglycan unit with the release of alanine.[402] Inhibition of trans-peptidation during peptidoglycan biosynthesis in cell-free preparations from *B. megaterium* has been brought about by penicillin G and cloxacillin, which inhibited the incorporation of [^{14}C]DAP. The effect appeared to be partially reversible after the antibodies were destroyed by treatment with penicillinase.[403] The resistance of cell walls of *B. cereus* to lysozyme has been attributed to the presence of over 70% of the 2-amino-2-deoxy-D-glucose residues in non-acetylated form, as determined by the identification from acidic hydrolysates of the peptidoglycan of the disaccharide 2-amino-2-deoxy-D-glucosyl-β-(1 $\rightarrow$ 4)-muramic acid 6-phosphate. Those cell walls that remained resistant to lysozyme after removal of *O*-acetyl groups, polysaccharide, and peptide components were converted into the lysozyme-sensitive form on *N*-acetylation.[404] Preliminary evidence has indicated that the insoluble residue remaining after temperature-mediated lysis of *B. psychrophilus* cell walls is primarily a peptidoglycan.[405]

Cell-wall preparations from *Micrococcus lysodeikticus* were shown to catalyse *in vivo* peptidoglycan synthesis from UDP-2-acetamido-2-deoxy-D-glucose, UDP-*N*-acetylmuramylpentapeptide, and glycine, which were partially cross-linked by a transpeptidation reaction with concomitant release of *C*-terminal D-alanine. Penicillin was shown to inhibit strongly the release of D-alanine and the incorporation of 2-acetamido-2-deoxy-D-glucose and *N*-acetylmuramylpentapeptide into preformed cell-wall peptidoglycan. It was suggested that the incorporation of newly synthesized strands of peptidoglycan and their attachment to older cell-wall polymer proceeds mainly by transpeptidation, and that transglycosylation is responsible only for part of the elongation of pre-existing glycan.[406] A study of the relationship existing between the condition of cell-wall mucopeptide and cell-division activity has been reported.[407]

Fractionation of the non-dialysable components from the lysozyme digestion of *M. lysodeikticus* cell walls revealed the presence of a polymer of D-glucose and 2-acetamido-2-deoxy-D-mannosyluronic acid linked by phosphate ester groups to short fragments of mucopeptide backbone, probably through muramic acid 6-phosphate.[408] A diffusible fraction from similarly treated *M. lysodeikticus* walls has been purified and identified as

[402] G. G. Wickus and J. L. Strominger, *J. Biol. Chem.*, 1972, **247**, 5297.
[403] G. G. Wickus and J. L. Strominger, *J. Biol. Chem.*, 1972, **247**, 5307.
[404] Y. Araki, T. Nakatani, and K. Nakayama, *J. Biol. Chem.*, 1972, **247**, 6312.
[405] S. J. Mattingly and G. K. Best, *J. Bacteriol.*, 1972, **109**, 645.
[406] D. Mirelman, R. Brancha, and N. Sharon, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3355.
[407] R. D. King and E. A. Grula, *Canad. J. Microbiol.*, 1972, **18**, 519.
[408] Y. Imanaga and J. T. Park, *Biochemistry*, 1972, **11**, 4006.

a tetrasaccharide containing two residues of 2-acetamido-2-deoxy-D-glucose, one residue of *N*-acetylmuramic acid, and one residue of muramic acid (probably as the internal amide). A disaccharide, which was also isolated, was identified as 2-acetamido-2-deoxy-D-glucosyl-(1 → 4)-D-*manno*-muramic acid.[409] The biosynthesis of a polysaccharide resembling the D-glucose and 2-acetamido-2-deoxy-D-mannosyluronic acid polymer of *M. lysodeikticus* cell walls was achieved using a particulate enzyme fraction that catalysed the incorporation of D-[^{14}C]glucose from UDP-D-[^{14}C]glucose. The *in vitro* biosynthesis of the polymer depended on the presence of a UDP-2-acetamido-2-deoxyhexosyluronic acid fraction, which was isolated from the organism, and UDP-2-acetamido-2-deoxy-D-glucose.[410] The nucleotide uronic acid fraction was identified as a mixture of UDP-2-acetamido-2-deoxy-D-glucosyluronic and -mannosyluronic acids.[411] A direct demonstration of the formation of a complex between bacitracin and the $C_{(55)}$-isoprenylpyrophosphate of *M. luteus* in the presence of metal ions has been reported.[412]

The effect of growth conditions on peptidoglycan structure and susceptibility to lytic enzymes of the cell walls of *M. sodonensis* has been reported. The walls from cells grown in a synthetic medium contained twice as many hexosamine residues as those from cells grown in a complex medium. The former walls were degraded to a greater extent by lysozyme and the overall peptidoglycan structure was more complex in walls of cells grown in the complex medium. Chemical analysis and electron microscopic examination led to the conclusion that, in both cases, the cell wall is composed of at least two peptidoglycan matrices, one of which is insensitive to lysozyme. This resistance results from a combination of an increased level of *O*-acetyl substitution and an increased complexity of cross-linked structures.[413]

Chemical analysis of the mucopeptides isolated from a *Staphylococcus aureus*, two *S. epidermidis*, and two *Micrococcus* strains showed differences in structure that are related partly to variations in the interpeptide bridges. Antibodies to the mucopeptides were isolated from human serum and colostrum. The Fab, but not the Fc, fragments of the immunoglobulin IgG class became fixed to the mucopeptides in a specific antibody–antigen reaction.[414] Peptidoglycans or cell walls of *S. epidermidis*, *Lactobacillus acidophilus*, *Micrococcus lysodeikticus*, and *Corynebacterium poinsettiae*, which had been solubilized by ultrasonic treatment, gave precipitin reactions with *Streptococcus* Group A-variant antisera. By means of inhibition studies with the pentapeptide L-Ala-D-Glu-γ-L-Lys-D-Ala-D-Ala and 2-acetamido-2-deoxy-D-glucose, it was demonstrated that the peptidoglycan

[409] O. Hoshino, U. Zehavi, P. Sinaÿ, and R. W. Jeanloz, *J. Biol. Chem.*, 1972, **247**, 381.
[410] R. L. Page and J. S. Anderson, *J. Biol. Chem.*, 1972, **247**, 2471.
[411] J. S. Anderson, R. L. Page, and W. L. Salo, *J. Biol. Chem.*, 1972, **247**, 2480.
[412] K. J. Stone and J. L. Strominger, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 3223.
[413] K. G. Johnson and J. N. Campbell, *Biochemistry*, 1972, **11**, 277.
[414] S. M. Helgeland and A. Grov, *Acta Pathol. Microbiol. Scand.*, 1971, **79B**, 819.

of *S. epidermidis* and the cell walls of *L. acidopholus* react with antibodies to the pentapeptide, whereas trichloroacetic acid extracts of the walls of *M. lysodeikticus* and *C. poinsettiae* precipitate predominantly antibodies directed against the glycan part of the peptidoglycan. This was considered to be due to the presence of peptide subunits ending in D-Ala-D-Ala in the peptidoglycans of *S. epidermidis* and *L. acidophilus*, whereas the other peptidoglycans lack this determinant.[415]

The biosynthesis of pentaglycine, the cross-linking peptide in the cell-wall peptidoglycan of *S. aureus*, has been shown to require glycyl-tRNA as a donor of glycine. Biosynthesis occurs by the sequential addition of the amino-acid on to the ε-amino-group of the L-lysine residue of the pentapeptide in a lipid-linked precursor.[416] The newly synthesized peptidoglycan is partially cross-linked by transpeptidation, as evidenced by the release of D-[^{14}C]alanine, and this reaction is strongly inhibited by small concentrations of penicillin G. The antibiotic also partially inhibited the incorporation of glycine into soluble, cell-wall peptidoglycan.[417] Hydrolysis of isolated cell walls of *S. aureus* with lysostaphin released the disaccharide 2-acetamido-2-deoxy-4-*O*-β-*N*-acetylmuramyl-D-glucose and an *O*-acetyl derivative. The purified disaccharides comprised 85% of the glycan component and the linkages were characterized as β-(1 → 4) by periodate oxidation and n.m.r. spectroscopy. Since the complementary disaccharide 4-*O*-(2-acetamido-2-deoxy-D-glucosyl)-*N*-acetylmuramic acid had been isolated previously in high yield from cell walls of the same organism, the glycan has a fully *N*-acetylated β-(1 → 4)-linked structure.[418] However, neither tRNA nor glycyl-tRNA synthetase appeared to be necessary for *in vitro* peptidoglycan synthesis from UDP-2-acetamido-2-deoxy-D-glucose and UDP-*N*-acetylmuramylpentapeptide. The regulation of the bacterial cell wall has been studied by characterization of the peptidoglycans from *S. aureus* mutants. From a mutant defective in the biosynthesis of peptidoglycan at non-permissive temperatures, it was observed that reduced incorporation of cell-wall precursors into peptidoglycan is accompanied by an accumulation of UDP-*N*-acetylmuramyl-L-alanyl-D-glutamate and a reduction in the specific activity of UDP-*N*-acetylmuramyl-L-alanyl-D-glutamate:L-lysine ligase. All of these effects, with the exception of that of the ligase, were reversed in the presence of molar sodium chloride.[419] Analytical results have indicated that every cross-linkage of a staphylococcal peptidoglycan is broken after dissolution of the walls in alkali.[420]

Peptidoglycan composition has been used as an aid in the division of members of an immunological group into sub-groups and to indicate the relationship between members of the same genus that are not related on an

[415] K. H. Schleifer and R. M. Krause, *European J. Biochem.*, 1971, **19**, 471.
[416] T. Kamiryo and M. Matsuhashi, *J. Biol. Chem.*, 1972, **247**, 6306.
[417] D. Mirelman and N. Sharon, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1909.
[418] D. J. Tipper, M. Tomoeda, and J. L. Strominger, *Biochemistry*, 1971, **10**, 4683.
[419] A. N. Chatterjee and F. E. Young, *J. Bacteriol.*, 1972, **111**, 220.
[420] A. R. Archibald, J. Baddiley, and J. G. Goundry, *Biochem. J.*, 1970, **116**, 313.

immunological basis. Thus, in Group D *Streptococcus*, *S. faecalis* and the closely related species *S. liquefaciens* and *S. zymogenes* were separated from *S. faecium* and *S. durans* on the basis of their peptidoglycan composition.[421] Group A Streptococci and their isolated cell walls, which are normally resistant to lysozyme and lysosomal enzymes of human phagocytes, were converted into lysozyme-sensitive forms by partial removal of the cell-wall carbohydrate, substitution of free amino-groups, and saponification of *O*-acetyl groups.[422] The cells of *S. lactis* bound increased levels of lysozyme when pretreated with either thioglycolic acid or propanol.[423] In the preparation of spheroplasts from *S. lactis*, stationary phase cells resisted lysis by lysozyme, but, in the presence of either sodium chloride or sodium dodecyl sulphate, prompt lysis occurred with the retention of a rhamnose wall polymer.[424]

Cell walls of Group A *S. pyogenes* have been solubilized by treatment with a purified preparation of a *Flavobacterium* enzyme. A high molecular weight fraction obtained from the cell digest contained rhamnose, 2-amino-2-deoxy-D-glucose, muramic acid, glutamic acid, alanine, and lysine as the major constituents, and seemed to be a complex of glycopeptide and group-specific C-polysaccharide. The pyrogenetic and related biological activities of this preparation were recorded.[425] On daily intraperitoneal injections into mice, the high molecular weight fraction induced cardiac lesions similar to those induced by cell-wall fragments obtained by sonic oscillation. This demonstrated that Group A streptococcal walls did not require an optimum particle size for manifestation of cardiotoxicity, but are active as a soluble complex of C-polysaccharide and glycopeptide.[426]

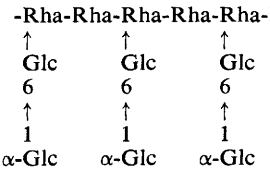
The peptidoglycan obtained on treatment of a filamentous form of *Proteus* P_{18} with small doses of penicillin was found to be identical with that of the non-treated form.[427] Extraction of *Shigella sonnei* cell walls with hot formamide solubilized a peptidoglycan, which was found to contain 2-amino-2-deoxy-D-glucose, muramic acid, glycine, alanine, glutamic acid, DAP, and valine.[428] No structural alterations of the peptidoglycans were detected on comparison of two types of filamentous cell-division mutants of *Agmenellum quadruplicatum* with the parent organism.[429] The cell-wall peptidoglycan of *Nocardia kirorani* has been shown to be composed of 2-acetamido-2-deoxy-D-glucosyl-β-(1 → 4)-*N*-glycolylmuramic acid units substituted by diamidated L-Ala-D-α-Glu-(L)-DAP tripeptides and diamidated L-Ala-D-α-Glu-(L)-DAP-(L)-D-Ala tetrapeptides.[430]

[421] H. D. Slade and W. C. Slamp, *J. Bacteriol.*, 1972, **109**, 691.
[422] A. D. Glick, J. M. Ranhand, and R. M. Cole, *Infection and Immunity*, 1972, **6**, 403.
[423] A. Hurst and H. Kruse, *Canad. J. Microbiol.*, 1972, **18**, 945.
[424] H. Kruse and A. Hurst, *Canad. J. Microbiol.*, 1972, **18**, 825.
[425] S. Hamada, T. Narita, S. Kotani, and K. Kato, *Biken J.*, 1971, **14**, 217.
[426] T. Narita, S. Hamada, S. Kotani, K. Hasegawa, and T. Ishida, *Biken J.*, 1971, **14**, 233.
[427] M. Mock, J. Fleck, and J. P. Martin, *Compt. rend.*, 1972, **275**, *D*, 611.
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[429] L. O. Ingram, C. Van Baalen, and W. D. Fisher, *J. Bacteriol.*, 1972, **111**, 614.
[430] M. J. Vacheron, M. Guinand, G. Michel, and J. M. Ghuysen, *European J. Biochem.*, 1972, **29**, 156.

Sacculus-like peptidoglycan structures have been obtained from growing cells of a marine Pseudomonad and were shown to contain 2-amino-2-deoxy-D-glucose, muramic acid, alanine, glutamic acid, and DAP, with almost half of the ε-amino-groups of DAP involved in cross-linkages.[431] The same components were found in the peptidoglycan of *P. aeruginosa*, which also contains protein covalently bound to the polymer.[432] The antibiotics enduracidin, moenomycin, and prasinomycin have been used to inhibit the incorporation of labelled L-alanine into the peptidoglycans of *Bacillus cereus* and *Staphylococcus aureus*, giving rise to an accumulation of radioactive UDP-*N*-acetylmuramyl-pentapeptides. The antibiotics similarly inhibited peptidoglycan synthesis by *B. stearothermophilus* particulate enzyme by strongly inhibiting the utilization of the 2-acetamido-2-deoxy-D-glucosyl-*N*-acetylmuramylpentapeptide–phospholipid complex.[433] The cell envelope of *Halobacterium halobium* was found to lack a peptidoglycan component. However, extraction with aqueous phenol solubilized a sulphated mannan.[434]

A homogeneous polymer (molecular weight 1.85×10^4) containing 2-amino-2-deoxy-D-galactose, acetate, and phosphorus (1 : 1 : 0.35) has been isolated from the cell wall of *Neisseria sicca*. Methylation analysis indicated that the main glycosidic linkages, probably in the α-configuration, are (1 → 4)- and (1 → 6)-linkages with a minimum of 10% branch points.[435]

Two antigenic glycans from the cell walls of *Streptococcus bovis* have been fractionated into a diheteroglycan component, containing glucose and rhamnose (1 : 1), and a triheteroglycan composed of galactose, rhamnose, and 6-deoxy-L-talose (2 : 1 : 1). On the basis of immunological, chemical, and enzymic evidence, it was suggested that the diheteroglycan is composed of a main chain of rhamnosyl units to which is attached many side-chains of isomaltosyl units; the latter are the immunodominant groups in the glycan and are responsible for the antigenic specificity of the polysaccharide (20).[436] On the basis of immunochemical data and the nature of the products of acidic hydrolysis, it was proposed that the triheteroglycan



(20)

[431] C. W. Forsberg, M. K. Rayman, J. W. Costerton, and R. A. MacLeod, *J. Bacteriol.*, 1972, **109**, 895.
[432] H. D. Heilmann, *European J. Biochem.*, 1972, **31**, 456.
[433] E. J. J. Lugtenberg, A. van Schijndel-Van Dam, and T. H. M. Van Bellegem, *J. Bacteriol.*, 1971, **108**, 20.
[434] M. Koncewicz, *Biochem. J.*, 1972, **130**, 40P.
[435] G. A. Adams and A. S. Chaudhari, *Canad. J. Biochem.*, 1972, **50**, 345.
[436] J. A. Kane, W. W. Karakawa, and J. H. Pazur, *J. Immunol.*, 1972, **108**, 1218.

consists of a main chain of alternating rhamnose and 6-deoxytalose residues with side-chains of digalactosyl units, and that it contains terminal α-linked galactosyl residues as the immunodeterminants.[437] Extracts from streptococcal Group E cells contained a wall-polysaccharide antigen composed of rhamnose and glucose (2 : 1). The reaction of specific group E rabbit antiserum with the polysaccharide was effectively inhibited by D-glucose and β-D-glucosides, whereas L-rhamnose was ineffective. Partial acidic hydrolysis of the antigen released a series of oligosaccharides. A study of these fractions by chemical analysis, inhibition of the antibody–antigen reaction, release of D-glucose by β-glucosidase, and other evidence indicated that β-D-glucose is the immunodominant sugar in the antigen. The most effective inhibitor was a trisaccharide having the structure β-D-Glc-(1 → 2)-α-L-Rha-(1 → 4)-L-Rha.[438] The group O streptococcal group antigen was shown to be a polysaccharide located in the cell wall; it is composed of 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, D-glucose, and D-galactose. Strong inhibition of the precipitin reaction was exerted by 2-acetamido-2-deoxy-D-glucose. The O-antigen is unusual for streptococcal polysaccharide antigens in that it does not contain L-rhamnose.[439]

An antigenic polysaccharide from *Mycobacterium tuberculosis*, which contains arabinose, galactose, and mannose, was enzymically depolymerized by cleavage of the D-arabinosidic linkages with concomitant loss of its ability to precipitate with rabbit antisera to the cells. A low molecular weight component, which effectively inhibited precipitation between undegraded polysaccharide and the antiserum, was found by methylation studies to be an arabinohexaose containing three residues of α-(1 → 5)-linked D-arabinofuranose units and two residues of α-(1 → 2)-linked D-arabinofuranose units.[440]

Lipopolysaccharides

Improved, rapid, quantitative procedures have been developed for the assay of the characteristic deoxy-sugars of Gram-negative bacterial lipopolysaccharides. The separation of sugars from the rest of the cell components was not necessary and mathematical processing of the results was carried out on a computer.[17] A procedure for determining the hexose and heptose contents of lipopolysaccharides has given increased sensitivity.[16]

Detailed methods for the isolation of lipopolysaccharides from Gram-negative bacteria have been reported.[441, 442] When lipopolysaccharides

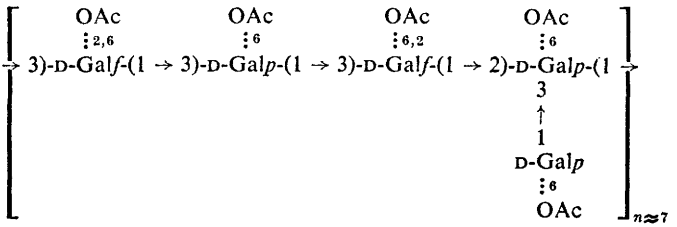
[437] J. H. Pazur, J. A. Kane, D. J. Dropkin, and L. M. Jackman, *Arch. Biochem., Biophys.*, 1972, **150**, 382.
[438] P. Soprey and H. D. Slade, *Infection and Immunity*, 1971, **3**, 653.
[439] H. Musaka and H. D. Slade, *Infection and Immunity*, 1972, **5**, 707.
[440] S. Kotani, T. Kato, T. Matsuda, K. Kato, and A. Misaki, *Biken J.*, 1971, **14**, 379.
[441] G. A. Adams, *Methods Carbohydrate Chem.*, 1972, **6**, 157.
[442] H. E. Conrad, *Methods Carbohydrate Chem.*, 1972, **6**, 154.

were treated with tyramine and sodium periodate, a tyraminyl polysaccharide was formed; this could be reduced and iodinated to provide a facile method for labelling the polysaccharide with radioactive iodine.[443] Additional evidence for the existence of a bilayer structure in Gram-negative liposaccharides has been obtained; both heating in an electric beam and cleavage of ester-bound fatty acids by alkali apparently split the bilayer structure into monolayers.[444] Lipopolysaccharides isolated from *Escherichia coli* and *Salmonella typhimurium* have been shown to be affected functionally and morphologically by polymyxin.[445] Bacterial lipopolysaccharides given orally to mice in relatively small amounts have produced significant amounts of circulating interferon.[446]

Studies on the *Klebsiella* O group 4 lipopolysaccharides have indicated that the O-specific side-chains are composed of the disaccharide repeating units → 2)-β-D-Rib*p*-(1 → 4)-α-D-Gal*p*-(1 → . It was not decided if the 'biological' repeating unit has this structure or the alternative → 4)-α-D-Gal*p*-(1 → 2)-β-D-Rib*f*-(1 → structure.[447] The structures of the O-specific side-chains in two *Klebsiella* O group 5 lipopolysaccharides have been studied by methylation and Smith degradation techniques. The results are consistent with the proposal that the side-chains are composed of repeating units containing five α-D-mannose residues, two of which are (1 → 3)-linked and three of which are (1 → 2)-linked. The side-chains are terminated by a 3-*O*-methyl-D-mannose residue.[448] A lipopolysaccharide was isolated from *Klebsiella* O group 9, the carbohydrate portion of which has been reported to be composed mainly of D-galactose with small amounts of 2-acetamido-2-deoxy-D-glucose and D-glucose.[449] The structure of the O-specific side-chains was investigated using a new technique involving methylation of the polysaccharide, partial acidic hydrolysis, reduction, and trideuteriomethylation, followed either by acidic hydrolysis and analysis of the sugars by g.l.c.–m.s. or by direct analysis of the methylated oligosaccharide alditols by g.l.c.–m.s. As a result of these studies, it was concluded that the side-chains are composed of pentasaccharide repeating units (21).[450]

O-Antigens extracted from the cells of *Vibrio parahaemolyticus* were characterized as lipopolysaccharides composed of glucose, galactose, 2-amino-2-deoxy-D-glucose, heptose, fatty acid ester, phosphorus, and nitrogen.[451] The effect of alkali and acid on the specificity of these O-antigens has been reported.[452] Studies of the inhibition of precipitin

[443] K. Keck, *Immunochemistry*, 1972, **9**, 359.
[444] J. W. Shands, *Infection and Immunity*, 1971, **4**, 167.
[445] M. Koike and K. Iida, *J. Bacteriol.*, 1971, **108**, 1402.
[446] J. S. Youngner, *Infection and Immunity*, 1972, **6**, 646.
[447] H. Bjorndal, B. Lindberg, J. Lonngren, K. Nilsson, and W. Nimmich, *Acta Chem. Scand.*, 1972, **26**, 1269.
[448] B. Lindberg, J. Lonngren, and W. Nimmich, *Acta Chem. Scand.*, 1972, **26**, 2231.
[449] W. Nimmich and G. Korten, *Pathol. Microbiol.*, 1970, **36**, 179.
[450] B. Lindberg, J. Lonngren, and W. Nimmich, *Carbohydrate Res.*, 1972, **23**, 47.
[451] M. Torii, T. An, K. Igarashi, K. Sakai, and K. Kuroda, *Biken J.*, 1969, **12**, 77.
[452] M. Torii and K. Igarashi, *Biken J.*, 1969, **12**, 149.



(21)

reactions of ten *V. parahaemolyticus* O-antigens indicated the existence of a variety of immunodominant sugars, including α-linked 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, D-glucose, and D-galactose.[453] Incubation of the K-antigen of *V. parahaemolyticus* with an enzyme preparation resulted in loss of the ability to precipitate homologous antisera and in the release of reducing sugar and an acidic 2-acetamido-2-deoxyhexose.[454]

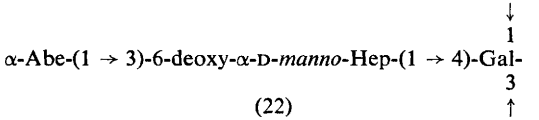
Electron microscopy studies of the endotoxin lipopolysaccharides of *Bacteroides*, *Fusobacterium*, and *Sphaerophorus* have been unable to detect any discernible effects on the morphology of the extracted particles, which had been purified in different ways and contained differences in chemical structures of the basic polysaccharide core.[455]

The polysaccharide moiety (molecular weight 1.24×10^4) of the lipopolysaccharide from *Escherichia coli* was shown to contain mannose (84%), glucose (6%), galactose (3%), heptose (5%), and 2-keto-3-deoxymannosulonic acid (1%). A related polysaccharide from the *rfa* mutant of the organism contains only mannose and has a molecular weight of 1.04×10^4. Both polysaccharides were permethylated, hydrolysed, reduced, and acetylated; the results of g.l.c.–m.s. indicated that two-thirds of the mannose units are substituted at C-2 and the remainder at C-3 in both polysaccharides. From the results of periodate oxidation and partial acidic hydrolysis, it was concluded that both polysaccharides contain twenty repeating units of α-mannosyl-(1 → 2)-α-mannosyl-(1 → 2)-mannose joined through α-(1 → 3)-linkages.[456] A core oligosaccharide (molecular weight 3.5×10^3) containing heptose, glucose, galactose, KDO, and phosphate was isolated on mild hydrolysis of the lipopolysaccharides of a rough strain of *E. coli*. Periodate oxidation, borohydride reduction, and methylation studies showed the presence of structural features similar to those already found for the core region of lipopolysaccharides from other Gram-negative bacteria.[457] Onion-like globular membrane structures, in addition

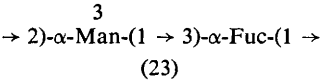
[453] M. Torii and K. Sakakibara, *Biken J.*, 1972, **15**, 17.
[454] K. Kuroda, G. Omori, M. Torii, and K. Imamura, *Biken J.*, 1972, **14**, 1.
[455] T. Hofstad, T. Kristoffersen, and K. A. Selvig, *Acta Pathol. Microbiol., Scand.*, 1972, **80B**, 413.
[456] K. Reske and K. Jann, *European J. Biochem.*, 1972, **31**, 320.
[457] J. J. Morton and J. C. Stewart, *European J. Biochem.*, 1972, **29**, 308.

to rod- and ribbon-like structures, have been observed for the purified lipopolysaccharide of *E. coli*.[458] Endotoxins have been reported in lipopolysaccharide preparations and the cell envelope of *E. coli*.[459] It has been suggested that the common enterobacterial antigenic determinant of R mutants of *E. coli* is either part of the lipopolysaccharide molecule or is strongly complexed with it.[460] Chemical analysis and phage typing of 2-keto-3-deoxyoctonate-lipid A from a heptose-deficient mutant of *E. coli* have suggested that the parent strain contains a core polysaccharide devoid of 2-amino-2-deoxy-D-glucose.[461] The effect of H_4edta, a non-ionic detergent, and lysozyme on the solubility of *E. coli* cell-wall polymers, including the lipopolysaccharide, has been reported.[462] Although the parental *E. coli* K12 strain could not attach *Salmonella typhimurium*-specific repeat units to its lipopolysaccharide core, it did have the capacity to attach *E. coli* O8-specific repeat units.[463]

Two O-somatic groups of antigens, termed free and bound antigens, from five types of *Pasteurella pseudotuberculosis* were found to be lipopolysaccharides differing in serological activity and lipid and sugar compositions. Both polymers contain glucose, mannose, xylose, heptose, and lipid B, but the bound antigen also contains KDO, lipid A, 2-amino-2-deoxy-D-glucose, and 3,6-dideoxyaldohexoses.[464] The O-specific side-chains of the cell-wall lipopolysaccharides, designated Group IIA and Group IIB, have been studied using the traditional techniques of methylation analysis and partial acidic hydrolysis. One of the sugar components of the Group IIA side-chains has been identified as a 6-deoxyheptose, possibly with the D-*manno*-configuration, in the tentatively proposed repeating unit (22).[465] The repeating oligosaccharide unit (23) of the Group



(22)



(23)

[458] Y. Katayama, H. Hattori, and A. Suganuma, *J. Bacteriol.*, 1971, **108**, 1412.
[459] J. Janda, *Biochem. J.*, 1972, **130**, 41P.
[460] H. Mayer, G. Schmidt, H. Y. Whang, and E. Neter, *Infection and Immunity*, 1972, **6**, 540.
[461] S. A. Rooney and H. Goldfine, *J. Bacteriol.*, 1972, **111**, 531.
[462] C. A. Schnaitman, *J. Bacteriol.*, 1971, **108**, 553.
[463] R. T. Jones, D. E. Koeltzow, and B. A. D. Stocker, *J. Bacteriol.*, 1972, **111**, 758.
[464] Y. S. Ovodov, R. P. Gorshkova, and S. V. Tomshich, *Immunochemistry*, 1971, **8**, 1071.
[465] C. G. Hellerqvist, B. Lindberg, K. Samuelsson, and R. R. Brubaker, *Acta Chem. Scand.*, 1972, **26**, 1389.

IIB side-chain was identified using similar techniques. Serological cross-reactions between this group and *Salmonella* Group B have been observed, and the lipopolysaccharides of the two groups have been shown to have a 3-*O*-α-D-abequosyl-α-D-mannose residue in common.[466]

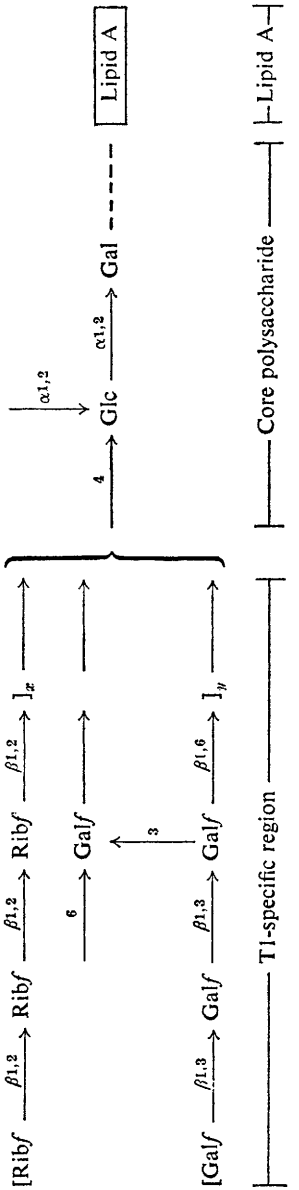
It has been shown that the lipopolysaccharide occurring in the cell wall of *Xanthomonas sinensis* contains a single molecule of KDO per molecule of lipopolysaccharide. Mild acidic hydrolysis of either the *Xanthomonas* polymer or authentic KDO resulted in the formation of 4,7-anhydro- and 4,8-anhydro-3-deoxy-octulosonic acid.[467]

The function of a soluble methyltransferase from *Mycobacterium phlei* in the biosynthesis of the 6-*O*-methyl-D-glucose residues of a mycobacterial lipopolysaccharide (J. A. Ferguson and C. E. Ballou, *J. Biol. Chem.*, 1970, **245**, 4213) has been reinvestigated. Methylation was not observed unless the D-gluco-oligosaccharides of DP 5-15 were partially acetylated. Digestion of an enzymically methylated octasaccharide with β-amylase gave products which suggested that methylation occurs only at sites more than four D-glucose residues from the non-reducing end. This is consistent with the fact that the section of the lipopolysaccharide containing 6-*O*-methyl-D-glucose begins four D-glucose units in from the non-reducing end. The structural similarity between the methylated octasaccharide and the mycobacterial lipopolysaccharide suggested that 6-*O*-methyltransferase is specific for methylation of a partially acetylated, or otherwise lipophilic, precursor of the lipopolysaccharide.[468]

Electron microscopic investigations have indicated that the cell-wall lipopolysaccharide of *Rhodopseudomonas capsulata* exists in filamentous and net-like aggregates. A lipid-protein–lipopolysaccharide complex was isolated from the outer layer of the cell wall of the organism.[469]

Cetavlon has been used for freeing the lipopolysaccharides of *Salmonella ngozi* and *S. dahlem* from such impurities as nucleic acids and acidic polysaccharides.[470] The lipopolysaccharide of *Salmonella* T1 forms has been reported to contain ribofuranose and galactofuranose units. From the results of mild acidic hydrolysis and periodate oxidation followed by alkaline hydrolysis, it was concluded that the specific polysaccharide of T1 lipopolysaccharide contains polyribose and polygalactose regions, as illustrated in (24).[471] Enzymic hydrolysis of *S. riogrande* lipopolysaccharide destroyed its ability to inhibit the agglutination of human A red cells by anti-*S. riogrande* rabbit serum. From the structures of the blood-group A-active oligosaccharides obtained from the lipopolysaccharide by mild acidic hydrolysis, it was assumed that the antigenic

[466] C. G. Hellerqvist, B. Lindberg, K. Samuelsson, and R. R. Brubaker, *Acta Chem. Scand.*, 1972, **26**, 1394.
[467] W. A. Volk, N. L. Salomonsky, and D. Hunt, *J. Biol. Chem.*, 1972, **247**, 3881.
[468] E. Grellert and C. E. Ballou, *J. Biol. Chem.*, 1972, **247**, 3236.
[469] J. Weckesser, G. Drews, and R. Ladwig, *J. Bacteriol.*, 1972, **110**, 346.
[470] B. Kedzierska and E. Mikulaszek, *Bull. Acad. polon. Sci., Sér. Sci. biol.*, 1972, **20**, 223.
[471] M. Berst, O. Lüdertiz, and O. Westphal, *European J. Biochem.*, 1971, **18**, 361.

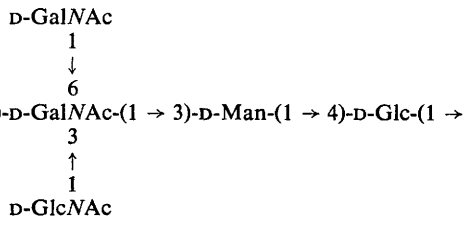

GlcNAc
α1,2
4
Glc
α1,2
Gal
Lipid A
[Ribf
β1,2
Ribf
β1,2
Ribf
β1,2
]x
6
Galf
3
[Galf
β1,3
Galf
β1,3
Galf
β1,6
]y
T1-specific region
Core polysaccharide
Lipid A

(24)

determinant of A specificity has the structure 2-acetamido-2-deoxy-α-D-galactosyl-(1 → 3)-D-mannosyl-D-glucosyl-(1 → 3)-2-acetamido-2-deoxy-D-galactosyl-D-glucose.[472]

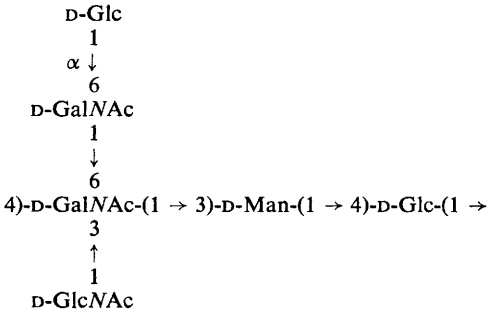
The cell envelope of *S. typhimurium* has been separated into cytoplasmic and outer membrane fractions of relatively high purity by density-gradient centrifugation of the total membrane fraction obtained by lysis of lysozyme–H_4edta spheroplasts. The outer membrane fraction was found to contain 90% of the lipopolysaccharide.[473] Evidence that the synthesis of the O-antigen occurs exclusively in the cytoplasmic membrane, before rapid transfer to the outer membrane, was obtained by pulse-chase experiments *in vivo* and by the assay of biosynthetic enzymes in isolated enzyme fractions. The mechanism of translocation of lipopolysaccharide is unknown.[474] Hypersensitivity in animals has been induced by smooth and rough strains of cell-wall lipopolysaccharides of *S. enteritidis* and *S. typhimurium* possessing a polysaccharide chain longer than that of a D-glucose-deficient mutant. The major antigenic group participating in this activity is presumed to be the rough core polysaccharide sequence, since fractions containing either O-side-chains or lipopolysaccharides of a heptose-deficient mutant were ineffective in provoking a hypersensitive reaction.[475]

The correlation between phage sensitivity, serological specificity, and chemical structure of the common core polysaccharide of the cell-wall lipopolysaccharides in rough mutants of *S. typhimurium* has been established.[476] Phage-converted *S. johannesburg* was found to have aquired a new specificity, which cross-reacted with all Factors 1 present on other *Salmonella*. The structures of the specific polysaccharides obtained from both wild (psR^-) and converted (psR^+) strains were established. The specific chain of psR^- contains residues of D-glucose and D-mannose, and a tetrasubstituted residue of 2-acetamido-2-deoxy-D-galactose on which residues of 2-acetamido-2-deoxy-D-glucose and -D-galactose are linked (25). The specific chain of psR^+ apparently differs by the addition of an α-D-glucose residue to a 2-acetamido-2-deoxy-D-galactose residue (26).[477]



(25)

[472] K. Furukawa, N. Kochibe, H. Takizawa, and S. Iseki, *Jap. J. Microbiol.*, 1972, **16**, 199.
[473] M. J. Osborn, J. E. Gander, E. Parisi, and J. Carson, *J. Biol. Chem.*, 1972, **247**, 3962.
[474] M. J. Osborn, J. E. Gander, and E. Parisi, *J. Biol. Chem.*, 1972, **247**, 3973.
[475] M. Kawakami, Y. Hara, and N. Osawa, *Infection and Immunity*, 1971, **4**, 519.
[476] A. A. Lindberg and C. G. Hellerqvist, *J. Bacteriol.*, 1971, **105**, 57.
[477] R. Girard and A. M. Staub, *Carbohydrate Res.*, 1972, **24**, 457.



(26)

A new type of auxotrophic mutant of *S. typhimurium*, which is defective in synthesizing the KDO region of the lipopolysaccharide, was found to require D-arabinose 5-phosphate for growth. Genetic and biochemical evidence indicated that the defect is due to an altered KDO-8-phosphate synthetase with an apparent K_m for arabinose 5-phosphate thirty-five times higher than that of the parental enzyme. As a result of this enzymic lesion, the mutant strain is dependent on exogenous arabinose 5-phosphate for growth and synthesis of the complete lipopolysaccharide.[478]

A two-enzyme segment of a membrane-bound series of glucosyl transferase enzymes from *S. typhimurium* has been reconstituted *in vitro* from the purified components. The reassembled structure contained glucosyl- and galactosyl-transferases as well as phosphatidylethanolamine and lipopolysaccharide. Since an isolated particle contained the four components, this was taken as a demonstration that the coupled activity of the two enzymes is restored and that the enzyme proteins are properly inserted into a common phospholipid–lipopolysaccharide matrix.[479]

Purified *Helix pomatia* A-hemagglutinin has been used to precipitate lipopolysaccharides of *S. typhimurium* rough mutants. It has been suggested that the structural element of the core region common to all reactive lipopolysaccharides is α-D-galactosyl-(1→6)-D-glucose and that these are the immunodominant sugars responsible for precipitation.[480]

The release of lipopolysaccharide from cells of *S. enteritidis* has been correlated with the change in permeability of the cells when they were treated with H_4edta.[481]

Both before and after phage conversion of a few *Salmonella* belonging to Group B, the structures of the polysaccharides present in the three groups differed only in the nature of the 3,6-dideoxyhexose present,

[478] P. D. Rick and M. J. Osborn, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3756.
[479] A. Hinckley, E. Muller, and L. Rothfield, *J. Biol. Chem.*, 1972, **247**, 2623.
[480] S. Hammarstrom, A. A. Lindberg, and E. S. Robertson, *European J. Biochem.*, 1972, **25**, 274.
[481] J. R. Chipley and H. M. Edwards, *Canad. J. Microbiol.*, 1972, **18**, 1803.

indicating that the differences observed in their structures are due to tyvelose, paratose, and abequose.[482]

Both protein and a lipopolysaccharide–protein complex have been extracted from the cell envelopes of *Pseudomonas aeruginosa* and were found to spread on aqueous subphases as monomolecular layers. The film of the complex is formed mainly from the protein, but is stabilized by lipopolysaccharide attached to the lower surface of the film.[483] Further studies on the chemical composition of the lipopolysaccharide of *P. aeruginosa* have established the presence of mannose, arabinose, and L-*glycero*-D-*manno*-heptose in addition to the sugars normally found in the polymer. Fractions of the degraded polysaccharide contained equal proportions of alanine and 2-amino-2-deoxy-D-galactose; at least 40% of the alanine residues are bound in an amide linkage, probably to the amino-sugar.[484] Examination of low molecular weight solutes released during acidic hydrolysis revealed the presence of ethanolamine triphosphate, which is considered to be attached to a polysaccharide fraction, but the possibility that some is attached to the lipid A moiety was not excluded.[485] The lipopolysaccharide of the wall of *P. fluorescens* has been shown to contain 3-amino-3,6-dideoxy-D-galactose.[486]

A group-reactive antigen of *Chlamydia psittaci* is considered to be an acidic polysaccharide, possibly a lipopolysaccharide.[487] It has been studied by electron microscopy using samples localized by silver–methenamine staining.[488] Characterization of this high molecular weight polymer indicated the presence of lipid and KDO. The ease with which the antigenicity was destroyed by periodate ion and mild acidic hydrolysis indicated that the acidic component is vital for antigenicity.[489]

The chemical composition of *Shigella flexneri* lipopolysaccharide and its serological activity were found to vary from culture batch to culture batch. The irregularity was traced to its biosynthesis; it appeared that, in addition to the properly formed S-specific units, other units containing D-glucose or too few repeating units are also formed.[490] In *S. flexneri* Type 2 and its Y variant O-antigenic side-chains, rhamnose is found to be (1 → 2)- and (1 → 3)-linked, but D-glucose also occurs in the Type 2 lipopolysaccharides.[491] Similarly, 2-acetamido-2-deoxy-D-glucose is found in (1 → 6)-linkage in both types, but it is also doubly linked through C-3 and C-6 in the Type 2 polymer.[492] The chemical structures of *S. flexneri* antigens have been reviewed.[493]

[482] A. M. Staub, *Immunochemistry*, 1972, **9**, 464.
[483] A. Suzuki and S. Goto, *Biochim. Biophys. Acta*, 1972, **255**, 734.
[484] I. R. Chester, G. W. Gray, and S. G. Wilkinson, *Biochem. J.*, 1972, **126**, 395.
[485] D. T. Drewry, G. W. Gray, and S. G. Wilkinson, *Biochem. J.*, 1972, **130**, 289.
[486] S. G. Wilkinson, *J. Gen. Microbiol.*, 1972, **70**, 365.
[487] S. P. Dhir, G. E. Kenny, and J. T. Grayston, *Infection and Immunity*, 1971, **4**, 725.
[488] S. P. Dhir and E. S. Boatman, *J. Bacteriol.*, 1972, **111**, 267.
[489] S. P. Dhir, S. Hakamori, G. E. Kenny, and J. T. Grayston, *J. Immunol.*, 1972, **109**, 116.
[490] G. Seltmann and W. Beer, *Z. allgem. Mikrobiol.*, 1971, **11**, 607.
[491] W. Beer and G. Seltmann, *Z. allgem. Mikrobiol.*, 1972, **12**, 97.
[492] G. Seltmann and W. Beer, *Z. allgem. Mikrobiol.*, 1972, **12**, 423.
[493] G. Seltmann, *Z. allgem. Mikrobiol.*, 1972, **12**, 497.

Capsular Polysaccharides and Exopolysaccharides

Immunoelectro-osmophoresis with rabbit anticapsular antibody has been used to detect type-specific pneumococcal polysaccharides in the serum of bacteremic patients with pneumococcal pneumonia. Using this technique, as little as 0.2—2 nanograms of polysaccharide from Types I, II, III, IV, V, VIII, XII, and XVIII could be detected.[494]

The structure of the capsular polysaccharide of *Diplococcus pneumoniae* Type II has been investigated. Methylation analyses of the original polysaccharide and of a product obtained from it on mild acidic hydrolysis, together with the characterization of an aldobiouronic acid, have suggested that the smallest possible repeating unit should be a hexasaccharide. It was concluded that the structure contains a terminal α-D-glucosyluronic acid-(1 → 6)-D-glucosyl and α-L-rhamnosyl-(1 → 4)-D-glucosyl groups.[495]

The binding of the hexasaccharide [3)-β-D-GlcUA-(1 → 4)-β-D-Glc-(1 →]$_3$ caused significant changes in the c.d. spectra of three homogeneous antibodies against pneumococcal Type III polysaccharide. This was ascribed to changes in the asymmetric environment of aromatic chromophores directly on the combining site and to changes in orientation inside or beyond the site.[496] High-zone tolerance to pneumococcal Type III polysaccharide in mice was demonstrated by complete inhibition of the direct plaque-forming response in the spleen.[497]

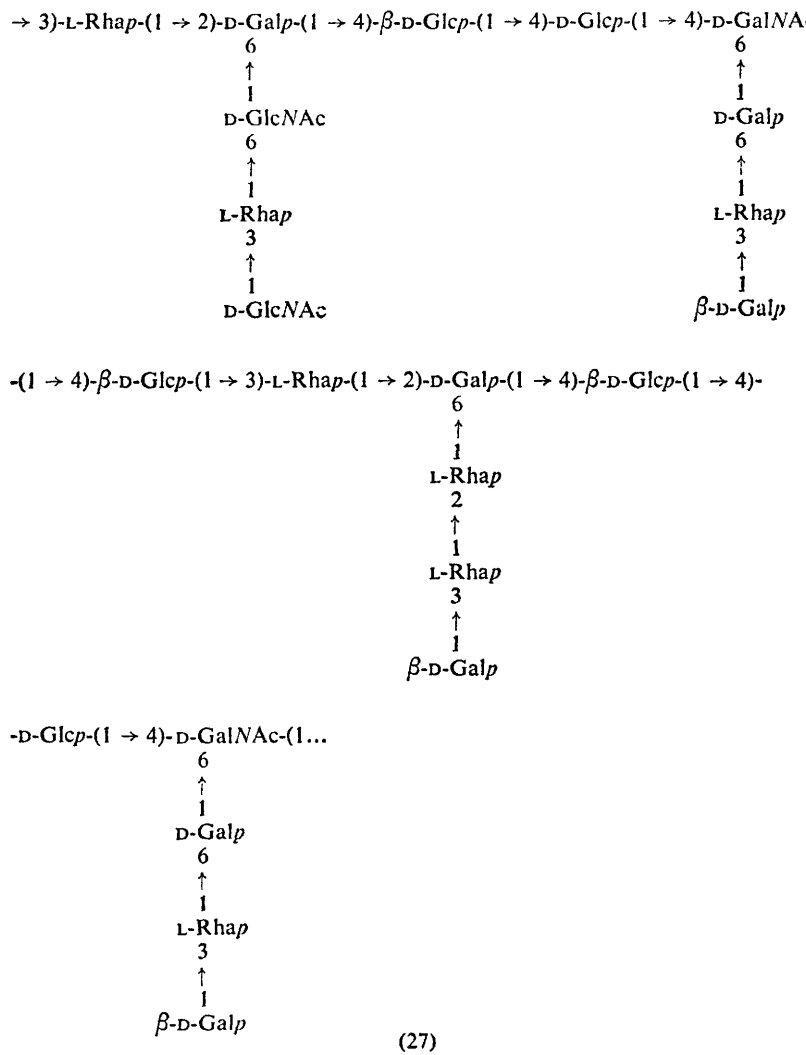
The specific, capsular pneumococcal Type IV polysaccharide has been shown to contain acetal-linked pyruvic acid, D-galactose, 2-amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-mannose, and 2-amino-2,6-dideoxy-D(?)-galactose (3 : 3 : 3 : 2 : 3), with the amino-sugars probably in the *N*-acetylated form.[498] Inhibition experiments with pneumococcal C and depyruvylated *Pneumococcus* Type IV polysaccharides indicated that phosphorylcholine is an important antigenic determinant of the C polysaccharide.[499]

Some of the structural features of the specific, pneumococcal Type VII polysaccharide have been revealed by methylation studies, time-lapse hydrolysis, periodate oxidation, and enzymic hydrolysis. The polysaccharide was found to be branched at D-galactose and 2-acetamido-2-deoxy-D-galactose residues. Non-reducing end-groups consist of D-galactose and 2-acetamido-2-deoxy-D-glucose, and the major components of the linear chains are (1 → 3)-L-rhamnose and (1 → 4)-D-glucose residues.

[494] G. E. Kenny, B. B. Wentworth, R. P. Beasley, and H. M. Foy, *Infection and Immunity*, 1972, **6**, 431.
[495] O. Larm, B. Lindberg, S. Svensson, and E. A. Kabat, *Carbohydrate Res.*, 1972, **22**, 391.
[496] D. A. Holowka, A. D. Strosberg, J. W. Kimball, E. Haber, and R. E. Cathou, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3399.
[497] J. G. Howard, G. H. Christie, and B. M. Courtenay, *Proc. Roy. Soc.*, 1971, **B178**, 417.
[498] J. D. Higginbotham and M. Heidelberger, *Carbohydrate Res.*, 1972, **23**, 165.
[499] M. Heidelberger, E. C. Gotschlich, and J. D. Higginbotham, *Carbohydrate Res.*, 1972, **22**, 1.

The results are in accord with structural features deduced from serological cross-reactions of the polysaccharide (27).[500]

In addition to previously identified components (*viz.* D-glucose, 2-acetamido-2-deoxy-D-glucose, and D-glucuronic acid), the immunologically specific, capsular polysaccharide of *Pneumococcus* Type IX has been found to contain 2-amino-2-deoxy-D-mannose and 2-amino-2-deoxy-D-galactose,



(27)

[500] A. S. Chaudhari, C. T. Bishop, and R. J. Fielder, *Carbohydrate Res.*, 1972, **25**, 161.

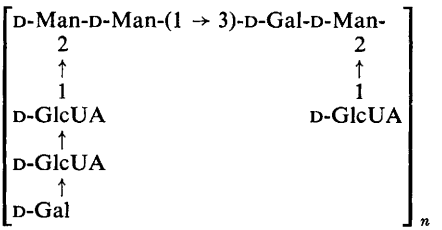
presumably as their *N*-acetyl derivatives. Partial hydrolysis yielded a series of oligosaccharides of which α-D-glucopyranosyluronic acid-(1 → 3)-D-glucose, D-glucopyranosyluronic acid-(1 → 3)-2-acetamido-2-deoxy-D-glucose, and D-glucopyranosyluronic acid-(1 → 3)-2-acetamido-2-deoxy-D-galactose have been identified.[501] The effects of periodate oxidation and borohydride reduction on the precipitation of the polysaccharide in antisera were interpreted in structural terms. A possible structure (28) was suggested

[α-D-GlcUA-(1 → 3)-α-D-Glc-(1 → 4)-D-GlcUA-(1 → 4)-D-GlcUA-(1 → 3)-D-Glc*N*Ac-(1 → 4)-D-Glc-(1 → 3)-D-Glc*N*Ac-(1 → 3)-D-Glc-(1 → ?)-D-Man*N*Ac-(1 → 3)-D-Glc-(1 → 3)-D-Man*N*Ac-(1 → 4)-D-Glc-(1 → 4)-D-GlcUA-(1 → 3)-D-Gal*N*Ac]$_n$

(28)

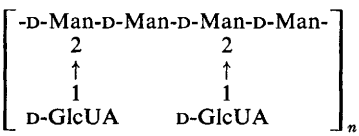
for the polysaccharide.[502] Pneumococcal polysaccharide Type XIV, which is composed of a main chain of D-galactose and 2-acetamido-2-deoxy-D-glucose residues, contains side-chains of α-D-galactose and lactose, with α-D-galactose at the terminal. On enzymic attack using galactose oxidase followed by oxidation of the 6-aldehyde group, a number of the terminal D-galactose units were converted into the corresponding D-galactosyluronic acid derivatives. By variations of these procedures,three different derivatives of the original polysaccharide were obtained that were able to precipitate different amounts of antibody from anti-pneumococcal Type XIV serum.[503]

Cross-reactions of the extracellular polysaccharides of *Lipomyces lipoferus* and *L. starkeyi* have been described. Non-reducing end-groups of D-glucuronic acid appeared to account for most of the reactions of the *L. lipoferus* polysaccharide, whereas non-reducing end-groups of D-galactose accounted for many of those of the *L. starkeyi* polysaccharide. Because of its strong cross-reaction with anti-pneumococcal Type II serum, the latter polysaccharide is also presumed to contain a portion of its D-glucuronic acid in the form of non-reducing end-groups, as indicated in the structure (29). The structure of the polysaccharide from *L. lipoferus* is represented by (30).[504]



(29)

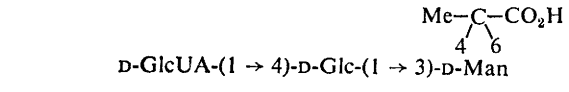
[501] J. D. Higginbotham, A. Das, and M. Heidelberger, *Biochem. J.*, 1972, **126**, 225.
[502] A. Das, J. D. Higginbotham, and M. Heidelberger, *Biochem. J.*, 1972, **126**, 233.
[503] S. Estrada-Parra and I. Gómez, *Immunochemistry*, 1972, **11**, 1095.
[504] M. Heidelberger and M. E. Slodki, *Carbohydrate Res.*, 1972, **24**, 401.



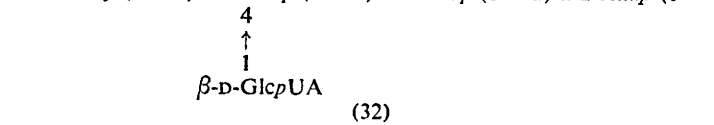
(30)

The capsular polysaccharides from several *Klebsiella* serotypes have been shown to contain pyruvic acid, probably in ketosidic linkage.[505] A phage-induced enzyme has been used to hydrolyse polysaccharides prepared from nine *Klebsiella* serotype 2 strains. In each case, the product was a tetrasaccharide having a chemical composition corresponding to the carbohydrate unit of the polysaccharide.[506] Methylation, periodate oxidation, and partial acidic hydrolysis techniques have been used to demonstrate the presence of 4,6-*O*-(1-carboxyethylidene)-D-mannopyranosyl units in the capsular polysaccharide of *Klebsiella* K-type 5. The structure of this polysaccharide differs from those known for other *Klebsiella* capsules by the lack of any carbohydrate side-chains. The repeating unit (31) (plus one unassigned *O*-acetyl group) is in accord with experimental data.[507]

Water-extractable type 6 *Klebsiella* antigens were separated into a type 6-specific acidic polysaccharide and a neutral polysaccharide (containing



(31)



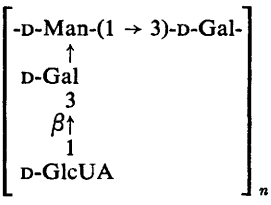
(32)

glucose, galactose, and mannose), which was devoid of type 6 activity although it was serologically active. The acidic polymer contains fucose, glucose, mannose, pyruvic acid, galacturonic acid, and possibly glucuronic acid.[508]

The polysaccharide of *Klebsiella* type 9 has been isolated and shown to be composed of pentasaccharide repeating units containing D-glucuronic acid, D-galactose, and L-rhamnose (1 : 1 : 3) in the proposed structure (32).[509]

[505] B. J. Gormus, R. W. Wheat, and J. F. Porter, *J. Bacteriol.*, 1971, **107**, 150.
[506] I. W. Sutherland, *J. Gen. Microbiol.*, 1972, **70**, 331.
[507] G. G. S. Dutton and M. T. Yang, *Canad. J. Chem.*, 1972, **50**, 2382.
[508] B. J. Gormus and R. W. Wheat, *J. Bacteriol.*, 1971, **108**, 1304.
[509] B. Lindberg, J. Lönngren, and J. L. Thompson, *Carbohydrate Res.*, 1972, **25**, 49.

The capsular polysaccharide from *Klebsiella* type 21 has been submitted to hydrolysis and an aldobiouronic acid so liberated has been characterized as 3-*O*-(D-glucopyranosyluronic acid)-D-mannose.[510] Methylation studies and partial acidic hydrolysis have been used to show that the capsular polysaccharide of *Klebsiella* K-type 20 is composed of a main chain of D-mannose and D-galactose units with the aldobiouronic acid, 3-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, attached as a side-chain [see (33)].[511]



(33)

Water-extractable and partially purified capsular polysaccharides from four selected strains of *Clostridium perfringens*, although similar in chemical composition, exhibited a high degree of specificity for the homologous antisera.[512] Fractions from two strains of *C. perfringens*, which were separated into polysaccharide and nucleoprotein, exhibited a serological activity proportional to the saccharide content of the polysaccharide fraction.[513] An *Escherichia coli* antigen that cross-reacted with the capsular polysaccharide of *Haemophilus influenzae* has been identified as a thermostable, acidic capsular polysaccharide.[514]

Methylation of *Aerobacter aerogenes* capsular polysaccharide and the derived 3-*O*-α-D-glucopyranuronosyl-D-mannose has been described.[515] Infection of *A. aerogenes* with a phage induced the *de novo* synthesis of a polysaccharide depolymerase that was active against the capsular polysaccharide of the host organism. The depolymerase was shown to be a highly specific glycanohydrolase, which randomly attacked the D-galactosyl-(1 → 3)-D-galactose linkages of the polysaccharide cleaving one bond in each tetrasaccharide repeating unit. The smallest oligosaccharide that the depolymerase would degrade was a dodecasaccharide composed of three tetrasaccharide repeating units.[516]

[510] G. G. S. Dutton and Y. M. Choy, *Carbohydrate Res.*, 1972, **21**, 169.
[511] Y. M. Choy and G. G. S. Dutton, *J. Bacteriol.*, 1972, **112**, 635.
[512] R. Cherniak and B. G. Henderson, *Infection and Immunity*, 1972, **6**, 32.
[513] A. Dworczynski and F. Meisel-Mikolajczyk, *Bull. Acad. polon. Sci., Sér. Sci. biol.*, 1971, **19**, 783.
[514] R. Schneerson, M. Bradshaw, J. K. Whisnant, R. L. Myerowitz, J. C. Parke, and J. B. Robbins, *J. Immunol.*, 1972, **108**, 1551.
[515] H. E. Conrad, *Methods Carbohydrate Chem.*, 1972, **6**, 361.
[516] E. C. Yurewicz, M. A. Ghalambor, D. H. Duckworth, and E. C. Heath, *J. Biol. Chem.*, 1971, **246**, 5607.

A highly purified glucan isolated and characterized from the oral microbe *Streptococcus salivarius* was found to have a wavelength for maximum absorption of the iodine complex, a specific rotation, and average exterior and interior chain lengths within the range reported for other microbial and animal glycogens.[517] The synthesis of glycogen by a mutant of *Escherichia coli*, which was devoid of D-glucose-phosphate isomerase and D-glucose-6-phosphate dehydrogenase activity, has been reported.[518] A glucan resembling glycogen in solubility, iodine complexing, and internal chain length has been isolated from *Bacillus stearothermophilus*. The product synthesized *in vitro* in the presence of acceptor ADP-D-glucose and cell-free extracts containing glucan synthetase had elongated external chains.[519] The rate of synthesis of glycogen by glycogen synthetase was increased when the branching enzyme was present and evidence of its ability to convert glycogen into a more highly branched product was presented.[520]

A water-insoluble glucan produced from sucrose by culture filtrates of *S. mutans* has been shown to contain α-(1 → 3)- and α-(1 → 6)-linkages in a ratio of 8 : 1. The high proportion of α-(1 → 3)-linkages was considered to be the cause of the insolubility of the glucan in water and its resistance to hydrolysis by dextranase.[521] Sucrose labelled with D-[^{3}H]fructosyl and D-[^{14}C]glucosyl moieties has been used to quantify the production and degradation of extracellular polysaccharide by cariogenic and non-cariogenic oral streptococci. All strains studied were able to produce α-glucan and fructan. The cariogenic streptococci were able to degrade the fructan produced by the non-cariogenic strains.[522] A mouse myeloma protein has been studied immunochemically and is specific for α-(1 → 3)-linked dextrans. Inhibition studies using α-(1 → 3)-linked glycosyl oligosaccharides indicated that the size of the combining site is that of a pentasaccharide. A related mouse myeloma protein reacted to a lesser extent with fructans. The best inhibitor was a terminal, non-reducing chain of two β-(2 → 1)-linked D-fructofuranosyl residues with an additional β-linkage either to C-6 of D-glucose or to C-1 of D-fructose.[523] Water-insoluble glucans synthesized by different glucosyltransferases produced by *S. mutans* and *S. sanguis* were investigated by chemical and physical methods. The polysaccharides are essentially α-linked and contain differing proportions of terminal 3-, 6-, and 3,6-linked D-glucose residues. The extent of hydrolysis by dextranase decreased with the content of (1 → 3)-linkages.[524] Glucan and fructan have been synthesized extracellularly from

[517] R. L. Khandelwal, T. N. Spearman, and I. R. Hamilton, *Canad. J. Biochem.*, 1972, **50**, 440.
[518] J. P. Chambost, A. Favard, and J. Cattanéo, *Carbohydrate Res.*, 1972, **24**, 379.
[519] S. H. Goldemberg, *Arch. Biochem. Biophys.*, 1972, **149**, 252.
[520] G. J. Walker and J. E. Builder, *European J. Biochem.*, 1971, **20**, 14.
[521] J. K. Baird and D. C. Ellwood, *Biochem. J.*, 1972, **127**, 88P.
[522] C. F. Schachtele, A. E. Loken, and M. K. Schmitt, *Infection and Immunity*, 1972, **5**, 263.
[523] A. Lundblad, R. Steller, E. A. Kabat, J. W. Hirst, M. G. Weigert, and M. Cohn, *Immunochemistry*, 1972, **9**, 535.
[524] M. Ceska, K. Granath, B. Norrman, and B. Guggenheim, *Acta Chem. Scand.*, 1972, **26**, 2223.

sucrose by three strains of *S. mutans*. The enzyme activity for polysaccharide formation is almost exclusively extracellular and is bound to the cell-free polymer itself.[525]

The detailed structure of a dextran produced from a cariogenic bacterium has been investigated using periodate oxidation and borohydride reduction techniques; the existence of a linear polymer of (1 → 6)-linkages with random (1 → 3)-branches was suggested. The general resistance to dextranases favoured the concept of a tree-like structure.[526] Two dextrans of different solubility elaborated by *Leuconostoc mesenteroides* were shown to contain branch points on C-2 and C-6 as well as (1 → 6)- and (1 → 3)-linkages.[527] A commercial dextran preparation has been hydrolysed with mineral acid and the products were separated by ultrafiltration through a series of graded anisotropic cellulose acetate membranes of decreasing pore sizes. Five well-defined, narrow-range, low molecular weight fractions were obtained. From molecular weight and sedimentation values, the frictional coefficient was calculated for each fraction and the asymmetry ratios were obtained.[528] The effective valency of rabbit anti-dextran immunoglobulins IgM and IgG has been found to be a function of the molecular size of the dextran used.[529] A detailed procedure for gel chromatography and analysis of the distribution of molecular weights of dextran in different biological fluids has been presented, together with results obtained on applying the method to studies of membrane structures.[530]

The blood-coagulant activity of dextrans sulphated with piperidine-*N*-sulphonic acid was generally found to be much lower than that of heparin.[531]

A gel-forming β-(1 → 3)-glucan obtained from culture filtrates of *Alcaligenes faecalis* has been studied by o.r.d. spectroscopy and viscosity and flow birefringence techniques. In solutions of low sodium hydroxide concentration, the glucan appeared to have an ordered conformation, whereas it appeared to behave as a random coil at high concentrations with a conformational transition occurring in a region between 0.19 and 0.24 mol l^{-1}.[532]

The predominance of iodophilic polysaccharide-synthesizing lactobacilli among the intraoral lactobacillary population of caries-active subjects has been described.[533] Biosynthesis of levan has been achieved enzymically on a solidified agar medium containing sucrose as a source of D-fructose.

[525] S. A. Robrish, W. Reid, and M. I. Krichevsky, *Appl. Microbiol.*, 1972, **24**, 184.
[526] L. W. Long and J. R. Edwards, *Carbohydrate Res.*, 1972, **24**, 216.
[527] E. J. Bourne, R. L. Sidebotham, and H. Weigel, *Carbohydrate Res.*, 1972, **22**, 13.
[528] S. C. Edberg, P. M. Bronson, and C. J. Van Oss, *Preparative Biochem.*, 1971, **1**, 249.
[529] S. C. Edberg, P. M. Bronson, and C. J. Van Oss, *Immunochemistry*, 1972, **9**, 273.
[530] G. Arturson and K. Granath, *Clinica Chim. Acta*, 1972, **37**, 309.
[531] K. Nagasawa, H. Harada, S. Hayashi, and T. Misawa, *Carbohydrate Res.*, 1972, **21**, 420.
[532] K. Ogawa, T. Watanabe, J. Tsurgi, and S. Ono, *Carbohydrate Res.*, 1972, **23**, 399.
[533] Y. Kobayashi and M. Takei, *Jap. J. Microbiol.*, 1972, **16**, 70.

A new method was described for assay of the enzyme (2,6-fructan-D-glucose 6-fructosyltransferase) based on its rate of diffusion through the agar medium.[534] A precursor of bacterial cellulose has been separated from cultures of *Acetobacter xylinum*. Electron microscopy demonstrated that the purified fraction was able to form cellulose microfibrils.[535] A Gram-negative, non-motile, bovine rumen bacterium was able to produce a starch-like polymer from D-xylose and xylan. The purified polysaccharide contained only D-glucose and was degraded by α-amylase and glucamylase.[536]

A review has dealt with crystal structure data for cyclohexa- and cyclohepta-amyloses and their derivatives.[537] The cyclohexa-amylose hexahydrate molecule has been shown to contain two water molecules inside the cyclodextrin ring, both being located on the mean molecular axis. The first is located approximately at a point where the mean plane through the six C-2 atoms intersects the axis, and the second is placed between the points where the mean planes through the six C-5 and C-6 atoms intersect the axis. The first water molecule is probably hydrogen-bonded to the second, which in turn is probably hydrogen-bonded to at least one O-6 oxygen atom.[538]

Anomalous chromatographic behaviour of cyclodextrins has been reported. Fractionation on Sephadex was considered to be by adsorption, rather than by molecular sieving.[539]

The heats of hydrolysis of cyclodextrins have been reported and thermodynamic considerations of the cyclization process were discussed. On the basis of enthalpy values known for α-(1 → 4)-D-glucosidic linkages of a non-cyclic nature, the energetic instability introduced by the cyclization has been evaluated. It was concluded that, although the cyclization is energetically unfavourable, the entropy term contributed more or less favourably to the stability of cycloamyloses in solution.[540]

The formation of inclusion complexes of cyclodextrins with iodine, tri-iodide, 4-nitrophenolate anion, and Congo Red have been investigated in aqueous solutions by c.d. and o.r.d. spectroscopy. The intensity of induced c.d. in the dextrin complexes was observed to change markedly with the combination of host dextrins and guest molecules, reflecting the different degrees of interaction between them owing to differing cavity diameters of the host dextrins.[541] Marked differences in the heats of reaction have been observed for cyclodextrins with tri-iodide and 4-nitrophenolate anion.[542] A change in the mode of reaction of anisole with

[534] M. Ceska, *Biochem. J.*, 1971, **125**, 209.
[535] J. R. Colvin and G. G. Leppard, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, **9**, 417.
[536] A. Oi, S. Mori, and K. Kitahara, *J. Agric. Chem. Soc. Japan*, 1971, **45**, 534.
[537] G. Strahs, *Adv. Carbohydrate Chem. Biochem.*, 1972, **25**, 53.
[538] P. C. Manor and W. Saenger, *Nature*, 1972, **237**, 392.
[539] J. H. Carter and E. Y. C. Lee, *Analyt. Biochem.*, 1971, **39**, 521.
[540] K. Takahashi and S. Ono, *J. Biochem.*, 1972, **72**, 679.
[541] K. Takeo and T. Kuge, *Stärke*, 1972, **24**, 281.
[542] K. Takeo and T. Kuge, *Stärke*, 1972, **24**, 331.

hypochlorous acid in the presence of cyclohexa-amylose has been reported. When anisole was bound into the cyclodextrin cavity, *o*-chlorination was completely suppressed, whereas *p*-chlorination was catalysed. In the complex with cyclohepta-amylose, *o*-chlorination was also blocked, but *p*-chlorination proceeded at the same rate as that in free solution. It was proposed that the cyclodextrin reacts with hypochlorous acid to form a hypochlorite before delivering the chlorine selectively to anisole bound in its cavity.[543]

Miscellaneous Polysaccharides.—A purified, serologically active polysaccharide from *Leptospira biflexa* was found to contain rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose.[544] The polysaccharide antigen from *Eubacterium saburreum* has been isolated by digestion of whole, acetone-dried cells with trypsin and fractionation by ion-exchange chromatography. Heptose and *O*-acetyl residues accounted for 90% of the molecule in which neither lipid nor amino-sugar is present.[545] Immunoelectrophoresis and sedimentation studies indicated homogeneity, while experiments with labelled antibodies suggested that this antigenic polysaccharide is located at the surface of the bacterial cell.[546] Deacetylation of the antigen with mild alkali reduced the serological activity and disclosed another antigenic specificity, as evidenced by its precipitation in agar gel.[547]

Vi antigen has been isolated from saline extracts of *Citrobacter* species by enzymic digestion; the product was up to two hundred times more protective in mice than preparations isolated by acidic hydrolysis.[548] The group-specific antigenic polysaccharide of *Mycobacterium leprae* has been found to be the same, or a very similar, polysaccharide to those of *Nocardia* and *Mycobacteria*.[549] The Type III antigen of group B Streptococci has been extracted and purified from the bacteria using hot acid, proteolytic digestion, and ion-exchange chromatography; the polysaccharide (molecular weight 1.56×10^4) contains galactose (39%), glucose (18%), and 2-amino-2-deoxy-D-glucose (23%), with D-glucuronic acid as one of the immunodominant components.[550]

A review of microbial exopolysaccharides has dealt with their isolation and properties as well as their potential as a source of emulsifiers and other rheological agents suitable for commercial purposes.[551] Environmental factors influencing the synthesis and excretion of exocellular bacterial and fungal macromolecules have been critically reviewed.[552]

[543] R. Breslow and P. Campbell, *Biol. Org. Chem.*, 1972, **1**, 140.
[544] T. Taniyama, Y. Yanagihara, I. Mifuchi, I. Azuma, and Y. Yamamura, *Infection and Immunity*, 1972, **6**, 414.
[545] T. Hofstad, *Acta Pathol. Microbiol. Scand.*, 1971, **79B**, 835.
[546] T. Hofstad and K. A. Selvig, *Acta Pathol. Microbiol. Scand.*, 1972, **80B**, 129.
[547] T. Hofstad, *Acta Pathol. Microbiol. Scand.*, 1972, **80B**, 609.
[548] K. H. Wong and J. C. Feeley, *Appl. Microbiol.*, 1972, **24**, 629.
[549] S. Estrada-Parra, *Infection and Immunity*, 1972, **5**, 258.
[550] H. Russell and N. L. Norcross, *J. Immunol.*, 1972, **109**, 90.
[551] I. W. Sutherland, *Process Biochem.*, 1972, **7**, 27.
[552] A. T. Bull, *J. Appl. Chem. Biotech.*, 1972, **22**, 261.

Fungal Cell Walls.—A review has appeared on the preparation, properties, structure, and uses of protoplasts from fungi.[553] The application of a purified extracellular β-(1 → 3)-glucanase to the analysis of yeast cell walls and to the formation of protoplasts has been demonstrated. The fact that this enzyme entirely dissolved the yeast cell walls made it an ideal tool for the analysis of carbohydrate formation in protoplasts, since it caused changes only in its specific β-(1 → 3)-glucan substrate.[554] Cells of *Saccharomyces cerevisiae* and *Hansenula anomala* were digested with enzymes to yield prospheroplasts. The surrounding envelope, which was isolated after lysis of the prospheroplasts, contained an alkali-soluble glucan as the major constituent. The prospheroplast envelope is considered to be part of the original cell wall of the yeast and is located in close apposition to the cytoplasmic membrane.[555] Yeast spheroplasts from both logarithmic- and stationary-phase cells have been formed by cell-wall degrading enzymes from a bacterial source.[556] The joint actions of two glucanases produced by *Arthrobacter* in spheroplast formation of *S. cerevisiae* have been reported. It was shown that the proportion of the individual glucanases is a significant factor in the efficient spheroplasting of the yeast cells.[557] Protoplasts of *S. cerevisiae*, which are capable of incorporating '2-deoxy-D-glucose', accumulated the sugar primarily as '2-deoxy-D-glucose 6-phosphate'. It was suggested that the high internal level of this phosphorylated sugar inhibits the synthesis of the cell-wall polysaccharide and glycoproteins.[558]

Chemical analysis of the cell wall of *Aspergillus niger* has shown that the main components are D-glucose and 2-acetamido-2-deoxy-D-glucose, with minor quantities of D-mannose, D-galactose, 2-amino-2-deoxy-D-galactose, protein, and lipid. Fractionation of the cell walls yielded an alkali-soluble α-(1 → 3)-glucan and an alkali-resistant fraction composed of chitin together with α-(1 → 4)- and β-(1 → 3)-linked glucans.[559] In a quantitative study of the changes in the α-(1 → 3)-glucan fraction during cleistothecium development in *A. nidulans*, it was demonstrated that the polysaccharide acts as the main reserve polymer. Cleistothecium formation started when D-glucose was depleted in the medium and enough α-(1 → 3)-glucan was present in the hyphal walls to serve as a carbon and energy source.[560]

The biochemical and morphological changes of the yeast-like (Y) form and the mycelial (M) form of *Paracoccidioides brasiliensis* have been examined. Glucans are predominant in both forms, the Y-form containing an

[553] J. F. Peberdy, *Sci. Progr.*, 1972, **60**, 73.
[554] H. Bauer, D. A. Bush, and H. Horisberger, *Experentia*, 1972, **28**, 11.
[555] S. Darling, J. Theilade, and A. Birch-Andersen, *J. Bacteriol.*, 1972, **110**, 336.
[556] J. W. Mann, C. E. Heintz, and J. D. MacMillan, *J. Bacteriol.*, 1972, **111**, 821.
[557] K. Doi, A. Doi, and T. Fukui, *J. Biochem.*, 1971, **70**, 711.
[558] S. C. Kuo and J. O. Lampen, *J. Bacteriol.*, 1972, **111**, 419.
[559] B. J. M. Zonneveld, *Biochim. Biophys. Acta*, 1971, **249**, 506.
[560] B. J. M. Zonneveld, *Biochim. Biophys. Acta*, 1972, **273**, 174.

α-(1 → 3)-linked glucan and the M-form a β-glucan having mainly β-(1 → 3)-glycosidic linkages with a few branches at the C-6 position. The incorporation of D-[^{14}C]glucose into the cell-wall glucans showed that the synthesis of α-glucan decreases rapidly after the temperature of the culture was changed from 37 to 20 °C, and that the synthesis of β-glucan is augmented at an early stage of the morphological change.[561]

Pseudonigeran, an α-linked glucan isolated from the mycelium of *Aspergillus niger*, was shown by methylation analysis, periodate oxidation, and Smith degradation to have a DP of 330 and to contain mainly (1 → 3)-linkages.[562] A purified mycodextranase, an endo-α-D-(1 → 4)-glucanase from *Penicilium melinii*, has been used in a study of the fine structure of nigeran and the general structure of isolichenin. From the data obtained, a general mechanism for the biosynthesis of nigeran was postulated.[563]

Analysis of trypsin-treated cell walls of two strains of *Blastomyces dermatitidis*, which exhibited differences in virulence towards mice, indicated that the highly virulent strain has a higher chitin content and a greater amount of phospholipid associated with an α-glucan than does the avirulent strain. The results suggested that the phospholipid–α-glucan complex could be one of the factors related to the virulence of this fungus.[564]

A mucilage present at the outer surface of the cell wall, and also in the culture medium, of the basidiomycete *Schizophyllum commune* was shown to consist of chains of (1 → 3)-linked β-D-glucosyl units with branches of single β-D-glucosyl units attached by (1 → 6)-linkage to every fourth unit along the chain. Located beneath the mucilage, and accounting for about 45% of the thickness of the wall, is an alkali-soluble glucan {$[\alpha]_D$ +242°} consisting of chains of α-(1 → 3)-linked D-glucose units. The glucan is present in the wall in a microcrystalline form, and both this and the chemically isolated glucan have a typical rodlet structure.[565]

Individual cellulose crystallites from the cell wall of *Valonia ventricosa*, when studied by electron diffraction techniques, showed crystalline zones running along the length of the fibrils for about 1000 Å without any longitudinal periodicity.[566]

A water-soluble β-glucan has been isolated from the mycelial wall of *Basidiomycete* QM806. Methylation, periodate oxidation, and enzymic degradation showed that the polysaccharide is a branched glucan with D-glucose units joined by a mixture of (1 → 6)-, (1 → 4)-, and (1 → 3)-linkages with approximately half of the β-(1 → 3)-linked D-glucose residues having branch points at C-6.[567] The resistance to degradation of

[561] F. Kanetsuna, L. M. Carbonell, I. Azuma, and Y. Yamamura, *J. Bacteriol.*, 1972, **110**, 208.
[562] M. Horisberger, B. A. Lewis, and F. Smith, *Carbohydrate Res.*, 1972, **23**, 175.
[563] K. K. Tung, A. Rosenthal, and J. H. Nordin, *J. Biol. Chem.*, 1972, **246**, 6722.
[564] R. A. Cox and G. K. Best, *Infection and Immunity*, 1972, **5**, 449.
[565] J. G. H. Wessels, D. R. Kreger, R. Marchant, B. A. Regensburg, and O. M. H. de Vries, *Biochim. Biophys. Acta*, 1972, **273**, 346.
[566] A. Bourret, H. Chanzy, and R. Lazaro, *Biopolymers*, 1972, **11**, 893.
[567] D. A. Bush and M. Horisberger, *Carbohydrate Res.*, 1972, **22**, 361.

sclerotan {a β-(1 → 3)-glucan} by enzymes from fungi parasitic on sclerotia has been attributed to structural 'errors' that impede the exo-glucanase or possibly to the presence of non-glycosidic linkages.[568]

In a study on the chitin–glucan complex of *Saccharomyces cerevisiae* cell walls, daughter cells without bud scars were found to contain lower levels of 2-amino-2-deoxy-D-glucose than the mother cells.[569] High-resolution autoradiography of *Choanephora curcurbitarum* conidial and hyphal walls, after incorporation of 2-acetamido-2-deoxy-D-[^{3}H]glucose, indicated that the incorporation occurred preferentially into the inner layer of the conidial wall and also into the wall of the growing hyphae. When this label was fed to cells infected with *Piptocephalis virginiana*, incorporation was observed in *P. virginina*; this indicated that the parasitic fungus is not capable of using the free chitin precursor for the synthesis of its own cell wall.[570] Autoradiography of intact colonies of *Trichoderma*, labelled with 2-acetamido-2-deoxy-D-[^{3}H]glucose as a marker of new cell-wall biosynthesis, has shown that the incorporation of the amino-sugar differs greatly from that of [^{3}H]uridine and [^{3}H]leucine as regards labelling pattern and susceptibility to enzymes.[571] The indirect effect of light on the encystment capacity of the aquatic fungus *Blastocladiella emersonii* was ascribed to a reduction of the total chitin synthetase activity associated with the γ-particles in the spores.[572] Like other chitinolytic bacteria, strains of *Cytophaga johnsonii* produced an extracellular chitinase and chitobiase. The metabolism of 2-acetamido-2-deoxy-D-glucose liberated from chitin was shown to involve de-*N*-acetylation to 2-amino-2-deoxy-D-glucose before further oxidation to D-glucosaminic acid occurs.[573] Mutants of *Aspergillus nidulans* having a blocked chitin synthesis underwent lysis and distortion along the hypha on continued incubation in growth media lacking an osmotic stabilizer. Pulse-labelling with 2-acetamido-2-deoxy-D-glucose and radiography showed that, in hyphae transferred to conditions of chitin synthesis, chitin was incorporated into the walls in older regions undergoing distortion.[574] An enzymic complex from cultures of *Streptomyces* released hexosamines (but no 2-acetamido-2-deoxy-D-glucose), neutral sugars, and uronic acids from purified walls of *Mucor rouxii*. The complex hydrolysed chitosan, but not chitin, and the walls from fungi belonging to the Mucorales.[575]

The levels of 2-amino-2-deoxyhexoses have been shown to be higher in the yeast form of *Blastomyces dermatitidis* than in the mycelial form.[576] In a study of cell-wall alterations associated with the hyperproduction of

[568] J. S. D. Bacon, A. H. Gordon, and D. Jones, *Biochem. J.*, 1972, **129**, 27P.
[569] K. Boran, Z. Holan, and J. Baldrian, *Folia Microbiol.*, 1972, **17**, 322.
[570] M. S. Manocha and K. Y. Lee, *Canad. J. Botany*, 1972, **50**, 35.
[571] E. Galun, *Arch. Mikrobiol.*, 1972, **86**, 305.
[572] E. C. Cantino and R. B. Myers, *Arch. Mikrobiol.*, 1972, **83**, 203.
[573] N. Sundarraj and J. V. Bhat, *Arch. Mikrobiol.*, 1972, **85**, 159.
[574] D. Katz and R. F. Rosenberger, *Arch. Mikrobiol.*, 1971, **80**, 284.
[575] S. Bartnicki-Garcia and E. Lippman, *J. Gen. Microbiol.*, 1972, **73**, 487.
[576] I. Roy and J. W. Landau, *Sabouraudia*, 1972, **10**, 107.

extracellular enzymes in *Neurospora crassa*, a marked decrease in the amount of 2-amino-2-deoxy-D-galactose was noted in the conidial walls of two strains.[577] A D-xylose-containing polysaccharide and one composed of 2-amino-2-deoxy-D-glucose (probably as chitin) have been isolated from the cell walls of both the carotenogenic and non-carotenogenic forms of *Blakeslea trispora*.[578]

The structural components of the cell walls of *Torulopsis aeris*, *Geotrichum lactis*, and *Oospora suaveolens* were shown to be mainly chitin and glucan. These polysaccharides appeared to be protected from the lytic action of enzymes by other polysaccharides.[579] The hyphal walls of the three chromomycosis agents *Phialophora verrucosa*, *P. pedrosoi*, and *Cladosporium carrionii* have similar chemical compositions, *viz.* D-glucose (17—31%), D-mannose (8—14%), 2-amino-2-deoxy-D-glucose (6—8%), and protein (19—42%). An alkali-soluble fraction was composed mainly of chitin and protein. It was suggested that the hyphal forms of these human pathogenic fungi have wall compositions intermediate between those of Euascomycetes and Hemiascomycetes.[580] D-Glucose and D-mannose were the only neutral sugars detected in the cell walls of a *Sepedonium* species. Both D-glucose and 2-acetamido-2-deoxy-D-glucose were liberated by a crude chitinase preparation, which also contained a β-(1 → 3)-glucanase.[581] Fractionation of the cell walls of *Dendryphiella vinosa* indicated the presence of glucans, with mainly β-(1 → 3)- and β-(1 → 6)-linkages, chitin, and proteins. D-Galactose and D-mannose were also shown to be present in low proportions and glycosidically linked to D-glucose in heteroglycans. The low level of these sugars, and the absence of polymers containing 2-amino-2-deoxy-D-galactose and uronic acid, differentiates this fungus from other Deuteromycetes.[582]

The effects of solubilization of structural polymers upon the cell-wall integrity of *Trichophyton mentagrophytes* have been reported. Alkali-soluble material was characterized as a galactomannan with (1 → 2)- and (1 → 6)-linkages, but no (1 → 4)-linkages, together with a β-glucan containing both (1 → 3)- and (1 → 6)-linkages. An acid-soluble glucomannan having long interwoven microfibrils appeared, on electron microscopic evidence, to be embedded in the alkali-soluble materials. On the outside of a fibrillar network there exist chitin microfibrils associated with a β-glucan containing mainly (1 → 3)- and small amounts of (1 → 6)-linkages.[583] The structure of the β-glucan component of the cell walls of *Piricularia oryzae* has been investigated. Controlled acetolysis released a

[577] A. G. Granzner, *J. Bacteriol.*, 1972, **111**, 443.
[578] M. S. Chenouda, *J. Gen. Appl. Microbiol.*, 1972, **18**, 143.
[579] J. P. G. Ballesta, *Trans. Brit. Mycol. Soc.*, 1972, **59**, 233.
[580] P. J. Szaniszlo, B. H. Cooper, and H. S. Voges, *Sabouraudia*, 1972, **10**, 94.
[581] J. E. Domer and R. D. Harman, *Sabouraudia*, 1972, **10**, 56.
[582] R. Bonaly, *Carbohydrate Res.*, 1972, **24**, 355.
[583] Y. Kitazima, Y. Banno, J. Noguchi, Y. Nozawa, and Y. Ito, *Arch. Biochem. Biophys.*, 1972, **152**, 811.

homologous series of laminaridextrins, whereas an exo-β-(1 → 3)-glucanase hydrolysed the polysaccharide to D-glucose and gentiotriose. These results, together with those of methylation, periodate oxidation, and fragmentation analysis, suggested that the polysaccharide contains a β-(1 → 3)-linked D-glucosyl backbone with side-chains attached through β-(1 → 6)-linkages and with part of the backbone containing β-(1 → 6)-linkages.[584] Presumptive evidence has been produced for the existence of a delicate balance between fungal-wall synthesis and wall lysis in apical growth. Circumstantial evidence supported the conclusions that the growing tips of fungi have a large lytic wall potential, that the release of this activity during growth is gradual and delicately co-ordinated with wall synthesis, and that the balance can be disturbed by a wide variety of external stimuli in such a way that the ensuing surge of lytic activity result in violent disintegration of the hyphal apex.[585]

Saccharomyces cerevisiae when grown in the presence of '2-deoxy-D-glucose' incorporated the sugar into the cell walls. After fractionation into mannan and glucan, it was shown that '2-deoxy-D-glucose' is incorporated mainly into the cell-wall mannan. Two oligosaccharides isolated from the mannan have been identified tentatively as 2-deoxy-3-*O*-α-D-mannopyranosyl-D-glucose and a trisaccharide containing an additional D-mannose unit at the non-reducing terminus of the disaccharide.[586]

The polymerized amino-sugar of the germ tube walls from the uredospores of wheat stem rust (*Puccinia graminis*) was identified as 2-acetamido-2-deoxy-D-glucose, which is probably present as chitin. In comparison, the spore walls contain polymerized 2-amino-2-deoxy-D-glucose, probably as chitosan, and a mannan.[587]

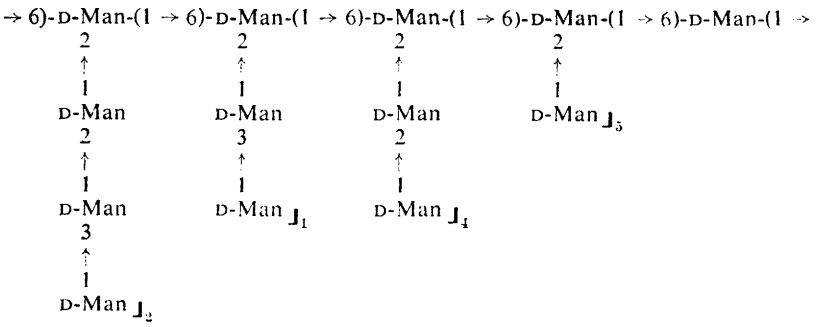
Three classes of subcellular particles from budding *Saccharomyces cerevisiae* cells contain glucanase, mannan, and protein.[588] The vesicles derived from the endoplasmic reticulum, which are secreted locally into the cell wall growing buds, have been isolated and shown to contain 50% mannan.[589] The specific staining of *S. cerevisiae* cell walls with fluorescein-conjugated concanavalin A was competitively inhibited by either unlabelled concanavalin A or methyl α-D-mannopyranoside. It was concluded that the staining of the cell wall results from a specific interaction of the fluorescein–concanavalin A conjugate with an α-mannan present in the cell wall of this yeast.[590]

A particulate membrane preparation isolated from broken cells of *S. cerevisiae* was found to be capable of incorporating D-mannose from

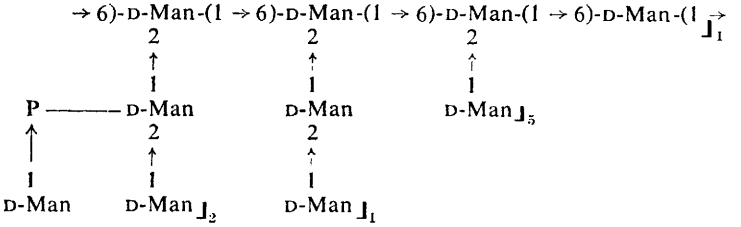
[584] T. Nakajima, K. Tamari, K. Matsuda, H. Tanaka, and N. Ogasawara, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 11.
[585] I. F. Ramírez-León and J. Ruíz-Herrera, *J. Gen. Microbiol.*, 1972, **72**, 281.
[586] P. Biely, Z. Krátký, and S. Bauer, *Biochim. Biophys. Acta*, 1972, **255**, 631.
[587] S. Joppien, A. Burger, and H. J. Reisener, *Arch. Mikrobiol.*, 1972, **82**, 337.
[588] P. Matile, M. Cortat, A. Wiemken, and A. Frey-Wyssling, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 636.
[589] M. Cortat, P. Matile, and A. Wiemken, *Arch. Mikrobiol.*, 1972, **82**, 189.
[590] J. S. Tkacz, E. B. Cybulska, and J. O. Lampen, *J. Bacteriol.*, 1971, **105**, 1.

GDP-D-[^{14}C]mannose into endogeneous acceptors of a lipid nature. From a study of the kinetics of incorporation of D-mannose from the nucleotide, it was concluded that GDP-D-mannose is not an immediate precursor of mannan and that a lipid intermediate is essential for the polysaccharide's synthesis.[591] The lipid intermediate was identified as a D-mannosyl 1-phosphorylpolyprenol.[592]

Two structurally and antigenically different *S. cerevisiae* cell-wall mannans have been isolated. One of the mannans was already known and yielded acetylated mannose, mannobiose, mannotriose, and mannotetraose on acetolysis of the (1 → 6)-linked backbone (34). The other, which occurs



(34)

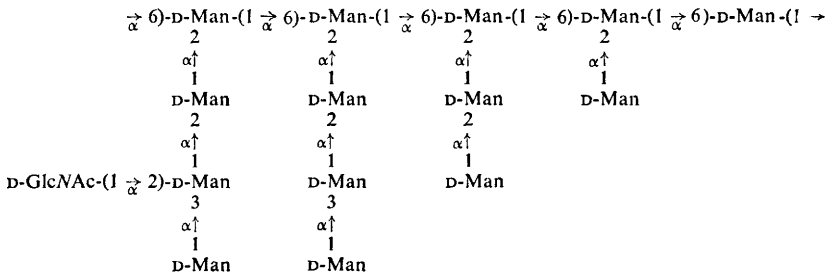


(35)

in *S. cerevisiae* brewer's strains, is a phosphomannan having a structure very similar to that of *Kloeckera brevis* mannan (35).[593] Bud scars prepared from *S. cerevisiae* cell walls by means of an exo-β-D-(1 → 3)-glucanase contained mannose (83%), glucose (4%), and 2-amino-2-deoxy-D-glucose. Electron microscopic examination of a mercury-labelled concanavalin A complex of the bud scars showed that the mannan is a true component of these scars.[594]

[591] R. Sentandreu and J. O. Lampen, *F.E.B.S. Letters*, 1971, **14**, 109.
[592] R. Sentandreu and J. O. Lampen, *F.E.B.S. Letters*, 1972, **27**, 331.
[593] T. N. Cawley and C. E. Ballou, *J. Bacteriol.*, 1972, **111**, 690.
[594] H. Bauer, M. Horisberger, D. A. Bush, and E. Sigarlakie, *Arch. Mikrobiol.*, 1972, **85**, 202.

The cell-wall mannan of the yeast *Kluyveromyces lactis* is characterized by a high content of 2-acetamido-2-deoxy-D-glucose, most of which is located in pentasaccharide side-chains containing four D-mannose units and one amino-sugar unit. The side-chains were released from the mannan by controlled acidic hydrolysis. The pentasaccharide structure was shown by methylation analysis to consist of a mannotetraose unit, α-D-Man-(1 → 3)-α-D-Man-(1 → 2)-α-D-Man-(1 → 2)-D-Man, having an α-linked 2-acetamido-2-deoxy-D-glucose residue attached to position 2 of the penultimate D-mannose residue in the chain. A representative structure (36) of the mannan was given.[595] The observed anaphylaxis-like reaction



(36)

induced by yeast mannans in non-sensitized mice appeared to depend on α-(1 → 2)- and α-(1 → 6)-linkages in the main chains.[596]

The cell-wall phosphomannan of *Kloeckera brevis* has been extracted and it was separated by means of ion-exchange chromatography into four components of differing phosphate content. From the results of Smith degradation, partial acidic hydrolysis, and ^{31}P n.m.r., it was demonstrated that a D-mannose unit is involved in a (1 → 6)-phosphodiester linkage to the middle D-mannose unit of a trisaccharide side-chain attached to the main polysaccharide.[597] The four phosphomannan fractions were resolved by gel-permeation chromatography into components of molecular weight 5×10^5, 1×10^5, and 2.5×10^4. The low molecular weight mannan is a subunit which appears to be cross-linked by protein to form the higher molecular weight complexes. Variation of the growth phase of the yeast was accompanied by variations in composition and extent of cross-linking of the mannan.[598] Immunochemical evidence has shown that antibodies were formed against the phosphorylated side-chains of *K. brevis* phosphomannan in preference to the neutral side-chains.[599]

[595] W. C. Raschke and C. E. Ballou, *Biochemistry*, 1972, **11**, 3807.
[596] L. S. Kind, P. K. Kaushal, and P. Drury, *Infection and Immunity*, 1972, **5**, 180.
[597] T. R. Thieme and C. E. Ballou, *Biochemistry*, 1971, **10**, 4121.
[598] T. R. Thieme and C. E. Ballou, *Biochemistry*, 1972, **11**, 1115.
[599] W. C. Raschke and C. E. Ballou, *Biochemistry*, 1971, **10**, 4130.

A galactomannan from the yeast form of *Cladosporium werneckii* released two types of carbohydrate chain on treatment with alkaline sodium borohydride; one of these was composed of small, reduced D-mannose-containing oligosaccharides and the other comprised high molecular weight phosphorylated galactomannans. The latter had a molecular weight of 6×10^4, the phosphate groups diesterified, and a portion of the D-galactose in the furanose form. From the results of mild acidic hydrolysis and identification of the fragments, a structure for the high molecular weight fraction was proposed in which the fragments are linked together by phosphodiester bonds to form highly branched chains which, together with the more numerous D-mannose-containing oligosaccharides, are linked to a peptide backbone through *O*-glycosidic linkages to L-serine and L-threonine.[600]

Modifications of the cell-wall structure of *Rhodotorula aurantiaca* on culturing in the presence of sulphur-containing amino-acids were manifested by increased lipid and mannan levels.[601] The behaviour of various fractions of polyphosphates and polysaccharides in the ontogenetic development of *Saccharomyces cerevisiae* has been described and correlations between the two types were examined. A connection between the rates of accumulation of cell-wall mannan and acid-insoluble polyphosphates, possibly in mannan and polyphosphate syntheses through the action of a pyrophosphorylase system, was confirmed.[602]

The hyphal and sclerotial walls of certain plant pathogenic fungi have been investigated by i.r. spectroscopy, monosaccharide composition, and lysis. The results indicated that the composition of the walls of *Sclerotium tuliparum* differs markedly from that of other species of *Sclerotium*, but is similar to *Rhizoctonia* in containing D-glucuronic acid and D-xylose in addition to D-glucose, D-mannose, L-fucose, and L-rhamnose.[603]

Mucoran, an acidic heteropolysaccharide isolated from *Mucor rouxii* and purified by ion-exchange chromatography, was shown to contain D-glucuronic acid, D-mannose, and L-fucose (5 : 3 : 2). The strong base used in the Hakomori methylation procedure precipitated the polysaccharide, which underwent extensive β-elimination with the release of unbranched glucuronomannan chains of different lengths. These soluble chains became fully methylated and could be separated into two fractions with DPs of 10 and 20. Mucoran (DP 600) was considered to have the structure (37).[604]

$$\left[\text{D-Man-(1} \rightarrow \text{4)-}\alpha\text{-D-GlcUA-(1} \rightarrow \text{3)-D-Man-(1}\right]_n \rightarrow$$

(37)

[600] K. O. Lloyd, *Biochemistry*, 1972, **11**, 3884.
[601] K. A. Killick, *Canad. J. Microbiol.*, 1972, **18**, 423.
[602] I. S. Kulaev, V. M. Vagabov, and A. B. Tsiomenko, *Doklady Akad. Nauk S.S.S.R.*, 1972, **204**, 734.
[603] D. Jones, V. C. Farmer, J. S. D. Bacon, and M. J. Wilson, *Trans. Brit. Mycol. Soc.*, 1972, **59**, 11.
[604] S. Bartnicki-Garcia and B. Lindberg, *Carbohydrate Res.*, 1972, **23**, 75.

The main polysaccharide of *Rhizopus nigricans* was found to be a highly heterogeneous glycan composed of L-fucose, D-mannose, D-galactose, and D-glucuronic acid. A minor polysaccharide was shown to contain D-glucuronic acid, 2-amino-2-deoxy-D-glucose, and amino-acids.[605]

Three homogeneous polysaccharides, each containing different amounts of D-xylose, D-mannose, and D-glucuronic acid, have been isolated from *Tremella fuciformis*.[606] The main chain of the polysaccharide isolated from *Rhodotorula flava* is composed of (1 → 3)-linked D-mannose units and side-chains of (1 → 4)-linked D-xylose.[607]

Fungal Exopolysaccharides

The yeast-like fungus *Pullularia pullulans* has been shown to utilize simple mono- and di-saccharides both in the production of cell mass and in the elaboration of the extracellular polysaccharide pullulan. A study was made of the utilization pattern of these sugars and the effect obtained by varying the pH. Pullulan appeared to be elaborated under conditions favourable to the accumulation of D-glucose or D-fructose metabolites within the cells.[608] When D-glucose was the sole carbon source, increase in cell mass preceded the elaboration of pullulan. A study was made of the conditions generated in the culture medium for activation of the secretion of the polysaccharide.[609] From the results of inhibition studies using cycloheximide, it was concluded that the production of pullulan by *P. pullulans* is not constitutive but inducible.[610]

A highly viscous glucan from *Monolinia fructicola* has been methylated and the products of hydrolysis identified by g.l.c.–m.s. of the partially acetylated 1-deuterioalditols. The presence of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,4,6-tri-*O*-methyl-D-glucose, and 2,6-di-*O*-methyl-D-glucose (1 : 2.1 : 1.1) indicated the existence of a highly branched polymer containing predominantly (1 → 3)- and lesser amounts of (1 → 4)-linkages.[611]

Periodate oxidation, acetolysis, and the action of α-mannosidase suggested that the cellular and exocellular mannans from *Hansenula holstii* are structurally distinct, with the exopolysaccharide resembling the branched structure normally associated with baker's yeast mannan.[612] The phosphomannan of *H. holstii* was shown to be the most potent inducer of interferon activity of the exocellular phosphomannans produced by four strains of *Hansenula* yeasts; the interferon induced was highly host-species-specific, preventing viral cytopathic effects only in the homologous cells.[613] A

[605] T. Miyazaki and T. Irino, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 2545.
[606] S. Ukai, K. Hirose, and T. Kiho, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 1347.
[607] G. A. Vitovskaya, N. P. Elinov, N. S. Kozhina, and G. Z. Mamatov, *Khim. prirod. Soedinenii*, 1972, **1**, 15.
[608] B. J. Catley, *Appl. Microbiol.*, 1971, **22**, 641.
[609] B. J. Catley, *Appl. Microbiol.*, 1971, **22**, 650.
[610] B. J. Catley, *F.E.B.S. Letters*, 1972, **20**, 174.
[611] M. S. Feather and A. Malek, *Biochim. Biophys. Acta*, 1972, **264**, 103.
[612] W. L. Cunningham and G S. Blas, *Biochem. J.*, 1971, **125**, 31P.
[613] S. Susuki, M. Suzuki, and M. Imaya, *Jap. J. Microbiol.*, 1971, **15**, 485.

polysaccharide containing 3-*O*-methylmannose has been isolated from culture filtrates of *Coccidiodes immitis*.[614]

The partial structure for the polysaccharide of *Physarum polycephalum* has been reported to contain (1 → 3)-, (1 → 4)-, and (1 → 6)-linked D-galactose units, together with sulphate and phosphate.[615]

The polysaccharides isolated from a number of species of *Rhizobium* all contained varying proportions of D-glucose, D-galactose, D-mannose, and D-glucuronic acid. It was concluded that polysaccharide analysis has limited value as a diagnostic tool for the classification of the *Rhizobium*.[616] However, a separate report has shown that there is a distinctive difference between *Rhizobium leguminosarum*, *R. phaseoli*, and *R. trifolii*, which have the same range of D-glucose (48—61%), D-galactose (86—14%), D-glucuronic acid (14—19%), pyruvate, and acetate, compared with *R. meliloti* and *Agrobacterium tumefaciens*, which have a higher D-glucose content (76—83%) and less than 1% D-glucuronic acid.[617] Analysis of the polysaccharides of some related strains of *R. trifolii* indicated that small differences in composition between the strains are unrelated to the ability to nodulate or to the capacity of the symbiotic organisms to fix nitrogen.[618]

The main polysaccharides produced by *Absidia cylindrospora* and *Mucor mucedo* were found to contain the same sugar components, *viz.* L-fucose, D-mannose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-galactose, in similar ratios. A similarity in structure was indicated on the basis of partial acidic hydrolysis and periodate oxidation studies.[619]

Aqueous extracts of cabbage leaves infected with the black rot pathogen *Xanthomonas campestris* contained a polysaccharide that was purified by precipitation with hexadecyltrimethylammonium bromide. The polysaccharide was shown to be similar to an extracellular polysaccharide fraction obtained from culture filtrates of *X. campestris* in liberating D-glucose, D-mannose, and D-glucuronolactone on acidic hydrolysis.[620, 621]

Miscellaneous Oligo- and Poly-saccharides

Evidence has been provided to suggest that, when sucrose is the main carbon source for growth of *Claviceps purpurea*, oligosaccharides act as substrates for an active β-fructofuranosidase produced by the fungus. In the first instance, the disaccharide is converted into D-glucose and an oligo-D-fructoside. Subsequent action of the enzyme on both sucrose and the oligofructoside led to the release of more D-glucose and the formation

[614] J. F. Porter, E. R. Scheer, and R. W. Wheat, *Infection and Immunity*, 1971, **4**, 660.
[615] D. R. Farr, H. Amster, and M. Horisberger, *Carbohydrate Res.*, 1972, **24**, 207.
[616] C. E. Clapp and R. J. Davis, *Soil Biol. Biochem.*, 1970, **2**, 109.
[617] L. P. T. M. Zevenhuizen, *J. Gen. Microbiol.*, 1971, **68**, 239.
[618] C. M. Hepper, *J. Microbiol. Serol.*, 1972, **38**, 437.
[619] T. Miyazaki and T. Irino, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 330.
[620] J. C. Sutton and P. H. Williams, *Canad. J. Botany*, 1970, **48**, 645.
[621] J. C. Sutton and P. H. Williams, *Canad. J. Botany*, 1971, **49**, 1049.

of additional oligosaccharides (38)—(42), which were characterized on the basis of enzymological and chemical evidence.[622]

β-D-Fru*f*-(2 → 6)-α-D-Glc*p*
(38)

β-D-Fru*f*-(2 → 6)-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*
(39)

β-D-Fru*f*-(2 → 1)-β-D-Fru*f*-(2 → 1)-α-D-Glc*p*
(40)

β-D-Fru*f*-(2 → 1)-β-D-Fru*f*-(2 → 6)-α-D-Glc*p*
(41)

β-D-Fru*f*-(2 → 1)-β-D-Fru*f*-(2 → 6)-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*
(42)

Glucans obtained from preparations of *Rhodotorula glutinis* have been shown to be glycogen-like polysaccharides with a high degree of branching. On injection into animals, the glucans caused an interferonogeneous effect and increased animal resistance to an influenza virus.[623] It has been demonstrated that yeast glycogen samples showing considerable variations in specific rotations and β-amylolysis limits were contaminated with a β-(1 → 6)-glucan, since after digestion of the glycogen with amyloglucosidase, the remaining material furnished gentiobiose and gentiotriose on partial acidic hydrolysis.[624] The paramorphogenic action of L-sorbose on polysaccharide synthesis in *Neurospora crassa* resulted from the inhibition of glycogen synthetase.[625] It has been demonstrated that the presence *in vivo* of adenosine 3′,5′-cyclic phosphate caused a rapid depletion of glycogen-storage material in the cellular slime mould *Dictyostelium discoideum*. It was suggested that one effect of this nucleotide is closely related to enzymic processes involved in the rapid conversion of glycogen into cell-wall material and other end products accumulating during sorocarp construction.[626]

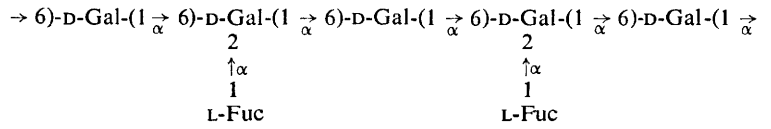
A soluble polysaccharide found in selected genera of Oomycetes has been characterized as a β-(1 → 3)-glucan.[627]

D-Glucose has been reported to be the sole precursor for the biosynthesis of intracellular glucan in *Clostridium botulinum*. The polysaccharide was found to function in the capacity of an endogenous substrate by supplying

[622] A. G. Dickerson, *Biochem. J.*, 1972, **129**, 263.
[623] N. P. Elinov, G. A. Vitovskaya, O. A. Aksenov, O. N. Ageeva, and A. E. Agnaeva, *Biokhimiya*, 1972, **37**, 590.
[624] R. B. Evans and D. J. Manners, *Biochem. J.*, 1971, **125**, 31P.
[625] N. C. Mishra and E. L. Tatum, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 313.
[626] P. A. Rosness, G. Gustafson, and B. E. Wright, *J. Bacteriol.*, 1971, **108**, 1329.
[627] S. Faro, *J. Gen. Microbiol.*, 1972, **72**, 393.

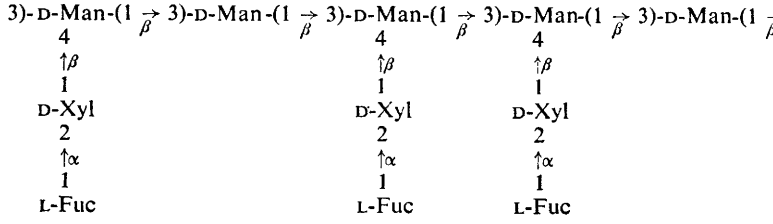
the cell with a readily available source of carbon and energy for spore production.[628]

A fucogalactan and a glucan have been isolated from aqueous extracts of the fruit bodies of *Fomes annosus*. From the results of methylation analysis, the fucogalactan was shown to consist of chains of α-(1 → 6)-linked D-galactopyranose units having 32% substituted in the 2-position (43). The glucan consists of β-(1 → 3)- and β-(1 → 6)-linked D-glucose



(43)

residues and branch points with (1 → 3)- and (1 → 6)-linkages. From the complex polysaccharide mixture contained in alkaline extracts of the fungus, a fucoxylomannan was successfully isolated by precipitation with the H-agglutinin from eel-serum. Methylation analysis of both the polysaccharide and an acid-degraded material indicated that about 40% of the (1 → 3)-linked D-mannose residues are substituted in the 4-position by side-chains terminating in α-L-fucopyranosyl-(1 → 2)-D-xylopyranosyl residues (44).[629]



(44)

Two water-soluble polysaccharides, which are not closely related immunologically, have been isolated from a variant strain and a typical virulent strain of *Cryptococcus neoformans*. Both polysaccharides were demonstrated to contain the same constituent monosaccharides, namely D-xylose, D-mannose, D-galactose, and D-glucuronic acid, but the virulent strain has a greater D-glucuronic acid content and a larger molecular weight than the variant strain.[630] Chromium chloride was used as a

[628] G. A. Strasdine, *Canad. J. Microbiol.*, 1972, **18**, 211.
[629] K. Axelsson, H. Björndal, S. Svensson, and S. Hammarström, *Acta Chem. Scand.*, 1971, **25**, 3645.
[630] T. R. Kozel and J. Cazin, *Infection and Immunity*, 1971, **3**, 287.

coupling agent for the conjugation of purified crytococcal polysaccharide to sheep erythrocytes. When the sensitized erthrocytes were used in a passive hemagglutination assay for the antigen, sub-microgram quantities of the soluble polysaccharide were detected.[631]

[631] T. R. Kozel and J. Cazin, *Infection and Immunity*, 1972, **5**, 35.

5

Glycoproteins, Glycopeptides, and Animal Polysaccharides

The continual and increasing advance of the chemistry of glycoproteins has been high-lighted by the recent publication of the second edition of the well-known book entitled 'Glycoproteins'.[632] Sections deal with the definition of glycoproteins, measurement of their physicochemical parameters, carbohydrate component analysis by various techniques, and the structural analysis of the carbohydrate moieties and glycopeptide linkages. A discussion of the occurrence and distribution of glycoproteins is followed by specialist treatment of the composition, structure, and function of virtually all the known glycoproteins. A general article on glycoproteins dealing with the occurrence, isolation, purification, composition, and structure contains a classification according to the number of carbohydrate moieties per protein unit of the macromolecule.[633] Recent advances in the chemical studies of mammalian glycoproteins, proteoglycans, and collagens, and plant and bacterial cell-wall glycoproteins have been reviewed,[634] as have the structures and functions of surface components of mammalian cells.[635]

A method reported for the identification of glycoproteins is based on periodate oxidation followed by reaction with metabisulphite and Alcian Blue.[636] The formation of bisulphite adducts with the liberated aldehyde groups increased the acidity of the glycoprotein and enhanced its reaction with Alcian Blue.

It has been reported that high yields of glycoproteins may be obtained by the use of lithium 3,5-di-iodosalicylate in their extraction from cells and tissues.[637] The use of immunoadsorbents in the purification of immunologically active species has been reviewed[638] and it has been shown that glycoproteins may be purified using disulphide-linked homologous antibodies as immunoadsorbents.[639]

[632] A. Gottschalk, 'Glycoproteins,' Parts A & B, Elsevier, Amsterdam, 1972.
[633] R. Montgomery, in 'The Carbohydrates', ed.: W. Pigman and D. Horton, Academic Press, New York, 1970, p. 628.
[634] K. Schmid, *Chimia* (*Switz.*), 1972, **26**, 405.
[635] V. Ginsburg and A. Kobata, in 'Structure and Function of Biological Membranes', ed. L. I. Rothfield, Academic Press, New York, 1971, p. 439.
[636] A. H. Wardi and W. A. Allen, *Analyt. Biochem.*, 1972, **48**, 621.
[637] V. T. Marchesi and E. P. Andrews, *Science*, 1971, **174**, 1247.
[638] O. Pihar, *Chem. listy*, 1971, **65**, 713.
[639] N. E. Crook, J. Stephen, and H. Smith, *Immunochemistry*, 1972, **9**, 945.

Studies on the hydrolytic conditions for analysis of the carbohydrate components of glycoproteins have shown that, for a fixed set of conditions, the extent of hydrolysis of glycosidic bonds is dependent upon the nature of the monosaccharides participating in the glycoside structure.[640]

Methods for the identification and quantitation of the monosaccharide components of glycoproteins as their alditol acetates[640, 641] and as trifluoroacetate derivatives of their methyl glycosides[642] have been reported; it was possible to obtain a complete analysis on 1 mg of a glycoprotein containing 6% carbohydrate.[641] Similar methodology has been employed for the quantitation and identification of monosaccharides released from glycoproteins and glycopeptides by enzymic hydrolysis.[643] The identification of fucose by g.l.c. of the trimethylsilylated methyl glycoside has been claimed to be more satisfactory than other recognized techniques.[644] A new method reported for the estimation of amino-sugars is based on chlorination with sodium hypochlorite, reduction of the excess of hypochlorite with sodium nitrite, and then allowing the chlorinated product to react with amylose–potassium iodide, the amount of blue starch formed being determined spectrophotometrically.[645] This method may be used for the differential determination of free and *N*-acetylated amino-groups. Techniques for the identification of glycopeptides in normal and pathological samples have been described.[646]

Methods for the structural analysis of the carbohydrate moieties of glycoproteins (*e.g.* periodate oxidation, and enzymic and partial acid hydrolysis) have been reviewed briefly[647] and the glycopeptides derived from glycoproteins have been classified systematically according to the type of glycopeptide linkage and the structure of the carbohydrate moiety.[648]

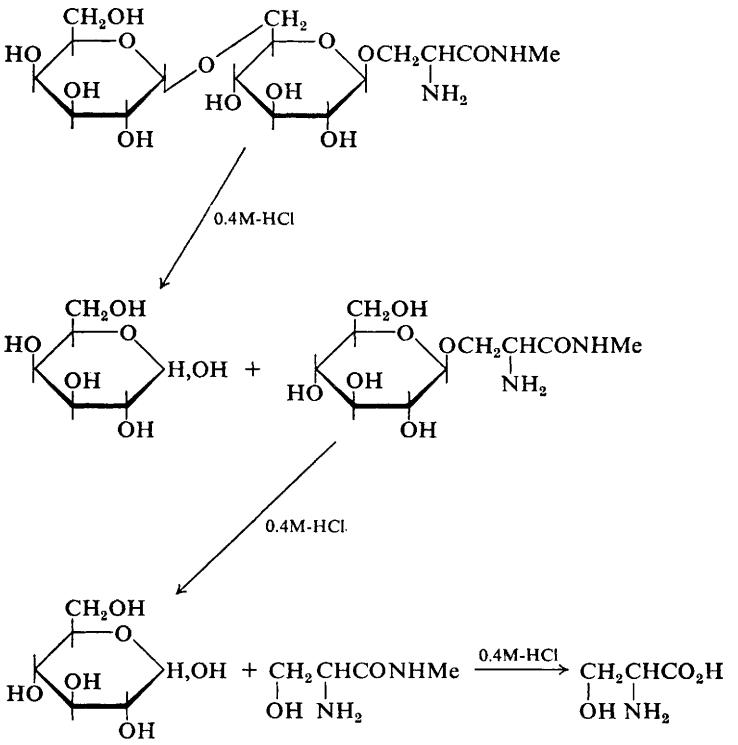
A gas-chromatographic method has been described for the estimation of the products (including ethylene glycol, propylene glycol, glycerol, erythritol, threitol, and 5-acetamido-3,5-dideoxy-L-*arabino*-2-heptulosonic acid) and surviving monosaccharides after periodate oxidation and borohydride reduction of glycopeptides and related materials.[649]

The mass spectra of acetylated *O*-glycosyl derivatives of L-serine and L-threonine methyl esters show characteristic features that allow identification of the amino-acid and carbohydrate residues as well as the type of linkage between them.[650] The spectra also provided information on the

[640] J. Metz, W. Ebert, and H. Weicker, *Chromatographia*, 1971, **4**, 345.
[641] W. Niedermeier, *Analyt. Biochem.*, 1971, **40**, 465.
[642] J. P. Zanetta, W. C. Breckenridge, and G. Vincendon, *J. Chromatog.*, 1972, **69**, 291.
[643] R. B. Carlsen and J. G. Pierce, *J. Biol. Chem.*, 1972, **247**, 23.
[644] R. Verga and A. Ghocchi, *J. Chromatog.*, 1970, **49**, 46.
[645] P. A. Sandford, A. J. Nafziger, and A. Jeanes, *Analyt. Biochem.*, 1971, **42**, 422.
[646] E. G. Brunngraber, B. D. Brown, and H. Hof, *Clinica Chim. Acta*, 1971, **32**, 159.
[647] J. R. Clamp, *Biochem. J.*, 1972, **128**, 116P.
[648] H. Sinohara, *Tohoku J. Exp. Med.*, 1972, **106**, 93.
[649] K. W. Hughes and J. R. Clamp, *Biochim. Biophys. Acta*, 1972, **264**, 418.
[650] O. S. Chizov, V. A. Derevitskaya, B. M. Zolotarev, L. M. Likhosherstov, O. S. Novikova, and N. K. Kochetkov, *Carbohydrate Res.*, 1971, **20**, 275.

monosaccharide sequence and the position of the glycosyl-glycose bond in glycopeptides.

The stabilities of monosaccharide–L-serine and disaccharide–L-serine *O*-glycosidic bonds to acid and alkali have been studied. Under acidic conditions, the glycosyl-glycose bond is more labile (*e.g.* Scheme 2, using

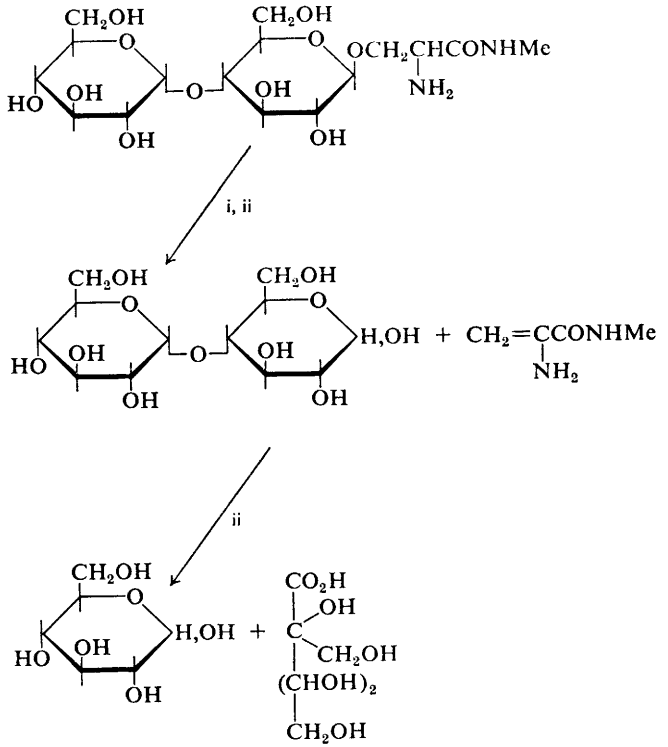


Scheme 2

O-L-seryl β-melibioside), whereas in alkaline media the glycosyl–amino-acid bond is cleaved first by the β-elimination mechanism (*e.g.* Scheme 3, using *O*-L-seryl β-maltoside).[651] Treatment of glycopeptides containing 2-acetamido-1-{(*N*-β-L-aspartyl)amino}-2-deoxy-D-glucosylamine units with alkaline borohydride at 100 °C resulted in complete cleavage of the glycosyl–amino-acid bond with concomitant production of terminal 2-amino-2-deoxy-D-glucitol.[652] Periodate oxidation, borohydride reduction, and hydrolysis of the carbohydrate fragment then gave an amino-polyol, the

[651] V. A. Derevitskaya, L. M. Likhosherstov, O. S. Novikova, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1971, 2246.

[652] Y. C. Lee and J. R. Scocca, *J. Biol. Chem.*, 1972, **247**, 5753.



Reagents: i, pH 11, 37 °C; ii, 0.05M-Na_2CO_3, 70 °C.

Scheme 3

nature of which is dependent upon the original substitution of the 2-acetamido-2-deoxy-D-glucose residue. This technique has been developed for general determination of the linkage position of 2-amino-2-deoxy-D-glucose attached to asparagine and has been used to show that the structure (45) is a common unit in the structure of many glycoproteins. The involvement of L-serine and L-threonine in glycopeptide linkages may be assessed quantitatively after β-elimination of the oligosaccharide by addition of sulphite ion, the dehydro-analogues forming sulphonic acid derivatives (Scheme 4).[653] The substitution of hydroxy-amino-acids by carbohydrate in a number of glycoproteins and proteoglycans has been investigated using [^{35}S]sulphite.

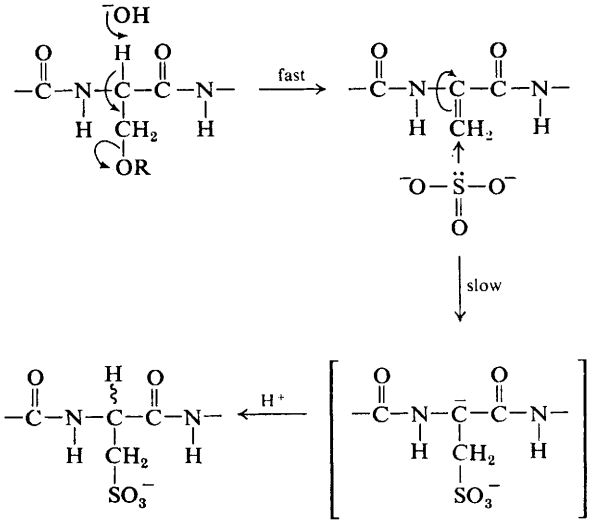
The amino-acid analyser has been adapted to monitor the extent and type of digestion of glycosyl–asparagine glycopeptides by purified glycosidases.[654] Such assessment of glycopeptides obtained from several

[653] D. L. Simpson, J. Hranisavljevic, and E. A. Davidson, *Biochemistry*, 1972, **11**, 1849.
[654] M. T. Kimmel and T. H. Plummer, *Analyt. Biochem.*, 1972, **49**, 267.

β-D-Man-(1 → 4)-β-D-Glc*N*Ac-(1 → 4)-β-D-Glc*N*Ac-Asn

(45)

glycoproteins containing glycosyl–asparagine linkages has shown structure (45) to be involved. The β-configuration of the D-mannosyl bond has been demonstrated in many cases by its susceptibility to β-mannosidase and by its resistance to α-mannosidase.[655, 656] Further proof that the D-mannosyl residues are β-linked to 2-acetamido-2-deoxy-D-glucose has been obtained



R = carbohydrate residue

Scheme 4

by i.r. and o.r.d. analyses of the derived 2-acetamido-2-deoxy-(D-mannosyl)-D-glucose disaccharide.[656]

Stability constants for the complexation of alkali-metal and alkaline-earth cations to *N*-acetylneuraminic acid have been reported.[657]

The biosynthesis, activation, and mechanism of assembly of monosaccharides in the biosynthetic pathway to glycoproteins and the subcellular sites of glycoprotein synthesis have been summarized,[658] and the involvement of glycopyranosyl esters of nucleoside pyrophosphates ('sugar nucleotides') in glycoprotein biosynthesis has been described in detail.[659] General aspects of the biosynthesis of glycoproteins have been reviewed

[655] T. Sukeno, A. L. Tarentino, T. H. Plummer, and F. Maley, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 219.
[656] T. Sukeno, A. L. Tarentino, T. H. Plummer, and F. Maley, *Biochemistry*, 1972, **11**, 1493.
[657] J. P. Behr and J. M. Lehn, *F.E.B.S. Letters*, 1972, **22**, 178.
[658] R. Schauer, *Biochem. J.*, 1972, **128**, 112P.
[659] H. Nikaido and W. Z. Hassid, *Adv. Carbohydrate Chem. Biochem.*, 1971, **26**, 351.

briefly [660] and the role of membranes in the biosynthesis of glycoproteins has been discussed.[661] Some relationships have been observed between glycoprotein and glycolipid biosyntheses.[662]

A new theory concerning the biological significance of glycoproteins has been discussed [663] and, among the possible functions of the carbohydrate moieties of glycoproteins, it is proposed that they impart a recognition signal to the protein to code for the topographical location of the glycoprotein.[664]

A general method has been described for the quantitative determination of the binding of glycoproteins and their derivatives in which certain carbohydrate residues have been removed enzymically, and it has been applied to the plasma membranes of rat liver.[665] The morphology of the macromolecular carbohydrate contents of mammalian cells has been reviewed.[666]

Plant and Algal Glycoproteins

Radioactivity from 2-amino-2-deoxy-D-[1-^{14}C]glucose was incorporated into ethanol-insoluble compounds of high molecular weight in a number of plant tissues, including germinating seedlings of barley (*Hordeum vulgare*), broad bean (*Vicia faba*), and corn (*Zea mays*), roots of corn, axenic cultures of duckweed (*Lemna minor*), and callus cells of sycamore (*Acer pseudoplatanus*).[667] Except in the case of duckweed, where some radioactivity was recovered as D-glucose, acid hydrolysis of these macromolecular materials released radioactive 2-amino-2-deoxy-D-glucose as the major radioactive species. On the basis of these results, it was concluded that 2-amino-2-deoxy-D-[1-^{14}C]glucose can be used as a specific precursor of the amino-sugar units of plant as well as animal glycoproteins.

Water-soluble glycoproteins containing hydroxyproline have been extracted with trichloroacetic acid and phenol from leaves of the broad bean (*Vicia faba*).[668] Fractionation of the extracts by electrophoresis and gel filtration revealed the presence of a spectrum of molecules with similar properties containing arabinose and galactose (10—90%) and uronic acid (10—70%). A high uronic acid content was associated with an appreciable rhamnose content and a molar ratio of arabinose : galactose of 2 : 1. Changes in the 13S-globulin of buckwheat seeds during germination have been studied and it appears that dissociation into subunits takes place.[669]

[660] W. Z. Hassid, in 'The Carbohydrates', vol. IIA, ed. W. Pigman and D. Horton, Academic Press, New York, 1970.
[661] M. J. Osborn, in 'Structure and Function of Biological Membranes', ed. L. I. Rothfield, Academic Press, New York, 1971, p. 377.
[662] P. W. Kent, *Biochem. J.*, 1972, **128**, 111P.
[663] D. H. Leaback, *Biochem. J.*, 1972, **128**, 127P.
[664] P. J. Winterburn, *Biochem. J.*, 1972, **128**, 118P.
[665] L. Van Lenten and G. Ashwell, *J. Biol. Chem.*, 1972, **247**, 4633.
[666] D. F. Parsons and J. R. Subjeck, *Biochim. Biophys. Acta*, 1972, **265**, 85.
[667] R. M. Roberts, A. B. Connor, and J. J. Cetorelli, *Biochem. J.*, 1971, **125**, 999.
[668] A. Pusztai, R. Begbie, and I. Duncan, *J. Sci. Food Agric.*, 1971, **22**, 514.
[669] M. A. Belozerskii, *Doklady Akad. Nauk S.S.S.R.*, 1971, **199**, 468.

An intact precursor of extensin has been isolated from carrot discs using its transient association with membranous organelles of the cytoplasm and the ease with which it can be extracted from the cell wall before incorporation into the cell-wall matrix.[670] The precursor is a hydroxyproline-rich 4.5S glycoprotein containing 40% carbohydrate which, based on incorporation studies, is L-arabinose.

Five glycoproteins have been extracted with hot water and dilute alkali from a seaweed (*Gliopeltis furcata*) and they were purified by precipitation with cetylpyridinium chloride.[671] Compositional analyses showed that they all contain galactose, 3,6-anhydrogalactose, and sulphate residues (6 : 3 : 4) in addition to variable amounts of uronic acid and protein. Glycolipoproteins isolated from fruit envelopes and leaves of the grape vine have been shown to contain 33% carbohydrate (consisting of arabinose, galactose, and glucose) and 50% lipid.[672]

Germinating seeds of the kidney bean (*Phaseolus vulgaris*) contain a water-soluble glycoprotein which can be purified to homogeneity by ion-exchange chromatography and disc electrophoresis.[673] The 6.1S glycoprotein has a molecular weight of 1.3×10^5 and on hydrolysis yielded mannose, 2-amino-2-deoxyglucose, and traces of pentose. The relationship of the polysaccharide composition of lupin (*Lupinus angustifolius*) hypocotyls to the content of the glycoprotein extensin has been examined.[674]

Crude and alkali-purified fractions of scales isolated from the haptophycean alga *Pleurochrysis scherffelii* contained a fibrillar alkali-resistant polysaccharide element, the predominantly cellulosic nature of which has been confirmed by *X*-ray diffraction, g.l.c. of a hydrolysate, and viscosimetric studies of the nitrated material.[675] Peptide material is covalently linked to this cellulosic species and remains associated with it throughout the purification process.

A γ-globulin (molecular weight 1.2×10^5) of rice embryo has been shown to contain pentose (4.4), hexose (15.0), and 2-amino-2-deoxyhexose (3.5 mole per mole).[676] Its behaviour on polyacrylamide gel electrophoresis in sodium dodecylsulphate indicated that the glycoprotein is composed of three identical major subunits (molecular weights 3.5×10^4) and one minor subunit (molecular weight 1.3×10^4). The α-cellulose fraction from the walls of sycamore (*Acer pseudoplatanus*) tissue-culture cells was shown to contain a water-insoluble glycoprotein.[677] Soluble glycopeptides were liberated by hydrazinolysis, one of which was shown to contain galactose (55%), arabinose (35%), and hydroxyproline (10%). Each hydroxyproline residue carries a carbohydrate moiety of average DP 9.

[670] M. M. Brysk and M. J. Chrispeels, *Biochim. Biophys. Acta*, 1972, **257**, 421.
[671] K. Izumi, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 653.
[672] A. S. Kadzhaya, B. I. Medvedev, and I. F. Medvedeva, *Biokhimiya*, 1972, **37**, 23.
[673] D. Racusen and M. Foste, *Canad. J. Botany*, 1971, **49**, 2107.
[674] J. A. Munro, R. W. Bailey, and D. Penny, *Phytochemistry*, 1972, **11**, 1597.
[675] W. Herth, W. W. Franke, J. Stadler, H. Bittiger, G. Keillich, and R. M. Brown, *Planta*, 1972, **105**, 79.
[676] Y. Morita and M. Horikoshi, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 651.
[677] M. F. Heath and D. H. Northcote, *Biochem. J.*, 1971, **125**, 953.

Microbial Glycoproteins

Cultures of *Corynebacterium insidiosum* produced an extracellular phytotoxic glycopeptide that possesses the ability to cause plant cuttings to wilt.[678] The purified glycopeptide (molecular weight 5×10^6 by light scattering and gel filtration) contains galactose (20.6), glucose (20.0), mannose (3.8), fucose (38.7%), and traces of rhamnose and unidentified reducing sugars. An unknown organic acid, chemically similar to a keto-deoxy-acid, comprises 8.8% of the glycopeptide, and the glycopeptide linkage appears to be glycosidic in nature, involving the hydroxy-group of threonine. The toxin was shown to be resistant to dissociation by either urea or pyridine. *C. sepedonicum* also produced a phytotoxic glycopeptide in culture and the glycopeptide is also found in potato plants infected with the organism.[679] The purest preparation of the glycopeptide contained as many as five very similar glycopeptides, the approximate composition of which was arabinose (0.7), ribose (3.8), galactose (4.5), glucose (18.8), mannose (33.4), rhamnose (1.1), 2-keto-3-deoxygluconic acid (9.9), and peptide (4.6%). Methylation studies showed that four of the six galactose units occur within the carbohydrate chain and that the other two are involved in branch points; thirteen of the twenty-two glucose units are within chains and nine are branch points, and of the forty-one mannose units, fourteen are within chains, eight are branch points, and the remaining nineteen occupy terminal positions. A fragment isolated from a pronasic digest of the glycopeptide contained a mannose residue linked glycosidically to a threonine residue.

Nucleoside diphosphate [^{14}C]monosaccharides were efficient precursors for the transfer of [^{14}C]monosaccharides to endogenous glycoproteins by homogenates of *Escherichia coli*.[680] The order of incorporation was shown to be UDP-D-[^{14}C]glucose > GDP-D-[^{14}C]mannose > UDP-2-[^{14}C]acetamido-2-deoxy-D-glucose > UDP-D-[^{14}C]galactose > GDP-L-[^{14}C]fucose > UDP-2-[^{14}C]acetamido-2-deoxy-D-galactose. Vi-antigen, an acidic polysaccharide which occurs as a surface component of several *Enteriobacteria* species, was more susceptible to hydrolysis by polygalacturonate lyase when in the form of a protein complex.[681]

A glycoprotein fraction from the cell envelope of *Halobacterium halobium* was readily resolved by polyacrylamide gel electrophoresis in sodium dodecylsulphate into periodic acid Schiff-positive bands of molecular weights 4.1×10^4, 5.6×10^4, and 7.9×10^4.[682] Molecular weight estimations of the products from exhaustive digestion with Pronase showed that the carbohydrate moieties have a molecular weight of 1.5×10^4. Mannose is the only hexose present in the glycoproteins in appreciable quantities,

[678] S. M. Ries and G. A. Strobel, *Plant Physiol.*, 1972, **49**, 676.
[679] G. A. Strobel, K. W. Talmadge, and P. Albersheim, *Biochim. Biophys. Acta*, 1972, **261**, 365.
[680] H. B. Bosmann, *Biochim. Biophys. Acta*, 1971, **252**, 369.
[681] L. A. McNicol and E. E. Baker, *Biochim. Biophys. Acta*, 1971, **229**, 233.
[682] M. A. Koncewicz, *Biochem. J.*, 1972, **128**, 124P.

and cell tests with concanavalin A suggested that the glycoproteins are surface components, the carbohydrate moieties being accessible to the phytohaemagglutinin.

A pharmacologically active glycoprotein extracted and purified from *Klebsiella pneumoniae* has been shown to contain galactose, glucose, and mannose (30% total).[683] The biosynthesis of pellicle glycoproteins of *Leptomonas collosoma* has been investigated by incubation of suspensions of trypanosomatid flagellate with 2-amino-2-deoxy-D-[1-^{14}C]glucose.[684] A protein–lipopolysaccharide complex extracted from the cell envelopes of *Pseudomonas aeruginosa* can be spread on aqueous surfaces as a monolayer.[685] The surface pressure, viscosity, and potential of the monolayer have been measured as a function of surface area and it was found that the film is stiffened and stabilized by lipopolysaccharide attached to the lower surface of the film.

Antigenic polysaccharide–peptide complexes extracted and purified from mycelia of *Trichophyton mentagrophytes* have been shown to be composed chiefly of glucose or mannose together with smaller amounts of galactose, 2-amino-2-deoxyglucose, and peptide.[686] Degradation of the peptide by proteolysis caused a loss of the delayed reaction in human skin tests, but had no effect on the immediate reaction. An antigen released into the medium by cultures of *Vibrio fetus* has been purified by polyacrylamide gel electrophoresis and gel filtration, and it was shown to be composed of carbohydrate and protein.[687] The glycoprotein possesses a molecular weight of 1.35×10^5 and a p*I* of 4.2.

Mannan-protein has been extracted from the walls of strains of yeast (*Saccharomyces cerevisiae* and *S. carlsbergensis*), and major differences were observed in the mannan side-chains between strains of a particular organism.[688] The capacity of the original cells to flocculate was found to be proportional to the number of phosphate groupings present in the outer layer of the mannan-protein. Gel filtration of phosphomannan-proteins from yeast (*Kloeckera brewis*) cells gave three broad peaks, which contained materials with molecular weights of 50×10^4, 10×10^4, and 2.5×10^4, as determined by sedimentation equilibria.[689] Digestion of these materials with Pronase yielded only one species (molecular weight $2.5—3.5 \times 10^4$) and it was suggested that the materials of higher molecular weight arise by cross-linking of protein by this carbohydrate. The single 2-amino-2-deoxyglucose residue present in each phosphomannan chain is considered to be involved in the glycopeptide linkage.

[683] A. M. Golovtchenko, R. Seval, J. C. Jardillier, J. P. Buret, and J. Agneray, *Compt. rend.*, 1972, **274**, *D*, 955.
[684] R. C. Hunt and D. J. Ellar, *Biochem. J.*, 1972, **128**, 125P.
[685] A. Suzuki and S. Goto, *Biochim. Biophys. Acta*, 1972, **255**, 734.
[686] Y. Nozawa, T. Noguchi, Y. Ito, N. Sudo, and S. Watanabe, *Sabouraudia*, 1971, **9**, 129.
[687] L. L. Myers, *Infection and Immunity*, 1971, **3**, 562.
[688] T. P. Lyons and J. S. Hough, *J. Inst. Brewing*, 1971, **77**, 300.
[689] T. R. Thieme and C. E. Ballou, *Biochemistry*, 1972, **11**, 1115.

Viral Glycoproteins

The envelope of Semliki Forest virus, a group A arbovirus, has been shown to contain a glycoprotein, the principal monosaccharides of which are galactose, mannose, and 2-acetamido-2-deoxyglucose.[690] The interaction of the glycoprotein with concanavalin A has been investigated.

Haemagglutinins

Plant Haemagglutinins.—Glutaraldehyde-cross-linked copolymers of bovine serum albumin and ovomucoid and of bovine serum albumin and peroxidase have been used for the isolation of phytohaemagglutinins by affinity chromatography.[691]

Concanavalin A from Jack-bean (*Canavalia ensiformis*) meal has been investigated for its stability in a wide range of environments by optical rotation, sedimentation, and turbidity measurements.[692] In the pH range 4.5—5.6 (I = 0.1), a single species of molecular weight 5.3×10^4 was shown to exist in solution, whereas dimerization occurred above pH 5.6, the extent of the reaction increasing with increasing pH. The results were discussed in terms of the environments suitable for carbohydrate binding. Multiple forms have been recognized in the subunit structure of concanavalin A,[693] and the isolation and proteolysis of an intact subunit (molecular weight 2.7×10^4) have been studied.[694] The aggregation of concanavalin A under conditions favouring binding of carbohydrate has been studied.[695]

An amino-acid sequence (238 residues) and three-dimensional structure have been reported for concanavalin A.[696] Interpretation of the X-ray crystallographic data indicated that the predominant structural element is an extended polypeptide chain arranged in two anti-parallel pleated sheets or β-structures, with residues not included in the β-structures arranged in regions of random coil. One of the pleated sheets contributes extensively to the interactions among the monomers to form both dimers and tetramers. The positions at which carbohydrates, Mn^{2+} ions, and Ca^{2+} ions are associated, and the point of cleavage for the formation of the naturally occurring fragments A_1 and A_2 have been tentatively assigned. The positions at which carbohydrates are bound are at least 20 Å from the two metal-binding sites, and the saccharide-binding site was shown to be a deep pocket of $6 \times 7.5 \times 18$ Å, the inner portion of which is occupied by

[690] J. D. Oram, D. C. Ellwood, G. Appleyard, and J. L. Stanley, *Nature New Biol.*, 1971, **233**, 50.
[691] S. Avrameas and B. Guilbert, *Biochimie*, 1971, **53**, 603.
[692] G. H. McKenzie, W. H. Sawyer, and L. W. Nichol, *Biochim. Biophys. Acta*, 1972, **263**, 283.
[693] Y. Abe, M. Iwabuchi, and S. Ishii, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 1271.
[694] B. A. Cunningham, J. L. Wang, M. N. Pflumm, and G. M. Edelman, *Biochemistry*, 1972, **11**, 3233.
[695] G. H. McKenzie, W. H. Sawyer, and L. W. Nichol, *Proc. Austral. Biochem. Soc.*, 1971, **4**, 116.
[696] B. A. Cunningham, G. N. Reeke, J. W. Becker, M. J. Waxdal, and J. L. Wang, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2580.

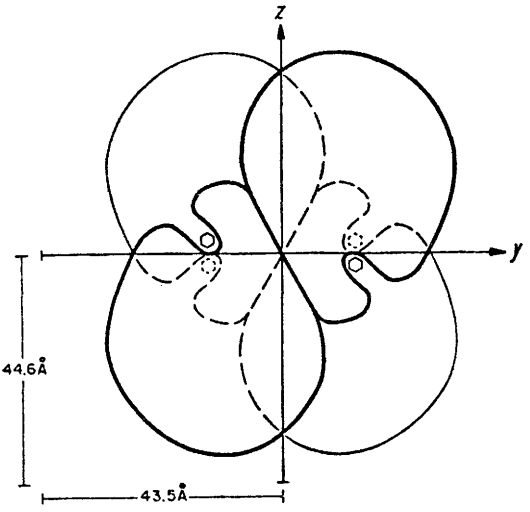
hydrophobic residues. A skeletal model of the concanavalin A molecule was presented in pictorial form. Further interpretations of electron-density maps of concanavalin A crystals indicate that the polypeptide chains in four asymmetric units cluster together to form a four-protomer unit (molecular weight 1.08×10^5) and that this molecule can be divided into two-protomer units (molecular weight 5.4×10^4).[697] There are notably more contacts joining protomers in the two-protomer unit than between the adjacent two-protomer units that compose the total molecule. Significant amounts of β-structure, but no helical structures, were apparent. Similar results have also been reported by other workers.[698] An *X*-ray crystallographic study has also been made of the demetallized haemagglutinin and it was demonstrated that the crystals could be reconverted into the native form by treatment with the appropriate metal ions.[699] Other data suggest that Ca^{2+} is bound by a cluster of two carboxy-groups and one amino-group.[700]

Crystals of concanavalin A containing 2-iodophenyl β-D-glucopyranoside have been prepared and difference electron-density projections have revealed the positions of the iodine atoms as being within prominent cavities on the surface of the lectin.[701] One glycoside molecule was found to be bound to each subunit (molecular weight 2.7×10^4) and thus four carbohydrate molecules are bound to symmetrically equivalent positions in the tetrameric concanavalin A molecule. The structures of the binding sites of the lectin have been determined from a difference Fourier map of the complex formed with *myo*-inositol, and a stereogram of the α-carbon positions of the amino-acid residues near to the *myo*-inositol-binding position has been produced.[702] The shape of the subunits and tetramer are represented in (46) (the hexagons indicate the inositol-binding positions and the *x*-axis intersects with the *y*- and *z*-axes at the centre of the tetramer, normal to the page).

A commercial sample of concanavalin A was shown to contain three types of protein, which could be separated by affinity chromatography on Sephadex G-50.[703] One of the proteins was found to be a demetallized species, another a protein with weak affinity for the gel, whilst the third possessed full binding activity. Treatment of the sample with Mn^{2+} and Ca^{2+} ions rendered it homogeneous with respect to its ability to bind carbohydrate.

D-Glucuronic acid has been demonstrated not to inhibit the binding of glycogen by concanavalin A; previous claims to the contrary were shown

[697] K. D. Hardman, M. K. Wood, M. Schiffer, A. B. Edmundson, and C. F. Ainsworth, *Cold Spring Harbor Symp. Quant. Biol.*, 1971, **36**, 271.
[698] G. N. Reeke and J. W. Becker, *Cold Spring Harbor Symp. Quant. Biol.*, 1971, **36**, 277.
[699] A. Jack, J. Weinzierl, and A. J. Kalb, *J. Mol. Biol.*, 1971, **58**, 389.
[700] G. Gachelin and L. Goldstein, *F.E.B.S. Letters*, 1972, **26**, 264.
[701] J. W. Becker, G. N. Reeke, and G. M. Edelman, *J. Biol. Chem.*, 1971, **246**, 6123.
[702] K. D. Hardman and C. F. Ainsworth, *Nature New Biol.*, 1972, **237**, 54.
[703] T. Uchida and T. Matsumoto, *Biochim. Biophys. Acta*, 1972, **257**, 230.



(46)

to be due to a pH effect.[704] It was also found that concanavalin A does not precipitate polymers containing non-reducing, terminal α-D-glucopyranosiduronic acid residues. On the other hand, it has been confirmed that heparin forms an insoluble complex with concanavalin A, but at a lower pH (5.4) than that which is optimal for complex formation with neutral polysaccharides.[705] The sensitivity of the reaction to other parameters and inhibitors was recorded.

The inhibition by more than fifty carbohydrates of haemagglutination by concanavalin A and the lectin from the pea (*Pisum sativum*) has been investigated using an automated quantitative technique.[706] A study of the concentrations at which haemagglutination occurred efficiently revealed a pattern similar to that established previously by precipitation techniques. Some oligosaccharides, although composed of monosaccharides known to be inhibitors, were shown to have a stimulatory effect upon both lectins.

An attempt has been made to quantify the agglutination of transformed cells by concanavalin A[707] and it has been shown that the binding of the lectin to normal and transformed cells may be detected by immunofluorescence[708] and microhaemadsorption.[709] Other methods reported for determination of normal and virus-transformed cell agglutination included labelling of concanavalin A and wheat-germ agglutinin with [^{125}I]iodine[710]

[704] J. Duke, I. J. Goldstein, and A. Misaki, *Biochim. Biophys. Acta*, 1972, **271**, 237.
[705] R. J. Doyle and T.-J. Kan, *F.E.B.S. Letters*, 1972, **20**, 22.
[706] G. Betail, L. Genaud, and M. Coulet, *Ann. Inst. Pasteur*, 1972, **123**, 731.
[707] P. de Micco and G. Meyer, *Compt. rend. Soc. Biol.*, 1971, **165**, 611.
[708] L. Mallucci, *Nature New Biol.*, 1971, **233**, 241.
[709] P. Furmanski, P. G. Phillips, and M. Lubin, *Proc. Soc. Exp. Biol. Med.*, 1972, **140**, 216.
[710] B. Ozanne and J. Sambrook, *Nature New Biol.*, 1971, **232**, 156.

and acetylation of concanavalin A with [^{3}H]acetic anhydride.[711] Differences in the binding of fluorescent concanavalin A to the surface membranes of normal and transformed cells have been reported [712] and the interaction of concanavalin A with fibroblast and lymphocyte plasma membranes has been investigated.[713] Studies on the agglutination by concanavalin A and wheat-germ agglutinin of normal cells following infection with non-oncogenic viruses have also been reported.[714] Differences in the agglutination by the two lectins of normal and Rous sarcoma virus-transformed chick-embryo cells have been attributed to the presence of different carbohydrates in the cell surfaces,[715] whereas others have reported that pre-treatment with hyaluronidase is necessary for agglutination of the transformed cells by the lectins.[716] Clustered binding sites, arising from proteolysis of 3T3 fibroblasts, have been reported to be involved in the formation of concanavalin A cross-bridges between agglutinated cells.[717] The stimulatory activity of concanavalin A and agglutinins from the lentil (*Lens culinaris*), kidney bean (*Phaseolus vulgaris*), and *Wistaria floribunda* in inhibition assays of [^{3}H]thymidine incorporation into human peripheral lymphocytes has been investigated.[718]

Data obtained for the interaction of concanavalin A with the cell wall of *Bacillus subtilis* have suggested that a rigid-rod conformation is necessary for the glucosylated teichoic acid.[719] The results demonstrated that concanavalin A can be used as a probe to study structural features of bacterial cell walls. The interaction of concanavalin A with a glycoprotein from the envelope of Semliki Forest virus has been investigated.[690]

A sample of concanavalin A purified and crystallized from nata beans (*Canavalia gladiata*) possessed biological characteristics similar to those of the lectin obtained from *C. ensiformis*, but differed in its physicochemical properties.[720] Isoelectric focusing was found to separate the sample into eight fractions. The effects of carbohydrates on the p*I*'s of several molecular species of concanavalin A from nata bean have been reported.[721] Pre-incubation with D-glucose, D-mannose, or methyl α-D-glucopyranoside resulted in extensive shifts of the p*I*'s to higher pH's, whereas D-galactose, which binds poorly to concanavalin A, had little effect. The shifts in p*I*'s occurred predominantly with those species whose original p*I* values lay within the range 6.6—7.1.

[711] M. J. Cline and D. C. Livingstone, *Nature New Biol.*, 1971, **232**, 155.
[712] J. Shaham and L. Sachs, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2479.
[713] P. M. Comoglio and R. Guglielmone, *F.E.B.S. Letters*, 1972, **27**, 256.
[714] G. Poste and P. Reeve, *Nature New Biol.*, 1972, **237**, 113.
[715] M. Kapeller and F. Doljanski, *Nature New Biol.*, 1972, **235**, 184.
[716] M. M. Burger and G. S. Martin, *Nature New Biol.*, 1972, **237**, 9.
[717] G. L. Nicolson, *Nature New Biol.*, 1972, **239**, 193.
[718] S. Toyoshima, M. Fukuda, and T. Osawa, *Biochemistry*, 1972, **11**, 4000.
[719] R. J. Doyle and D. C. Birdsell, *J. Bacteriol.*, 1972, **109**, 652.
[720] H. Akedo, Y. Mori, Y. Tanigaki, K. Shinkai, and K. Morita, *Biochim. Biophys. Acta*, 1972, **271**, 378.
[721] H. Akedo, Y. Mori, M. Kobayashi, and M. Okada, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 107.

A purified phytohaemagglutinin from the seeds of *Bauhinia purpurea* var. *alba* was shown to possess an $S_{20,w}$ value of 7.5S and a molecular weight of 1.95×10^5.[722] It was also found to contain xylose (1.1), galactose (0.3), glucose (0.9), mannose (4.9), fucose (0.5), and 2-amino-2-deoxyglucose (3.4%), and to agglutinate erythrocytes irrespective of their ABO and MN blood-group types. A number of mono- and oligo-saccharides showed low minimum concentration for inhibition of cell agglutination by the lectin. The inhibition of cell agglutination by lectins from *Iberis amara*, *Phaseolus vulgaris*, castor bean (*Ricinus communis*), and *Wistaria floribunda* was also investigated.

Properties of the haemagglutinin isolated from *Lathyrus odoratus* have been reported.[723] Haemagglutinins have been isolated from the seeds of *Phaseolus vulgaris*, *Cytisus sessilifolius*, and *Ulex europaeus* by affinity chromatography on matrices to which their carbohydrate inhibitors had been attached.[724] Commercially available haemagglutinin from red kidney beans (*Phaseolus vulgaris*) has been shown to contain glycoproteins with lymphocyte-stimulating and red and white blood-cell agglutinating activities.[725] By a series of steps involving SE- and QAE-Sephadex chromatography and gel filtration, two lymphocyte-stimulating agglutinins were isolated in homogeneous form. One of these possessed erythroagglutinating and leucoagglutinating activities, whereas the other possessed only the latter activity. Molecular weights of 1.5×10^4 and 1.4×10^4 and p*I*'s of 6.5 and 5.0, respectively, were derived for the two molecules, while dissociation studies showed that both agglutinins are composed of four subunits of similar size held together by non-covalent bonds. Isoelectric focusing of the subunits revealed that the leucoagglutinin contains subunits of only one type, whereas the erythroagglutinin contains two types, one being identical to the subunit of the leucoagglutinin and the other being specific for erythroagglutination. The lymphocyte-stimulating activity of both agglutinins could be abolished by periodate oxidation, but such treatment only slightly affected the agglutinating capacities.

Two agglutinins have been isolated and purified from *Ricinus communis* beans by affinity chromatography on agarose gel using a solution of D-galactose as eluent.[726] Both of the agglutinins were inhibited by carbohydrates containing β-D-galactose residues and the smaller agglutinin (molecular weight 6×10^4) was also inhibited by 2-acetamido-2-deoxy-D-galactose. The larger agglutinin was used to agglutinate specifically several normal and transformed cell lines. The agglutination of erythrocytes by an agglutinin from a *Ricinus* species was accompanied by proteolysis.[727]

[722] T. Irimura and T. Osawa, *Arch. Biochem. Biophys.*, 1972, **151**, 475.
[723] O. Delachambre, G. Betail, J. Guillot, I. Bernard-Griffiths, and M. Coulet, *Compt. rend. Soc. Biol.*, 1971, **165**, 1903.
[724] I. Matsumoto and T. Osawa, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1810.
[725] T. H. Weber, H. Aro, and C. T. Nordman, *Biochim. Biophys. Acta*, 1972, **263**, 94.
[726] G. L. Nicolson and J. Blaustein, *Biochim. Biophys. Acta*, 1972, **266**, 543.
[727] E. Waldschmidt-Leitz, L. Keller, and C. Sigrist, *Z. physiol. Chem.*, 1972, **353**, 227.

Columns of red blood cells fixed on polyacrylamide particles have been used in the purification of haemagglutinins from *Ricinus communis*, *Russula nigricans*, *Tubiporus erythropus*, *Xerocomus chrysenteron*, and *X. subtomentosus*.[728] Purification of the agglutinin from soybean meal has been achieved by affinity chromatography on a Sepharose matrix containing β-D-galactopyranosylamine residues;[729] the product was homogeneous on elution with D-galactose and its composition was the same as that of a previously reported preparation.

Injection of phytohaemagglutinin into rats resulted in the formation of a hyaluronic acid–protein complex in the peritoneal fluid.[730]

Insoluble derivatives of some of the foregoing agglutinins have been reported. Concanavalin A insolubilized by cross-linking with glutaraldehyde has been used in the purification of enzymes and glycoproteins.[691] Concanavalin A insolubilized covalently on Sepharose has been shown to interact with hormonal glycoproteins and was used for their purification;[731] it was also used for the purification of lymphocyte membrane glycoproteins [732, 733] and for the separation of cells.[734] The interaction of concanavalin A insolubilized covalently on acrylic polymers with rat lymphocytes has been investigated,[735] and an agglutinin from *Lens culinaris* insolubilized covalently on Sepharose has been used for the isolation of glycoproteins from porcine lymphocyte plasma membranes.[736] Concanavalin A–ferritin and *Ricinus communis* agglutinin–ferritin covalent conjugates have been prepared and were used to investigate saccharide residues on cell-membrane fragments.[737]

Microbial Haemagglutinins.—A haemagglutinin has been purified from *Pseudomonas aeruginosa* by affinity chromatography on Sepharose.[738] Polyacrylamide gel electrophoresis of the product, which was easily desorbed from the column with D-galactose, showed it to be homogenous. The haemagglutinin was found to exhibit a broad spectrum of specificity for cells and preferentially agglutinated papain- or neuraminidase-treated erythrocytes.[739] The haemagglutinin was eluted from the cells by heating at 56—70 °C and subsequent acidification of the aqueous environment; its action was inhibited by D-galactose, 2-acetamido-2-deoxy-D-galactose, and

[728] G. Betail, M. Coulet, J. Guillot, and M. Scandariato, *Compt. rend. Soc. Biol.*, 1971, **165**, 1304.
[729] J. A. Gordon, S. Blumberg, H. Lis, and N. Sharon, *F.E.B.S. Letters*, 1972, **24**, 193.
[730] H. Greiling, W. Mohr, and G. Beneke, *Z. Naturforsch.*, 1972, **27b**, 414.
[731] M. L. Dufau, T. Tsuruhara, and K. J. Catt, *Biochim. Biophys. Acta*, 1972, **278**, 281.
[732] D. Allan, J. Auger, and M. J. Crumpton, *Biochem. J.*, 1972, **126**, 6P.
[733] D. Allan, J. Auger, and M. J. Crumpton, *Nature New Biol.*, 1972, **236**, 23.
[734] G. M. Edelman, U. Rutishauser, and C. F. Millette, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 2153.
[735] I. Betel and K. J. van den Berg, *European J. Biochem.*, 1972, **30**, 571.
[736] M. J. Hayman and M. J. Crumpton, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 923.
[737] H. Hirano, B. Parkhouse, G. L. Nicolson, E. S. Lennox, and S. J. Singer, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2945.
[738] N. Gilboa-Garber, L. Mizrahi, and N. Garber, *F.E.B.S. Letters*, 1972, **28**, 93.
[739] N. Gilboa-Garber, *F.E.B.S. Letters*, 1972, **20**, 242.

lactose. A partially purified form of *P. aeruginosa* haemagglutinin has been reported to agglutinate erythrocytes and to resemble the phytohaemagglutinins; it was considered that the molecule is a glycoprotein having a molecular weight smaller than the haemagglutinin obtained from *Phaseolus vulgaris*.[740]

Many mono- and oligo-saccharides and their derivatives have been tested as inhibitors of the agglutination of erythrocytes by *Salmonella typhimurium* and *Shigella flexneri* bacteria bearing type-1 fimbriae.[741] D-Mannose and certain of its derivatives were found to be strongly inhibitory, whereas D-fructose moderately inhibited agglutination. Modification of any of the hydroxy-groups at the C-2, -3, -4, or -6 positions of the D-mannopyranosyl residue caused inhibition failure, indicating the necessity of these groups for binding to the active sites of the fimbrial haemagglutinins. The α-configuration of the D-mannopyranosyl residues was found to be important since carbohydrates containing α-linked D-mannose residues were much more active inhibitors than those containing β-linked D-mannose, which were either poor inhibitors or non-inhibitors. The fimbrial reaction was also inhibited by yeast mannan and attention has been drawn to the remarkable degree of similarity of the inhibition pattern of type-1 fimbrial haemagglutinin and that of concanavalin A.

Viral Haemagglutinins.—It has been concluded that the haemagglutinin of influenza virus is a glycoprotein, since L-[^{14}C]fucose and 2-amino-2-deoxy-D-[1-^{14}C]glucose were incorporated into the molecule during biosynthesis.[742] However, syntheses of the haemagglutinin and the viral neuraminidase were found to be inhibited by '2-deoxy-D-glucose' and 2-amino-2-deoxy-D-glucose.

Animal Haemagglutinins.—Haemagglutinins have been separated from haemolysins in extracts of the albumin gland of a snail (*Helix aspersa*) by ion-exchange chromatography and gel filtration.[743] Agglutination of cells by the products has been investigated and it was found that they react preferentially with the α-anomers of carbohydrates. One of the two agglutinins obtained from a snail (*Eobania vermiculata*) behaved like an anti-2-acetamido-2-deoxy-α-D-galactopyranose, whereas the other reacted with human red blood cells of any ABO blood group.[744] Studies on the specificity and binding properties of a blood-group-A-reactive haemagglutinin from *Helix pomatia* have revealed that the molecule can be precipitated with macromolecules having terminal, non-reducing, α-linked 2-acetamido-2-deoxy-D-galactose residues, but not with those having β-linked 2-acetamido-2-deoxy-D-glucose residues.[745]

[740] N. Gilboa-Garber, *Biochim. Biophys. Acta*, 1972, **273**, 165.
[741] D. C. Old, *J. Gen. Microbiol.*, 1972, **71**, 149.
[742] S. S. Ghandi, P. Stanley, J. M. Taylor, and D. O. White, *Microbios*, 1972, **5**, 41.
[743] S. Khalap, C. F. Phelps, H. H. Freudenberg, and E. R. Gold, *Vox Sanguinis*, 1972, **23**, 218.
[744] M. Bizot, *Vox Sanguinis*, 1971, **21**, 465.
[745] S. Hammarstrom and E. A. Kabat, *Biochemistry*, 1971, **10**, 1684.

Blood-group Substances

The structures of oligosaccharides from human milk and their relationship to the blood-group substances have been reviewed.[746, 747] The structure, genetics, and biosynthesis of blood-group-specific glycoproteins have been discussed briefly [748] and the biosynthesis of their carbohydrate moieties from glycopyranosyl esters of nucleoside pyrophosphates ('sugar nucleotides') has been reviewed.[659]

Glycoproteins and glycopeptides containing galactose, mannose, fucose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and sialic acid were released from the membranes of human blood-group A erythrocytes by treatment with chymotrypsin and they were purified by ion-exchange chromatography and gel filtration.[749] One of the glycoproteins isolated had a potent inhibitory activity against wheat-germ agglutinin, which was markedly diminished on treatment of the glycoprotein with β-acetamido-deoxyglucosidase. Pronase digestion of the other glycoproteins yielded glycopeptides which, in some instances, were inhibitory against human anti-A serum and against haemagglutinins from lentil (*Lens culinaris*), pea (*Pisum sativum*), and *Vicia faba* seeds. Evidence was presented for the involvement of mannose residues in the inner core of one of the glycopeptides in the binding to agglutinins.

The degradation of the carbohydrate moieties of human blood-group A and B substances and the stability of the glycopeptide linkages have been examined with regard to the production of the corresponding peptides.[750] The immunochemical potencies of human blood-group A- and B-specific urinary difucosyl pentasaccharides have been compared with those of the mono- and di-fucosyl type-2 oligosaccharides of soluble blood-group A and B substances.[751] The urinary difucosyl oligosaccharides were as active as the corresponding difucosyl compounds from the soluble materials, but were less effective inhibitors than the reduced, monofucosyl oligosaccharides in most serum and lectin systems tested. The difucosyl oligosaccharides from soluble material that are specific for blood-group B gave slight inhibition in a Le^b–anti-Le^b system, whereas the corresponding urinary compound was inactive.

Human erythrocyte membrane glycoproteins can be recovered in high yields in the aqueous phase after extraction of the membranes with chloroform–methanol.[752] Sodium dodecylsulphate–polyacrylamide gel electrophoresis revealed the presence of four glycoprotein fractions (I—IV) of molecular weights 2.4×10^4, 3.7×10^4, 5.0×10^4, and 5.8×10^4, respectively. These fractions contained the blood-group I-, MN-, and

[746] H. Wiegandt and H. Egge, *Fortschr. Chem. org. Naturstoffe*, 1970, **28**, 404.
[747] A. Kobata, *Seikagaku*, 1970, **42**, 829.
[748] W. M. Watkins, *Biochem. J.*, 1972, **128**, 114P.
[749] Y. Akiyama and T. Osawa, *Z. physiol. Chem.*, 1972, **353**, 323.
[750] S. D. Goodwin and W. M. Watkins, *Biochem. J.*, 1971, **125**, 79P.
[751] A. Lundblad and E. A. Kabat, *J. Immunol.*, 1971, **106**, 1572.
[752] H. Hamaguchi and H. Cleve, *Biochim. Biophys. Acta*, 1972, **278**, 271.

Ss-active substances and some of the blood-group A, B, and H activities. Gel filtration revealed that the M and most of the N activities were possessed by fraction IV and that fraction I contained the highest A, I, and S and some of the N activities. Glycoprotein II was associated with A activity. Human ovarian cyst linings and fluids have been examined for glycosyltransferases associated with the biosynthesis of the blood-group A- and B-active glycoproteins.[753] An α-acetamidodeoxygalactosyltransferase, which transferred 2-acetamido-2-deoxy-D-galactose from UDP-2-acetamido-2-deoxy-D-galactose to low-molecular-weight acceptors containing the H-active structure at the non-reducing terminal, was detected in both linings and particle-free fluids of cysts from group A subjects. An α-galactosyltransferase with the same acceptor specificity occurs in cysts from group B subjects. β-Galactosyltransferase activity was detected in both linings and fluids irrespective of the ABO group of the donor.

Blood-group A, B, H, Le^a, Le^b, and I substances, and the products arising from periodate oxidation and Smith degradation, have been examined for their susceptibility to alkaline borohydride under various conditions.[754] Procedures for the identification and quantitation of possible degradation products (including hexenetetrols, 3-deoxygalactitol, galactitol, 2-amino-2-deoxygalactitol, and 2-amino-2-deoxyglucitol) were reported. Conditions that cleaved the glycosidic linkages of the oligosaccharide chains to serine and threonine residues with reduction of the exposed 2-acetamido-2-deoxygalactose residues, but with minimal degradation of the oligosaccharides, were established. The structural implications of the free and oligosaccharide-bound 2-acetamido-2-deoxygalactitol released were discussed.

Smith degradation has been carried out on blood-group A + H substances from porcine stomach linings.[755] The first cycle of reactions gave evidence for the presence of fucosyl-(1 → 4)-galactose linkages, whereas the second cycle provided evidence for the branched character of the carbohydrate moieties. By use of glycosidases and six cycles of Smith degradation, 90% of the carbohydrate chains of the blood-group substances could be removed.[756] Smith and alkaline degradations also provided evidence for the existence of sequences of 2-acetamido-2-deoxyglucopyranosyl-(1 → 3)-2-acetamido-2-deoxygalactose.[757] Culturing of porcine cardiac gastric mucosal fragments in the presence of D-[^{14}C]galactose permitted labelling of the water-soluble mucins, gel filtration of which gave three

[753] V. M. Hearn, C. Race, and W. M. Watkins, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 948.
[754] B. Anderson, L. Rovis, and E. A. Kabat, *Arch. Biochem. Biophys.*, 1972, **148**, 304.
[755] L. M. Likhosherstov, L. S. Bogdashova, V. A. Derevitskaya, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1971, 366.
[756] V. A. Derevitskaya, L. M. Likhosherstov, M. D. Martynova, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1972, **203**, 700.
[757] L. M. Likhosherstov, L. S. Bogdasheva, and V. A. Derevitskaya, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1972, 2568.

radioactive fractions.[758] The fractions, all containing hexose (28), fucose (10), 2-amino-2-deoxyhexose (28), and sialic acid (1%), were found to be polydisperse on ultracentrifugation and to possess similar blood-group A and H activities.

Sulphated glycoproteins have been isolated from tryptic digests of porcine stomach mucosa by precipitation with ethanol and cetylpyridinium chloride, electrophoresis, and ion-exchange chromatography.[759] All the sulphated fractions exhibited strong blood-group A + H activity, the extent of which depended on the sulphate content. A study of one fraction showed that it contained galactose, fucose, 2-acetamido-2-deoxygalactose, and 2-acetamido-2-deoxyglucose (molar ratios 3.0—4.0 : 1.0—2.1 : 1.0 : 1.9—2.0) and sulphate (2.4—5.4 moles per mole of 2-amino-2-deoxyhexose). 2-Acetamido-2-deoxyglucose 6-sulphate was the only sulphated component detected. Two groups of carbohydrate chains were separated from the glycoproteins; one contained eighteen and the other fourteen monosaccharide units, and a partial, common structure (47) was suggested for them.

A glycoprotein containing blood-group AB and MN antigenic groups, as well as receptors for influenza virus and various phytohaemagglutinins, has been extracted from human blood red-cell membranes with lithium 3,5-di-iodosalicylate.[637] A homogeneous fraction, obtained by ion-exchange chromatography, contained 60% carbohydrate and represented 75% of the total glycoprotein of the red-cell membrane. A human salivary glycoprotein exhibiting blood-group activity in the ABO and Lewis systems, and which also inhibited the haemagglutinins of influenza virus, has been investigated.[760] Preparations from a non-secretor (O, Le a^+b^-) and a secretor (O, Le a^-b^+) contained different amounts of carbohydrate comprising galactose (4.8 and 4.7), glucose (1.2 and 1.2), mannose (0.4 and 0.5), fucose (1.7 and 3.6%), and molar ratios of 2-amino-2-deoxygalactose : 2-amino-2-deoxyglucose of 0.39 and 0.40 : 1.00, respectively; the latter preparation contained a greater amount of 2-amino-2-deoxyhexose. The presence of α-L-fucosyltransferases in human sera of different ABO secretors and Lewis blood-group phenotypes was investigated using GDP-L-fucose as the sugar donor and low-molecular-weight oligosaccharides derived from the macromolecular glycoprotein as acceptors.[761] In contrast to the occurrence of 2-α-L-fucosyltransferases in milk, submaxillary glands, and stomach mucosa of ABH secretors only, an enzyme that transferred L-fucose to the C-2 position of non-reducing β-D-galactosyl residues was found in the sera of all normal human ABO donors, irrespective of their ABH secretor–nonsecretor status. A 3-α-L-fucosyltransferase which conveyed L-fucose to the C-3 positions of D-glucose and 2-amino-2-deoxy-

[758] D. Snary and A. Allen, *Biochem. J.*, 1971, **123**, 845.
[759] B. L. Slomiany and K. Meyer, *J. Biol. Chem.*, 1972, **247**, 5062.
[760] T. Sønju and G. Rølla, *Acta Pathol. Microbiol. Scand.*, 1971, **79**, *B*, 95.
[761] H. Schenkel-Brunner, M. A. Chester, and W. M. Watkins, *European J. Biochem.*, 1972, **30**, 269.

Fuc
1
↓
2
GalNAc-(1 → 3)-Gal-(1 → 3 or 4)-GlcNAc-(1 → 4 or 6)-Gal
Fuc
1
↓
?
Gal-(1 → 3 or 4)-GlcNAc60S-(1 → 3)-Gal-(1 → 3 or 4)-GalNAc-(1—
serine
or
threonine
Fuc
1
↓
2
Gal-(1 → 3 or 4)-GlcNAc-(1 → 4 or 6)-Gal
Fuc
1
↓
?
1
↓
?

(47)

D-glucose was found in all sera examined, whereas 4-α-L-fucosyltransferase activity was absent.

A soluble glycoprotein fraction extracted from human blood-group B erythrocytes with butanol–water showed strong B activity.[762] Treatment with α-galactosidase caused the disappearance of this activity and the liberation of D-galactose, demonstrating the terminal, non-reducing positions of these units. Human ovarian cyst blood-group B substance pre-treated with acid yielded a series of dialysable oligosaccharides on degradation with alkaline borodeuteride.[763] Five of these oligosaccharides had been isolated previously from blood-group B, I, or Le^a substances. The structures of four other oligosaccharides (48)—(51), which had not

α-D-Gal*p*-(1 → 3)-D-galactitol

(48)

β-D-Glc*pN*Ac-(1 → 6)-2-acetamido-2-deoxy-D-galactitol

(49)

α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*pN*Ac-(1 → 6 ?)-3-hexenetetrol(s)

(50)

α-L-Fuc*p*
1
↓
2
α-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Glc*pN*Ac-(1 → 3 or 4)-D-galactitol

(51)

been isolated previously, were found to fit well with the proposed overall structure of the megalosaccharide from ovarian cyst blood-group substances. Immunochemical data confirmed that α-D-galactopyranosyl-(1 → 3)-D-galactose is immunodominant in the structure of blood-group B substance. α-Galactosyltransferase from human milk has been found to catalyse the biosynthesis of B character on blood-group O erythrocytes, with erythrocytes from secretors and non-secretors participating in this reaction to the same extent.[764] Substances possessing H-active structures inhibited the reaction and it was shown that the enzyme also transferred D-galactose to water-soluble substances from erythrocytes with H-activity from secretors. Galactosyltransferases, associated with the biosynthesis of blood-group B-specific glycoproteins, in human submaxillary glands and stomach mucosa have also been investigated.[765]

Treatment of porcine gastric blood-group substance with α-fucosidase rapidly destroyed its type H specificity.[766] The structures of oligosaccharides

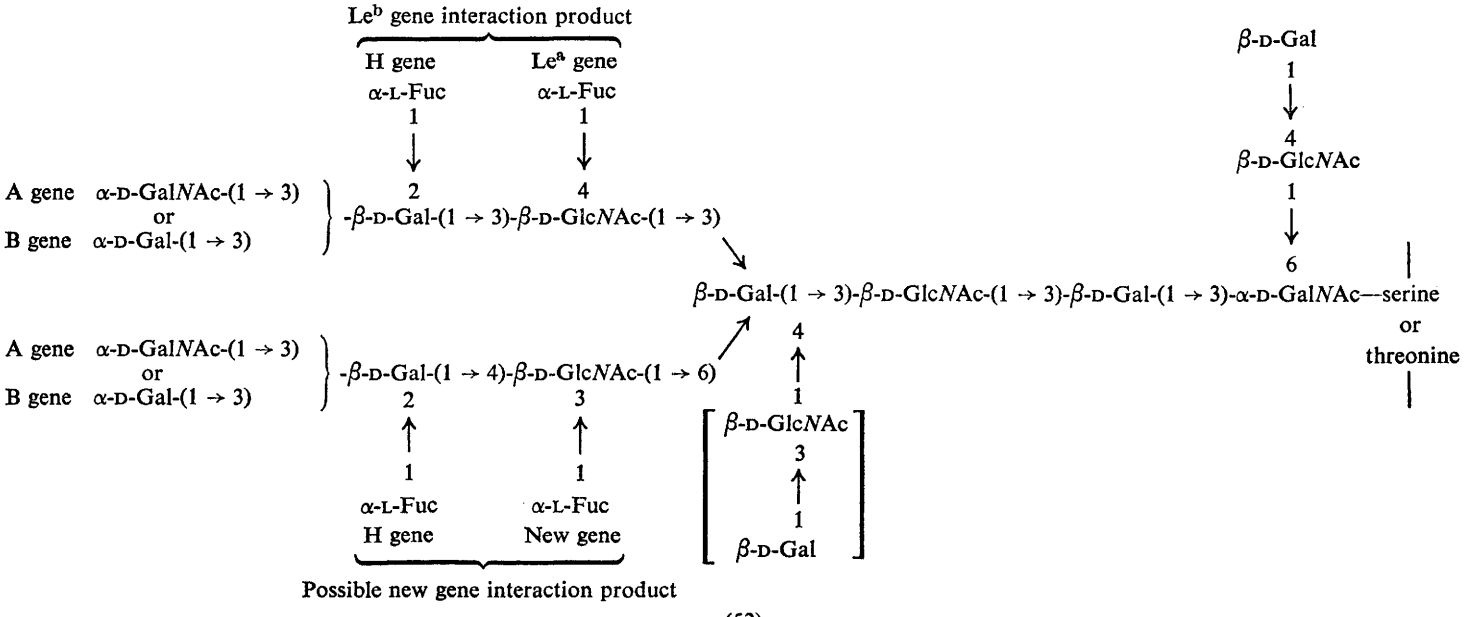
[762] S. Yativ and H. M. Flowers, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 514.
[763] A. Lundblad, S. Hammarström, E. Licerio, and E. A. Kabat, *Arch. Biochem. Biophys.*, 1972, **148**, 291.
[764] J. Pacuszka and J. Košcielak, *European J. Biochem.*, 1972, **31**, 574.
[765] R. D. Portez and W. M. Watkins, *European J. Biochem.*, 1972, **25**, 455.
[766] Y. Iijima and T. Muramatsu, *Arch. Biochem. Biophys.*, 1971, **145**, 50.

from blood-group A^-H^--inactive mucins secreted by porcine submaxillary glands have been investigated in an attempt to explain the serological relationships of the mucin to H^+-active mucin.[767] A fucosyltransferase which transferred L-fucose from GDP-L-fucose to glycoprotein and oligosaccharide acceptors has been isolated from rat small intestinal mucosa.[768] The enzyme attached L-fucose in an α-(1 → 2)-linkage to a terminal β-D-galactosyl residue, the structure formed being the determinant for human blood-group H(O) activity.

Quantitative precipitin studies with three anti-I sera showed that they were distinctly different in specificity, as revealed by their reactions with Le^a substances, bovine blood-group substances, and periodate-oxidized and Smith-degraded blood-group A, B, and H substances.[769] The various I specificities are concealed in the interior structures of the blood-group A, B, H, Le^a, and Le^b substances, but could be exposed either by stepwise periodate oxidations and Smith degradations of A, B, and H substances or by mild acid hydrolysis of B substance. Inhibition assays showed that one of the anti-I sera was best inhibited by a terminal, non-reducing *O*-β-D-galactopyranosyl-(1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 6)-structure, which is present in the precursors of blood-group substances. A composite megalosaccharide structure (52) showing the relation of the various blood-group determinants was proposed and the genetic implications of these findings were discussed. Gel filtration of a non-dialysable material arising from treatment of a phenol–saline extract of human erythrocytes with ficin yielded four fractions (I—IV).[770] The main I-type activity was found in fraction I (*N*-acetylneuraminic acid content 21%), while fractions I—III contained different amounts of MN activity. Fraction II (22% *N*-acetylneuraminic acid) was Pr_1-active but Pr_2-inactive, whereas these activities were reversed for fraction III (20% *N*-acetylneuraminic acid). Fraction IV contained serologically inactive glycoprotein. The ficin-catalysed fragmentation of glycoproteins from human erythrocyte membranes also yielded a number of glycopeptides; these have been separated and their carbohydrate components determined.[771] These compositions were discussed in terms of the I, MN, Pr_1, and Pr_2 activities of the molecules.

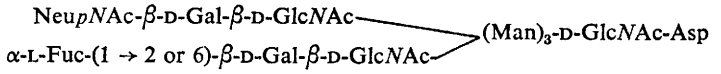
A mixture of glycosphingolipids has been obtained from bovine blood-group J substance and it was concluded from this and other data that the substance occurs in serum as a glycolipoprotein.[772] Extraction of haemoglobin-free stroma of human erythrocytes with hot 75% ethanol yielded a blood-group substance with M and N specificity containing hexose (12.5%), fucose (0.6%), 2-acetamido-2-deoxyhexose (13.2%), and sialic acid

[767] M. M. Baig and D. Aminoff, *J. Biol. Chem.*, 1972, **247**, 6111.
[768] A. Bella and Y. S. Kim, *Arch. Biochem. Biophys.*, 1972, **147**, 753.
[769] T. Feizi, E. A. Kabat, G. Vicari, B. Anderson, and W. L. Marsh, *J. Immunol.*, 1971, **106**, 1578.
[770] D. Roelcke, W. Ebert, J. Metz, and H. Weicker, *Vox Sanguinis*, 1971, **21**, 352.
[771] W. Ebert, J. Metz, H. Weicker, and D. Roelcke, *Z. physiol. Chem.*, 1971, **352**, 1309.
[772] O. W. Thiele and J. Koch, *Fette, Seifen, Anstrichm.*, 1972, **74**, 161.


Le^b gene interaction product
H gene
Le^a gene
α-L-Fuc
α-L-Fuc
1
1
2
4
A gene α-D-GalNAc-(1 → 3)
or
B gene α-D-Gal-(1 → 3)
-β-D-Gal-(1 → 3)-β-D-GlcNAc-(1 → 3)
β-D-Gal
1
4
β-D-GlcNAc
1
6
β-D-Gal-(1 → 3)-β-D-GlcNAc-(1 → 3)-β-D-Gal-(1 → 3)-α-D-GalNAc—serine
or
threonine
A gene α-D-GalNAc-(1 → 3)
or
B gene α-D-Gal-(1 → 3)
-β-D-Gal-(1 → 4)-β-D-GlcNAc-(1 → 6)
4
2
3
1
β-D-GlcNAc
3
1
β-D-Gal
1
1
α-L-Fuc
H gene
α-L-Fuc
New gene
Possible new gene interaction product

(52)

(13.8%).[773] Glycopeptides possessing very weak M and N haemagglutination-inhibitory activity were released from intact human erythrocytes and stroma by the action of trypsin.[774] Variations in the chromatographic and electrophoretic behaviour of the glycopeptides indicated differences in the molecular weights and net electric charges. The compounds were found to contain up to four neutral sugars (galactose, glucose, mannose, and fucose), 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and sialic acid, and it appeared that the glycopeptides released from the surface of the red blood cells represent the free, terminal portions of the peripheral zone glycoproteins. The oligosaccharide moieties of the alkali-stable, glycopeptide-linkage regions of sialoglycopeptides derived from human erythrocytes have been studied.[775] One of the four glycopeptides isolated after treatment with alkaline borohydride and digestion with pronase of MN-active sialoglycopeptides possessed a molecular weight of 2330 and was composed of galactose (3), mannose (3), fucose (1), 2-acetamido-2-deoxyglucose (4), sialic acid (1), and aspartic acid (1 residue(s) per mole). On the basis of experiments with glycosidases, a structure (53) was reported.



(53)

The action of blood-group B gene-specified α-galactosyltransferase from human serum and stomach mucosal extracts on human group O and 'Bombay' O_h erythrocytes has been studied.[776]

Collagens

Various aspects of the collagen of the vertebrate eye have been reviewed.[777]

It has been shown that the glycosylated hydroxylysine from collagens may be determined directly after hydrolysis using an amino-acid analyser.[778] Using this method, the glucosylgalactosyl- and galactosyl-hydroxylysines from the collagens from species of Vertebrata, Mollusca, Annelida, Arthropoda, and Prochordata have been measured. During the interaction of chondroitin–keratan sulphate hybrid proteoglycan with procollagen, tight packing of the procollagen in the developing complex was observed.[779] The complex displayed greater ability than free procollagen, but lesser ability than the proteoglycan, to absorb water.

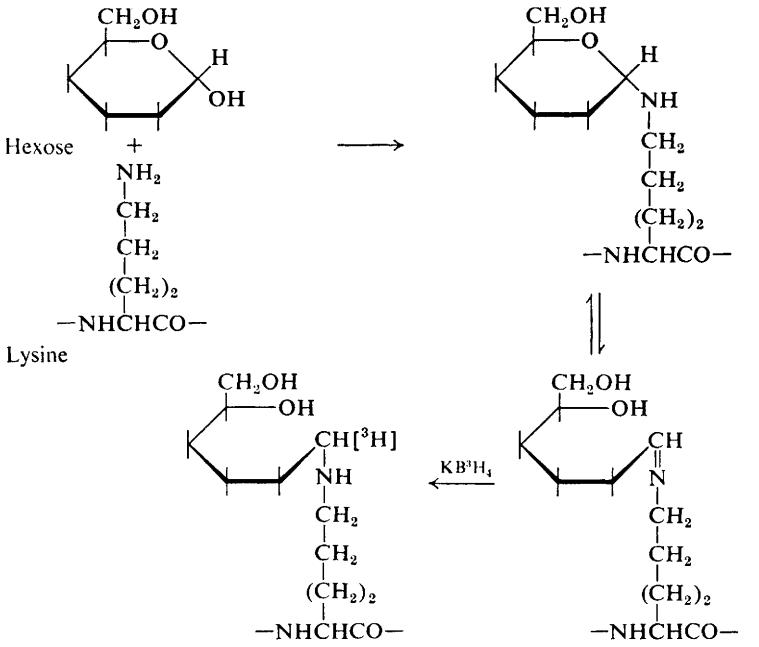
[773] M. A. Fletcher and B. J. Woolfolk, *J. Immunol.*, 1971, **107**, 842.
[774] L. J. Jackson and G. V. F. Seaman, *Biochemistry*, 1972, **11**, 44.
[775] D. B. Thomas and R. J. Winzler, *Biochem. J.*, 1971, **124**, 55.
[776] C. Race and W. M. Watkins, *Vox Sanguinis*, 1972, **23**, 385.
[777] Z. Dische, 'International Review of Connective Tissue Research', vol. 5, Academic Press, New York, 1971.
[778] S. Kimura, *J. Biochem. (Japan)*, 1972, **71**, 367.
[779] S. M. Bychov, S. S. Nikolaeva, and V. N. Kharlamova, *Biull. eksp. Biol. Med.*, 1971, **62**, 38.

The yields of collagen obtainable from human skin by the α-amylase, edta, and lyotropic relaxation methods of extraction were similar.[780] Age-related changes in the stability of human polymeric collagen have been reported.[781] Collagen contents greater than normal have been found in connective tissue of the atherosclerotic intima and media of human aorta.[782] Gel filtration of the products of papain digestion of human intervertebral disc separated the collagenous and non-collagenous materials.[783] The collagenous glycopeptides contained only galactose and glucose, while those from *annulus fibrosus* contained smaller amounts of 2-amino-2-deoxyhexose than those from *nucleus pulposus*. Changes in the carbohydrate contents related to age were investigated. The neutral carbohydrate compositions of collagens from human and rat costal cartilage have been reported.[784] Reducible cross-linkages in hydroxylysine-deficient collagens of a heritable disorder of connective tissue have been investigated.[785]

The carbohydrate contents of collagen and related glycopeptides of the baboon (*Cyanocephalus papio-papio*) corneal stroma have been determined.[786]

Reducible lysine–carbohydrate condensation products have been identified in bovine skin collagen.[787] Borotritide reduction of the tissue followed by acid hydrolysis and ion-exchange chromatography yielded a radioactive fraction, which was then separated into further fractions using an autoanalyser. Two of these fractions produced non-radioactive lysine on periodate oxidation and it was argued that they must have been condensation products involving the ε-amino-group of lysine. The mass spectra of the trifluoroacetylated derivatives were consistent with the compounds being N^{ε}-1-(1-deoxyhexitol)lysine or its anhydro-derivative; on comparison with synthetic compounds, it was shown that the original compounds were N^{ε}-(glucitol)lysine, N^{ε}-(mannitol)hydroxylysine, and N^{ε}-(glucitol)hydroxylysine, and their anhydro-derivatives arising through treatment with acid. Since these compounds had been isolated from reduced tissue, it was considered that they must be present originally as N^{ε}-lysine glycosylamines, which arise according to Scheme 5, but which are not necessarily naturally occurring. A similar report has described the release of a reduced N^{ε}-hexosylhydroxylysine from calf skin collagens after borohydride reduction and acid hydrolysis.[788] Structural identification was

[780] M. J. O. Francis and D. C. Macmillan, *Biochim. Biophys. Acta*, 1971, **251**, 236.
[781] M. J. O. Francis, R. Smith, and D. C. Macmillan, *Biochem. J.*, 1972, **128**, 122P.
[782] T. Anastassiades, P. A. Anastassiadis, and O. F. Denstedt, *Biochim. Biophys. Acta*, 1972, **261**, 418.
[783] C. H. Pearson, F. Happey, A. Naylor, R. L. Turner, J. Palframan, and R. D. Shentall, *Ann. Rheumatic Disease*, 1972, **31**, 45.
[784] K. Y. T. Kao, W. E. Hitt, and J. G. Leslie, *Biochim. Biophys. Acta*, 1972, **279**, 431.
[785] D. R. Eyre and M. J. Glimcher, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2594.
[786] M. Moczar and E. Moczar, *Comp. Biochem. Physiol.*, 1972, **43B**, 261.
[787] S. P. Robins and A. J. Bailey, *Biochem. Biophys. Res. Comm.*, 1972, **48**, 76.
[788] M. L. Tanzer, R. Fairweather, and P. M. Gallop, *Arch. Biochem. Biophys.*, 1972, **151**, 137.

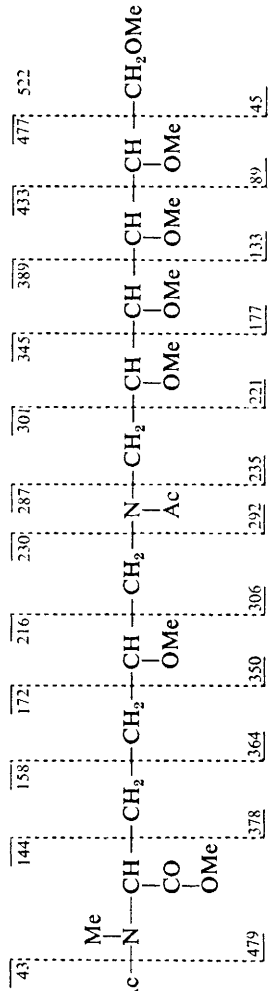


Scheme 5

achieved by mass spectrometry of its acetylated, permethylated derivative (54). The compound appeared to arise by reduction of the Schiff base formed between the carbonyl group of a hexose and the ε-amino-group of hydroxylysine; the parent compound was considered to act as a cross-link between collagen and proteoglycans or glycoproteins. The effects of u.v. light on the chemical, physical, and immunological properties of acid-soluble and enzyme-treated calf skin have been studied.[789]

The collagen fibres of bovine lung parenchyma and pleura have been found to be associated with dermatan sulphate.[790] Fractionation of the soluble glycoprotein components of the *S*-carboxymethylated basement membrane of bovine renal glomeruli gave species of molecular weights 3.0—70 × 10^4.[791] Some of these contained glucosylgalactose units (up to 35 per 1000 amino-acid residues) and were therefore considered to be collagenous. One of the two glycoprotein fractions obtained from bovine Achilles tendon by extraction with sodium and magnesium chlorides contained collagen.[792] The binding of collagen by the two fractions

[789] T. Miyata, T. Sohoe, A. L. Rubin, and K. H. Stenzel, *Biochim. Biophys. Acta*, 1971, **229**, 672.
[790] F. S. Wusteman, *Experientia*, 1972, **28**, 887.
[791] B. G. Hudson and R. G. Spiro, *J. Biol. Chem.*, 1972, **247**, 4239.
[792] J. C. Anderson and D. S. Jackson, *Biochem. J.*, 1972, **127**, 179.



(54)

suggested that the glycoproteins may maintain the structural stability of collagen fibrils.[793]

Incubation of bovine articular cartilage with papain followed by several ionic washes removed most of the bound 2-amino-2-deoxyhexose, uronic acid, sialic acid, and non-collagen-bound hexose.[794] Subsequent extraction with sodium chloride solubilized the collagen, while glycosaminoglycans still present could be removed by repeated re-precipitation. The purified collagen resembled the α1-chain from calf skin collagen on denaturation, but differed from it in its high content of hydroxylysine and glycosidically bound carbohydrate (14 D-galactose and 8 or 9 D-glucose residues per α-chain). The collagen also contained two 2-amino-2-deoxyhexose residues per chain. The preparation and properties of an alkali-soluble collagen from demineralized bovine bone have been described [795] and the influence of glycoprotein on the formation of bovine nasal cartilage collagen fibrils in the presence of chondroitin sulphate proteoglycan has been investigated.[796]

A carbohydrate-rich fraction of the glycopeptide mixture obtained from porcine skin collagen has been separated into four components, one of which contained 50% of the carbohydrate content of the fraction and was composed of galactose, glucose, glycine, and hydroxylysine in equimolar amounts.[797] It was concluded from a number of studies that the carbohydrate moieties possess a β-D-glucopyranosyl-D-galactopyranosyl structure and that the glycopeptide linkages are situated originally in the polar areas of the collagen molecule.

A glycoprotein, which could not be precipitated with ethanol, has been isolated from rabbit skin.[798] The glycoprotein was found to contain 50% carbohydrate consisting of galactose, mannose, 2-amino-2-deoxyglucose, and small amounts of fucose and sialic acid; it also contains hydroxyproline.

Oxidation at C-6 of the D-galactopyranosyl residues of the oligosaccharide side-chains of guinea-pig collagen with galactose oxidase completely abolished platelet–collagen cohesion.[799] Reduction of the oxidized material with sodium borohydride normalized the characteristics of the collagen. It was suggested that the integrity and accessibility of the D-galactose-receptor may be crucial for the formation of a tertiary collagen–enzyme–platelet membrane complex that must precede platelet aggregation. Guinea-pig collagen from which D-glucose units had been removed was shown to act as receptor for the transfer of D-glucose from UDP-D-[^{14}C]glucose by a glycosyltransferase from *Escherichia coli*.[680] An investi-

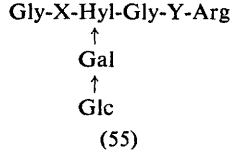
[793] J. C. Anderson and D. S. Jackson, *Biochem. J.*, 1972, **128**, 122P.
[794] E. Strawich and M. E. Nimni, *Biochemistry*, 1971, **10**, 3905.
[795] G. D. Kemp and G. R. Tristram, *Biochem. J.*, 1971, **124**, 915.
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gation of the three-dimensional ultrastructure of native rat collagen has provided some evidence of a carbohydrate matrix.[800]

Comparative electron-microscope studies have been reported for collagens extracted from chick skin, cartilage, and bone.[801] The biosynthesis of collagen in embryonic chick lens has been studied both *in vivo* and *in vitro*.[802] The data obtained indicate that the addition of glucose and galactose residues to form the glucosylgalactosyl unit is initiated considerably before the molecule is secreted and that glycosylation occurs shortly after the synthesis of hydroxylysine in the molecule. The conversion of precursor collagen into α-chains in the intact lens was also studied.[803]

Although hydroxylysine-linked carbohydrate moieties were found to be absent from sawfly (*Nematus ribesii*) silk collagen, incubation of the collagen with UDP-D-[^{14}C]galactose and a UDP-galactose:hydroxylysine galactosyltransferase gave rise to radioactive D-galactosylhydroxylysine residues.[804] It was concluded that the absence of such residues *in vivo* is not due to steric hindrance by the collagen molecule, but to a lack of the appropriate transferase. The chemistry of the collagen was compared with those of rat skin, squid fin, and earthworm cuticle.

Comparative immunological studies on fish and invertebrate collagens have been reported.[805] The sequences of several glycopeptides derived from cuttlefish (*Sepia subaculeata*) by digestion with pronase have been established and compared with those derived from vertebrate collagens;[806] each of the cuttlefish glycopeptides was found to contain a glucosylgalactosylhydroxylysine moiety. The overall results indicated that a common amino-acid sequence (55) in the collagen structures acts as a recognition sign for the glycosyltransferase to attach the carbohydrate moiety to the polypeptide chain.



(55)

Pepsin-solubilized collagen from the body-wall connective tissue of a sea anemone (*Metridium dianthus*) contained 1% carbohydrate (mannose, fucose, and 2-amino-2-deoxyglucose) in the form of a heteropolysaccharide moiety.[807] Polyacrylamide gel electrophoresis of the collagen gave an

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[803] M. E. Grant, N. A. Kefalides, and D. J. Prockop, *J. Biol. Chem.*, 1972, **247**, 3545.
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α-chain and a β-component. The major glycopeptide from the collagen of the sponge *Hippospongia gossypina* was shown to have the structure *O*-α-D-glucopyranosyl-(1 → 2)-*O*-β-D-galactopyranosyloxy-(1 → 5)-lysine; direct evidence for the assignment of the anomeric configurations was obtained from ^{1}H n.m.r. studies.[808] The glucosylgalactosylhydroxylysine compounds isolated from the collagens of sea anemone (*Metridium dianthus*), sea cucumber (*Thyone briareus*), and bovine cornea were found to be identical with that of the sponge glycopeptide by a number of tests, including treatment with galactose oxidase. A simple procedure for the isolation of large amounts of the glycopeptide from the common household sponge was described.

The absence of glycosaminoglycans from the connective tissue of *Rhizostroma pulmo* is considered noteworthy in that it represents an example where the presence of glycosaminoglycans is unnecessary for fibrillogenesis and stabilization of collagen.[809]

Collagen insolubilized by covalent attachment to Sepharose has been used for affinity chromatography of collagenase.[810]

Glycogens

The occurrence, isolation, structure, and enzymic degradation of glycogen have been reviewed.[811] The biosynthesis of glycogen has been reviewed briefly [660] and recent advances in the field have been summarized.[812] Aspects of both the biosynthesis and metabolism of glycogen have been described [813] and the involvement of glycopyranosyl esters of nucleoside pyrophosphates ('sugar nucleotides') in glycogen biosynthesis has been reviewed.[659]

A fluorometric method based on the anthrone reaction, which can be used to measure as little as 20 pg glycogen in tissue, has been described;[814] the accuracy of the method compares favourably with that of other techniques. The procedure is more specific for glycogen than the anthrone colorimetric method, since the fluorescence afforded by D-glucose 6-phosphate, D-fructose 6-phosphate, and D-fructose 1,6-diphosphate is one-half of that arising from an equal quantity of glycogen. A fluoroenzymic method, based on the conversion of glycogen into D-gluconic acid 6-phosphate with amylo-α-1,4-α-dextrin-1,6-glucosidase hexokinase and glucose 6-phosphate dehydrogenase, has also been described for the measurement of glycogen.[815]

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Fluorometric determination of the increase in NADPH permits determination of 200 μg glycogen.

The effects of D-glucose and glycogen concentrations on the synthesis and turnover of glycogen in human hormone-producing cytotrophoblastic cells have been examined by studying the incorporation of D-[6-^{3}H]-glucose.[816] It was concluded that the concentration of glycogen plays an important role in regulating the metabolic functions of the cell. The metabolism of glycogen in human gestational choriocarcinoma cell lines in continuous culture has been studied and a BeWo glycogen phosphorylase was characterized.[817] Normal human fibroblasts cultured in the presence of $\alpha\alpha$-trehalose contained more glycogen than those cultured in the absence of the disaccharide.[818] It was therefore possible to simulate the increased half-life of glycogen of fibroblast lines from patients with Type II glycogen-storage disease in which the α-glucosidase active at pH 4 is absent. A marked persistence of glycogen in cells from both Type II and Type III patients was evident from labelling studies.

The anthrone reaction has been used in the determination of glycogen in hepatic needle-biopsy specimens from humans.[819] An assay for the diagnosis of Type I glycogen-storage disease has been based upon differences in the incorporation of uniformly labelled D-[^{14}C]glucose and D-[2-^{3}H]-glucose.[820] An incompletely branched form of glycogen of low molecular weight has been isolated from liver, heart, and kidney tissues of a case of glycogen-storage disease.[821] Digestion with pullulanase and β-amylase, periodate oxidation, Smith degradation, end-group analysis, osmotic pressure measurements, and spectral studies of the iodine complexes gave results indicating a molecular-weight range of 8—15 $\times$ 10^3 and an average chain length of 17 or 18 D-glucose units per branch. The branching enzyme involved in the biosynthesis of the glycogen was also investigated. The action of the branching enzyme α-1,4-glucan:α-1,4-glucan 6-glycosyltransferase on glycogen present in the liver of cases of Type IV glycogen-storage disease has been studied.[822] The product was subjected to structural analysis by stepwise degradation with glycogen phosphorylase and oligo-α-1,4 $\rightarrow$ 1,4-glucantransferase-dextrin-1,6-glucosidase and the results indicated that the branching enzyme had branched both the outer side-chains and main chains to nearly the same extent. The Type IV polysaccharide is characterized by its long, unbranched, outer chains. Furthermore, the structural data of the modified glycogen are consistent with results that could be predicted if the branching enzyme is assumed to place each new

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[822] W. B. Gibson, B. I. Brown, and D. H. Brown, *Biochemistry*, 1971, **10**, 4253.

outer branch point at an average position four D-glucose units away from the nearest pre-existing outer branch point of the parent polysaccharide.

The ability of the lysosomal 'acid' α-glucosidase from human placenta and rat and mouse liver to hydrolyse glycogen and to catalyse the glycosylation of glycogen by maltose was inhibited by antibodies to the enzyme.[823] These antibodies could not be detected in tissues from patients with Type II glycogenosis.

Glycogen and glycogen synthetase D (UDP-D-glucose:α-1,4-glucan α-4-glucosyltransferase) have been purified from human polymorphonuclear leukocytes, and the influence of both UDP-D-glucose and glycogen on the action of the enzyme have been studied.[824] The results indicated that the influence of glycogen on the reaction rate is apparent only at low concentrations of glycogen. The diurnal rhythm of glycogen in human blood neutrophils under various conditions has been investigated.[825] It has been reported that glycogen can form a complex with normal human serum amylase and that such complexes may be responsible for the occurrence of macroamylase species in the sera of cases of macroamylasemia.[826]

Purified glycogen synthetases I and D from porcine brain were found to be associated with glycogen and were shown to be active in the absence of added glycogen primer.[827] Removal of endogenous glycogen, however, caused a decrease in their activities.

The determination of glycogen by turbidimetry with tannin has been reported and hydrolysis of rabbit liver glycogen with acid led to an increase in its reactivity with tannin.[828] On the basis of this reactivity and further data, a model was proposed for the liver glycogen in which polysaccharide subunits are bound through a glycoprotein. Fractionation of glycogen from rabbit liver with aqueous phenol on DEAE-cellulose yielded six fractions.[829] The fractions of higher molecular weight were eluted with higher concentrations of lithium chloride and were less stable, being degradable to the fractions of lower molecular weight. The properties of the fractions were examined by a number of physicochemical techniques. The glycogen-debranching enzyme oligo-α-1,4-glucan:α-1,4-glucan-4-glycosyltransferase-amylo-1,6-glucosidase has been purified from rabbit liver. The enzyme preparation was shown to possess both transferase and glucosidase activities. In view of the fact that the enzyme moved as a single band on gel electrophoresis and possessed the two activities in constant ratio throughout its purification, it was concluded that one molecule exhibits

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[824] L. Plesner, E. S. Leroy, P. Wang, M. R. Perez, and V. Esmann, *Biochim. Biophys. Acta*, 1972, **268**, 344.
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[829] L. N. Bobrova and B. N. Stepanenko, *Biokhimiya*, 1972, **37**, 697.

both activities.[830] The role of 'acid' α-glucosidase in the lysosomal catabolism of rabbit liver glycogen has been discussed.[831]

Glycogen synthesis and degradation in cultured hepatocytes of hamsters have been investigated.[832] Treatment with glucoamylase was shown to depress the levels of liver glycogen in mice and it was suggested that the breakdown of glycogen by the enzyme may play a part in the secretion of insulin.[833] Glycogen metabolism of isolated livers of normal and genetically obese-hyperglycaemic (ob/ob) mice has been studied.[834]

The glycogen contents of tissues from normal and glucagon-treated rats, and the ability of the glycogens to complex with iodine, have been examined.[835] The effects of oxotremonine, atropine, and propanolol on the glycogen contents of various parts of rat brain have been studied.[836] A number of changes in the glycogen contents and concentrations, as well as in the activities of a number of enzymes involved in glycogen metabolism, have been observed in the rat hemidiaphragm muscle after unilateral denervation.[837]

The finding that a rat liver enzyme preparation catalysed the transfer of D-glucose from UDP-D-glucose to endogenous protein or protein-bound oligosaccharides to give α-1,4-glucans was considered to be related to the initiation of glycogen synthesis.[838] Developmental changes in the biosynthesis and metabolism of foetal and neonatal rat liver have been monitored.[839] The contents of glycogen and glycogen-degrading enzyme of normal and fasting rat livers have been determined[840] and glycogen synthesis in the perfused liver of starved rats has been studied.[841] The activities of rat liver enzymes responsible for glycogen metabolism after whole-body irradiation with *X*-rays or mixed γ-ray–neutron radiation have been examined.[842] The role of precursors in the synthesis of rat liver glycogen during cortisol administration[843] and the effects of acetylcholine on glycogen formation and glycogen synthetase activity in perfused rat liver[844] have been studied. Intermediates in the biosynthesis of rat epiphysial cartilage glycogen have been studied.[845]

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[845] C. J. Handley and C. F. Phelps, *Biochem. J.*, 1972, **126**, 417.

Glycogen levels in the tissues of chicks during the perinatal and post-hatching periods have been determined [846] and factors influencing the levels of glycogen in the brain during these periods have also been studied.[847] Glycogen formation from butylene glycol, propylene glycol, and ethylene glycol and their esters have been examined in chick liver.[848]

Statistically significant rhythms of glycogen contents in frogs (*Rana pipiens*) have been observed.[849] The glycogen of the tapeworm *Hymenolepis diminuta* has been labelled *in vivo* using uniformly labelled D-[^{14}C]glucose.[850] Extraction of the glycogen (by a cold buffer method) and fractionation according to molecular weight (by rate-zonal centrifugation) revealed that the highest specific activities occurred in glycogen forms of intermediate molecular weight, although these fractions were present in lower concentrations than the fractions of higher and lower molecular weight. The age dependence of incorporation of the precursor was also investigated.

The solubilization of shell-fish glycogen in organic solvents on induction with gelatin has been shown to be mediated through polysaccharide–protein hydrogen-bonding.[851] The induced solubilization was partially inhibited by raising the temperature above 0 °C, while water inhibited glycogen–glycogen interactions in acidified methanol, ethanol, n-propanol, and t-butyl alcohol. In general, it was found that the most hydrophobic alcohols were good solvents and that the presence of strong acids tended to inhibit complex formation, whereas weak acids promoted solubility. Factors affecting glycogen biosynthesis and metabolism in the oyster (*Ostrea edulis*) have been studied.[852]

During the direct development of *Pieris brassicae* glycogen, the levels were observed to vary, but animals undergoing a diapause contained smaller amounts of glycogen.[853] Glycogen synthetase and the control of glycogen biosynthesis in the cellular slime mould *Dictyostelium discoideum* have been examined during the growth (myxamoebal) phase [854] and during cell differentiation.[855] Glycogen metabolism during the development of the Coleoptera *Tenebrio molitor* has been studied by the incorporation of uniformly labelled D-[^{14}C]glucose.[856]

Glycosaminoglycuronans, Glycosaminoglycans, and their Protein and Peptide Derivatives

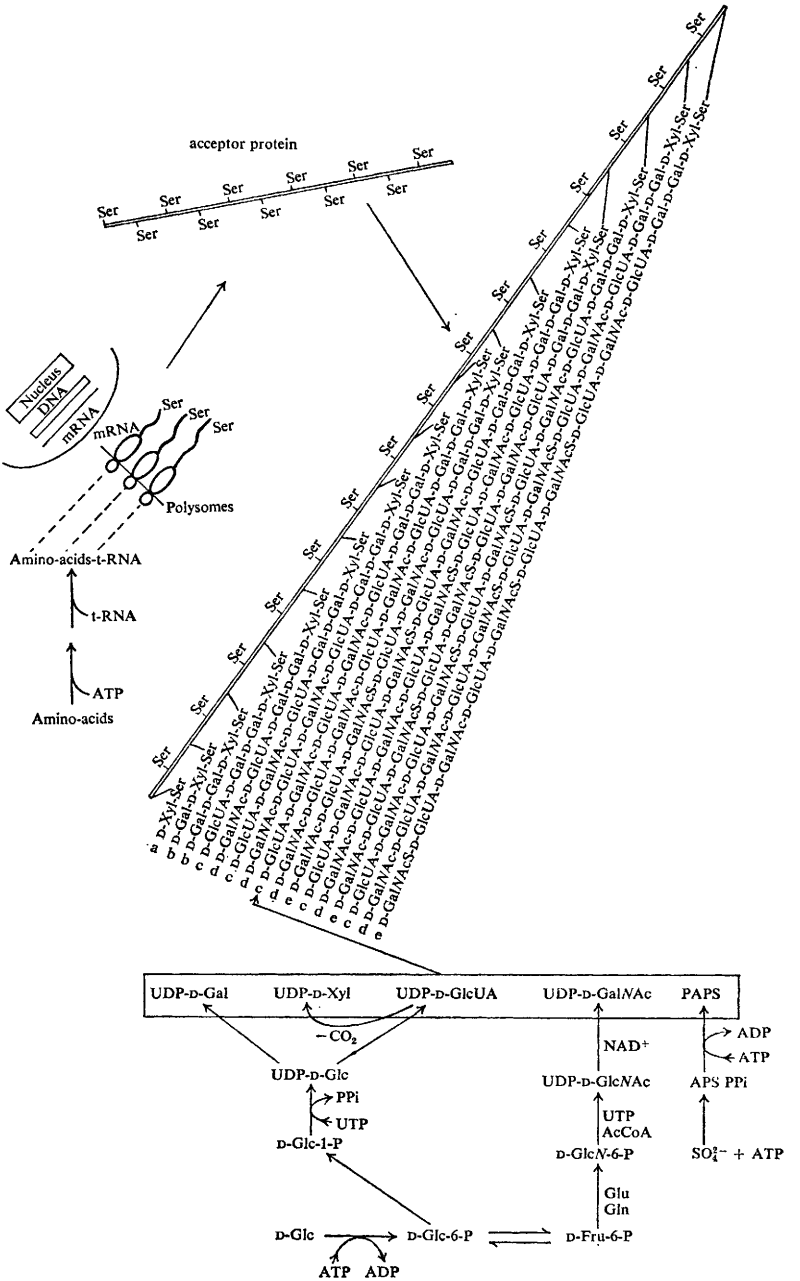
The occurrence, determination, isolation, properties, structure, and enzymic degradation of hyaluronic acid, chondroitin, the chondroitin sulphates,

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[848] M. Yoshida and H. Ikumo, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 497.
[849] S. Mizell, H. Moskow, and S. Warren, *Comp. Biochem. Physiol.*, 1971, **39A**, 465.
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[854] G. Weeks and J. M. Ashworth, *Biochem. J.*, 1972, **126**, 617.
[855] B. D. Hames, G. Weeks, and J. M. Ashworth, *Biochem. J.*, 1972, **126**, 627.
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dermatan sulphate, heparin, heparan sulphate, and keratan sulphate of higher animals have been reviewed.[857] Particular attention has been accorded to the glycosaminoglycans of connective tissues in a review of the chemistry of the vertebrate eye.[777] The distribution and function of the glycosaminoglycans of blood-vessel walls have been summarized.[858] In a review of the structure, biosynthesis, and heterogeneities of chondroitin sulphate–protein, the chemical and metabolic heterogeneities of the molecule have been explained by differences in size and turnover rates of acceptor protein pools as well as by differences in activities and specificities of monosaccharide- and sulpho-transferases, probably localized in different cell compartments.[859] Collation of the data on the various stages of the biosynthetic pathway to chondroitin sulphate permitted a scheme (Scheme 6) to be drawn up for the building of simple precursors into the macromolecule. The physical chemistry of the glycosaminoglycans has been reviewed.[860]

Occurrence, Isolation, Measurement, and Structure.—*General.* The effects of acid hydrolysis on the measurement of the 2-amino-2-deoxyhexose and amino-acid contents of proteoglycans, and the separation of the products of hydrolysis, have been studied.[861] The acidic hydrolysis of glycosaminoglycuronans has been found to be facilitated by their reaction with carbodiimides followed by reduction with sodium borohydride.[862] The products of the initial reaction appeared to be lactones or intramolecular esters, so that reduction replaced the original uronic acid unit with the corresponding hexose unit. When hyaluronic acid, chondroitin sulphate, and heparin were subjected to the reaction sequence, more than 90% of the original glycuronosyl bonds were cleaved on hydrolysis with acid. The remaining, acid-resistant 2-amino-2-deoxyglycosyl linkages were cleaved with nitrous acid to complete an essentially quantitative, non-destructive depolymerization of the glycosaminoglycuronans. The applications of the reaction sequence to component analyses were discussed. The sodium hypochlorite–amylose–potassium iodide spectrophotometric method for the determination of 2-amino-2-deoxy- and 2-acetamido-2-deoxy-sugars has been applied to the measurement of these units in hyaluronic acid, chondroitin sulphate, and heparin without depolymerization.[863] Studies of the effects of pH, reaction time, and temperature indicated that the colour yield is influenced by the identity of the amino-sugars present as well as by the position and type of glycosidic linkages.

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[863] P. A. Sanford, A. J. Nafziger, and A. Jeanes, *Analyt. Biochem.*, 1971, **44**, 111.



a = UDP-D-Xyl + D-xylosyltransferase
b = UDP-D-Gal + D-galactosyltransferase
c = UDP-D-GlcUA + D-glucuronosyltransferase
d = UDP-D-Gal*N*Ac + 2-acetamido-2-deoxy-D-galactosyltransferase
e = PAPS + sulphotransferase

Scheme 6

The qualitative Alcian Blue test for glycosaminoglycans has been quantitated.[864] Maximum interaction of glycosaminoglycans in standard solutions and in urine occurred at an Alcian Blue concentration of 0.05% in sodium acetate buffer containing magnesium chloride. The complex formed, after removal by centrifugation, may be dissociated with the dibutyl ester of sulphosuccinic acid, the absorbance of the resultant clear-blue solution being determined. The procedure was shown to be relatively specific, although, under optimum conditions, Tamm–Horsfall glycoprotein was found to react with Alcian Blue to a significant extent. A further colorimetric method for the quantitative determination of glycosaminoglycuronans is based on the solubilization of Toluidine Blue–glycosaminoglycuronan complexes from cellulose acetate membranes.[865] The method was adapted to the determination of individual glycosaminoglycuronans after electrophoresis on cellulose acetate, and hyaluronic acid, chondroitin sulphate, heparin, and keratan sulphate could be determined separately.

Methods have been described for the identification of individual glycosaminoglycans in tissue sections; hyaluronate lyase was used for the identification of hyaluronic acid, while chondroitin sulphate lyase ABC was used for the chondroitin and dermatan sulphates.[866] The methods were applied to a number of tissues from various species. The periodate–Schiff reaction has been adapted for the measurement of dermatan sulphate; the modification is specific for this glycosaminoglycuronan provided that glycogen and glycoproteins are removed.[867] Results using this method were in good agreement with those obtained by an enzymic technique.

The use of Bio-Gel P (cross-linked polyacrylamide) as a matrix for the gel filtration of glycosaminoglycuronans (using water as eluent) revealed the operation of adsorption phenomena, which could be overcome by using an ionic medium as eluent.[868] Similar investigations with porous glass as the matrix indicated the operation of repulsion (negative adsorption) effects in water and demonstrated the association of protein with the glass, even in the presence of an ionic medium. Possible causes of the adsorptions were discussed and compared with those previously reported for Sephadex (cross-linked dextran) gels.

A comparative study of the efficiencies of the most frequently used column-chromatographic techniques for the separation of glycosaminoglycans resulted in the claim that cetylpyridinium chloride–cellulose and DE-52 cellulose are superior to Dowex 1 X2, Zerolit H-IP, SRA 132, DEAE-Sephadex A-25, QAE-Sephadex A-50, and ECTEOLA-cellulose.[869] However, in no case could a complete separation of a mixture containing

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[869] R. Bohn and D. A. Kalbhen, *J. Chromatog.*, 1971, **62**, 399.

hyaluronic acid, chondroitin 4- and 6-sulphates, dermatan sulphate, heparan sulphate, heparin, and keratan sulphate be achieved. A combined method employing fractionation on cetylpyridinium chloride–cellulose, DE-52 cellulose, and Dowex 1 X2 gave the best results for the complete separation of these glycosaminoglycans, the amounts of polysaccharide recovered ranging from 66% for heparan sulphate to 90% for heparin.[870] An alternative procedure for the separation of milligram quantities of glycosaminoglycans involved fractionation on Sephadex G-50 followed by refractionation of the material of high molecular weight on DEAE-cellulose.[871] In this case, the resolution depends principally on the degree of sulphation and to a certain extent on molecular weight.

The thin-layer chromatographic separation of standard and urinary preparations of glycosaminoglycans on cellulose has been reported.[872, 873] Sequential runs using six solvents in one direction gave a complete separation of urinary glycosaminoglycans and the R_f values were greater than those previously reported.[873]

An improved method for the electrophoretic separation of chondroitin 4- and 6-sulphates, dermatan sulphate, and their abnormally sulphated derivatives on cellulose acetate strips has been reported.[874] Conventional and gelatinized cellulose acetate strips have been examined for their suitability in the electrophoretic assessment of microgram quantities of heparin.[875] From results obtained using a series of electrolytes, gelatinized strips were found to be more satisfactory; their use with commercial samples of heparin gave two bands. A combination of gel and paper electrophoreses in conjunction with the action of chondroitin sulphate lyases ABC and AC and heparinases has been used for the identification of glycosaminoglycans.[876] Hyaluronic acid, the chondroitin sulphates, heparan sulphate, and heparin could be distinguished by this procedure.

The molecular structure of hyaluronic acid has been elucidated from the *X*-ray fibre diffraction patterns of a number of oriented fibres, films, and salts of hyaluronic acid.[877] Sodium hyaluronate was found to crystallize in two distinct hexagonal unit cells, but the molecular conformation is a three-fold helix in each case. In the free-acid form, the conformation is tightened to a two-fold helix. It was suggested that the alternation of 1*eq*,3*eq* and 1*eq*,4*eq* glycosidic linkages along the molecular chain is necessary to position all the important substituent groups on both sides of the chain in the free-acid form. The importance of humidity and temperature in the promotion of crystal structure changes in sodium

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[875] T. C. Box, J. N. Miller, and R. J. Stretton, *J. Chromatog.*, 1972, **64**, 193.
[876] C. P. Dietrich and S. M. C. Dietrich, *Analyt. Biochem.*, 1972, **46**, 209.
[877] E. D. T. Atkins and J. K. Sheehan, *Biochem. J.*, 1971, **125**, 92P.

hyaluronate has been demonstrated by *X*-ray analyses.[878] Humidity is important for annealing, and crystallization may be speeded up by annealing at high temperatures. Two crystalline forms of the same sample of the salt could be obtained by variation of the conditions used for crystallization.

In conformational studies of hyaluronic acid, values for the ratio of unperturbed to free-rotational, root-mean-square, end-to-end distances (σ) were calculated for chains composed of the unsolvated disaccharide repeating unit.[879] The variation of σ with various parameters was predicted.

A study of the degradation of hyaluronic acid by ferrous ions under atmospheres of nitrogen, air, and oxygen has shown the necessity of oxygen for the reaction.[880] Degradation was demonstrated by a decrease in specific viscosity of the solution, by an increase in the number of acidic groups, and by the formation of residues that yielded β-formylpyruvic acid on periodate oxidation. Digestion of the degraded material with hyaluronidase, β-acetamidodeoxyglucosidase, and β-glucuronidase, followed by ion-exchange chromatography and gel filtration, yielded an unsaturated compound identified as 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-glucose. The degradation of hyaluronic acid by ferrous ions rendered the polymer incompletely hydrolysable by the three enzymes.

The inhibition of peptide hydrolysis by hyaluronic acid, chondroitin sulphate, and heparin,[881] and the effect of hyaluronic acid on the stimulation of lymphocytes[882] have been investigated. Maximum interaction between chondroitin 6-sulphate and poly-L-lysine in dilute solution occurred when the sulphate and ε-amino-groups were present in equimolar proportions.[883]

The interaction of heparin with Toluidine Blue, sodium chloride, and bovine albumin has been shown to occur by a reversible semipolar or ionic bond reaction and it was concluded that heparin possesses cation-exchange properties.[884] Heparin has been confirmed to form an insoluble complex with concanavalin A, but at a lower pH (5.4) than that which is optimal for complexing with neutral polysaccharides.[705] The sensitivity of the reaction to other parameters and its inhibition were studied. The anticoagulant and lipaemia-clearing activities of heparin have been compared with those of a number of heparinoids.[885] Heparin was shown to be a necessary activator

[878] E. D. T. Atkins and W. Mackie, *Biopolymers*, 1972, **11**, 1685.
[879] R. L. Cleland, *Biopolymers*, 1971, **10**, 1925.
[880] J. F. Kennedy and H. Cho Tun, *Carbohydrate Res.*, 1972, **22**, 43.
[881] C. Andre, A. Berand, and R. Lambert, *Compt. rend. Soc. Biol.*, 1970, **164**, 853.
[882] Z. Darżynkiewicz and E. A. Balazs, *Experimental Cell Res.*, 1971, **66**, 113.
[883] R. A. Gelman, W. B. Rippon, and J. Blackwell, *Biochem. Biophys. Res. Comm.*, 1972, **48**, 708.
[884] S. Ohkuma and T. Furuhata, *Proc. Japan Acad.*, 1970, **46**, 884.
[885] K. Nagasawa, A. Ogamo, and S. Hayashi, *Arch. Biochem. Biophys.*, 1972, **150**, 451.

of tyrosine hydroxylase, but no other glycosaminoglycuronan acted as an activator.[886]

Treatment of keratan sulphates with alkali has been shown to form 2-amino-3,6-anhydro-2-deoxy-D-glucose units and a similar reaction occurred with heparin and heparan sulphate, although to a lesser extent.[887] The structure of the anhydro-sugar was confirmed by isolation and comparison with authentic material; its formation illustrates the production of 3,6-anhydro residues in polysaccharides having the 6-position sulphated and the 3-position unsubstituted. Under the conditions used, up to 52% of the 2-amino-2-deoxyhexose units of keratan sulphates were converted in up to 93% yield, whereas heparin and heparan sulphate gave corresponding figures of 19 and 29%. The use of the reaction for structural studies was discussed. During the interaction of keratan sulphate–chondroitin proteoglycan with procollagen, tight packing of the procollagen was observed in the developing complex.[779] The complex was less able to absorb water than the proteoglycan.

The interaction between glycosaminoglycuronans and human plasma lipoproteins has been studied using gels consisting of cross-linked hyaluronic acid or sulphated glycosaminoglycuronans covalently attached to Sepharose.[888] Whereas lipoproteins showed no interaction with the cross-linked hyaluronic acid, equilibration of the same lipoproteins with the other gels showed that they could be bound to insolubilized chondroitin 4-sulphate, dermatan sulphate, heparin, and heparan sulphate at near neutral pH. These interactions were attributed to ionic bindings between negatively charged groups on the glycosaminoglycuronans and positively charged amino-groups on the lipoprotein molecules. The range of ionic strengths at which the complexes could be dissociated was relatively narrow and depended on the type of polysaccharide involved. The charge densities of the molecules involved in the electrostatic binding were considered to regulate the strength of the ionic bond and, at equal charge density, polysaccharides containing L-iduronic acid appeared to react more strongly than those containing only D-glucuronic acid.

Particular Sources. Glycosaminoglycans, particularly hyaluronic acid, have been examined as inducers of cell aggregation, since the factors that induce aggregation are inactivated by hyaluronidase.[889] Conversely, human umbilical cord hyaluronic acid was found to promote aggregation. An assessment of the glycosaminoglycans of normal human fibroblasts by culturing the cells in the presence of radioactive precursors and by employing cellulose acetate electrophoresis has been reported.[890]

In a study of the relationship between human dermal glycosaminoglycans and ageing, it was found that up to 80% of the hyaluronic acid and up to

[886] R. J. Kuczenski and A. J. Mandell, *J. Neurochem.*, 1972, **19**, 131.
[887] P. Sampson and K. Meyer, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 2329.
[888] P. Iverius, *J. Biol. Chem.*, 1972, **247**, 2607.
[889] B. Pessac and V. Defendi, *Science*, 1972, **175**, 898.
[890] J. K. Herd, *Analyt. Biochem.*, 1972, **48**, 103.

50% of the dermatan sulphate is soluble in a saline extractant.[891] The separation of the glycosaminoglycans by two-dimensional electrophoresis on cellulose acetate in a pyridine–formate buffer was sensitive down to 0.5 μg polysaccharide and the separation of chondroitin 4- and 6-sulphates, hyaluronic acid, and chondroitin was achieved by the inclusion of cupric acetate in the electrolyte.[892] Application of the method to identification of the glycosaminoglycans in human skin demonstrated the presence of hyaluronic acid and dermatan sulphate as the major components, while chondroitin 4- and 6-sulphates and heparan sulphate occur as minor components.

The glycosaminoglycan contents of normal human brain have been investigated.[646, 893] Normal human prostate has been shown to contain hyaluronic acid, dermatan sulphate, and chondroitin 4- and 6-sulphates.[894] The glycosaminoglycans of the intima and media of human aorta have been analysed [782] and a glycosaminoglycan–lipoprotein complex has been isolated from the fatty streaks of aortic tissue.[895] The purification and characterization of the proteoglycans of human, sheep, porcine, and rat spleens obtained during pregnancy has been described; the proteoglycans were shown to resemble those found in Kurloff cells.[896]

The carbohydrate compositions [783] and the degradation [897] of the proteoglycans of human intervertebral discs have been studied. A new technique of non-disruptive extraction, followed by fractionation in caesium chloride gradients, has provided a means of preparing from cartilage relatively pure proteoglycan aggregate, subunit, and link fractions.[898] Immunological studies of the fractions from human, bovine, porcine, rat, and chick cartilage have led to the identification of an antigen associated with the proteoglycan subunit, which is common to several species, and to the demonstration of additional specific antigens in aggregate and link fractions. The results of polyacrylamide gel–sodium dodecyl-sulphate electrophoresis are consistent with the currently accepted concept that cartilage proteoglycans are composed of subunits and core proteins with covalently attached sulphated polysaccharide side-chains, which form aggregates by non-covalent association with the link protein. The principal neutral sugar of the protein polysaccharide light (PPL) fraction of human and rat costal cartilage has been identified as D-galactose.[784] The material from humans was found to possess higher D-mannose

[891] R. Fleischmajer, J. S. Perlish, and R. I. Bashey, *Biochim. Biophys. Acta*, 1972, **279**, 265.
[892] R. Hata and Y. Nagai, *Analyt. Biochem.*, 1972, **45**, 462.
[893] C. Haberland, E. G. Brunngraber, L. A. Witting, and H. Hof, *Neurology*, 1972, **22**, 305.
[894] C. S. Sato and F. Gyorkey, *Proc. Soc. Exp. Biol. Med.*, 1972, **140**, 79.
[895] S. R. Srinivasan, P. Dolan, B. Radhakrishnamurthy, and G. S. Berenson, *Prep. Biochem.*, 1972, **2**, 83.
[896] M. F. Dean, H. Muir, A. H. E. Marshall, P. A. Revell, and B. Vernon-Roberts, *F.E.B.S. Letters*, 1971, **16**, 183.
[897] C. H. Pearson, F. Happey, A. Naylor, J. M. Osborn, and R. L. Turner, *Biochem. J.*, 1972, **129**, 44P.
[898] H. Keiser, H. J. Shulman, and J. I. Sandson, *Biochem. J.*, 1972, **126**, 163.

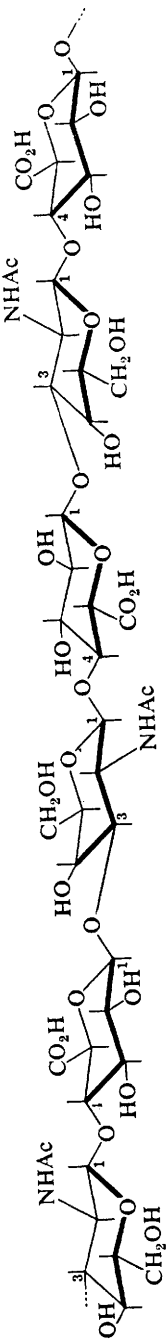
and L-fucose contents, but lower D-xylose and D-glucose contents, than the rat material. Alkaline borohydride reduction suggested that the glycopeptide linkages in both materials involve principally *O*-D-xylosyl-L-serine(L-threonine) residues and that L-fucose may be involved in the glycopeptide linkages to a minor extent. It has been suggested that lysozyme occurs in human hyaline cartilage as a counterion to the glycosaminoglycans.[899]

X-Ray diffraction patterns have been obtained for hyaluronate from human umbilical cord and bovine vitreous humour.[900] Sodium hyaluronate gave a pattern that indexed on a hexagonal unit cell with dimensions $a = 1.17$ nm and a fibre repeat distance of 2.85 nm. Annealing of the salt gave a different form that indexed on a hexagonal unit cell of unchanged fibre repeat distance, but with $a = 1.87$ nm. The existence of the monosaccharide residues in the chair conformation throughout the chain resulted in a three-fold helix. Analysis of the diffraction patterns led to two tentative structures for sodium hyaluronate based on different packing of the polysaccharide chains. Hyaluronic acid gave a different *X*-ray pattern from its salt, the fibre repeat distance being 1.96 nm and the unit cell appearing to be monoclinic. The chair conformation gives rise to a two-fold helix, and conformational changes between the free acid and monovalent salt were discussed. From the results of these experiments and from model building, it was concluded that the main substituent groups are equally distributed on either side of the chain (56) and that a left-handed helix is more favourable, since the acetamido- and carboxy-groups rotate away from each other, thus providing space for the cation to enter.[901] It was also concluded that hydrogen-bonding between acetamido-groups is only possible (Scheme 7) if the chains are antiparallel. The physicochemical properties of hyaluronic acid were discussed in terms of the structures identified.

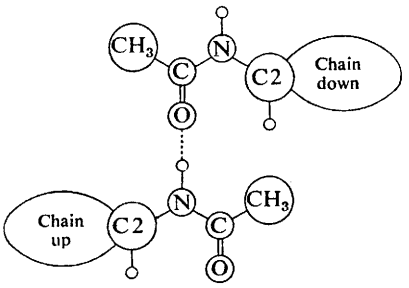
Chondroitin 4-sulphate–chondroitin 6-sulphate–protein has been obtained from intact human platelets,[902] and heparin and chondroitin sulphate have been identified among the products released from the platelets by thrombin and polystyrene latex particles.[903] The method of measuring human plasma glycosaminoglycans by selective adsorption has been modified to measure materials according to their binding by weak or strong anion-exchangers.[904]

The concentrations of hyaluronic acid and chondroitin sulphate in normal human synovial fluids have been determined.[905] A method involving caesium chloride density-gradient ultracentrifugation has been reported

[899] R. A. Greenwald, A. S. Josephson, H. S. Diamond, and A. Tsang, *J. Clin. Invest.*, 1972, **51**, 2264.
[900] E. D. T. Atkins, C. F. Phelps, and J. K. Sheehan, *Biochem. J.*, 1972, **128**, 1255.
[901] E. D. T. Atkins and J. K. Sheehan, *Nature New Biol.*, 1972, **235**, 253.
[902] A. J. Barber and G. A. Jamieson, *Biochemistry*, 1971, **10**, 4711.
[903] I. Hagen, *Biochim. Biophys. Acta*, 1972, **273**, 141.
[904] G. Neri, N. DiFerrante, and R. Hrgovcic, *Clinica Chim. Acta*, 1971, **35**, 359.
[905] P. O. Seppälä, J. Kärkkäinen, A. Lehtonen, and P. Mäkisara, *Clinica Chim. Acta*, 1972, **36**, 549.



(56)



Scheme 7

for the isolation of hyaluronic acid–protein from human synovial fluid.[906] Studies of the susceptibility of the molecule to hyaluronidase and trypsin, as well as the persistent co-chromatography of carbohydrate and protein, confirmed that hyaluronic acid is combined with a small quantity of protein in normal synovial fluid.

A simple method for the measurement of human urinary glycosaminoglycans has been based on their adsorption on to ECTEOLA-cellulose.[907] The method gave results comparable to those obtained by other techniques. The urinary output of sulphated and non-sulphated glycosaminoglycans by normal individuals has been measured.[908] Human urinary glycosaminoglycans have been isolated and identified by precipitation with cetyltrimethylammonium bromide, cellulose acetate electrophoresis, and hydrolysis with hyaluronidase and chondroitin sulphate lyases AC and ABC.[909] The principal glycosaminoglycans of both adults and children have been identified as chondroitin sulphates, with heparan sulphate as a minor component. The urinary glycosaminoglycans of children contain appreciable amounts of a hyaluronidase-resistant, chondroitin sulphate lyase AC-susceptible compound. The molecular weights ($\bar{M}_w$) of non-ultrafilterable glycosaminoglycans isolated from normal human urine have been reported to be as follows: hyaluronic acid + chondroitin (2 : 1) 8 × 10^3, dermatan sulphate 16 × 10^3, chondroitin sulphate 8—13 × 10^3, and heparan sulphate 8—30 × 10^3.[910] The ratio of $\bar{M}_w$ to $\bar{M}_n$ did not exceed 1.5 in any of the fractions, and the calculated Stokes' radii for the largest forms of chondroitin sulphate and heparan sulphate were 36 and 60 Å, respectively. All the sulphated glycosaminoglycan fractions contained slightly more than one residue of D-xylose per chain, whereas the non-sulphated polysaccharides contained one residue of D-xylose and two residues of D-galactose per chain. A crystallizable glycopeptide isolated

[906] I. Scher and D. Hamerman, *Biochem. J.*, 1972, **126**, 1073.
[907] A. Calatroni, *Clinical Chem.*, 1972, **18**, 266.
[908] P. D. Mier and E. Urselmann, *Dermatologica*, 1970, **141**, 34.
[909] J. M. Goldberg and E. Cotlier, *Clinica Chim. Acta*, 1972, **41**, 19.
[910] Å. Wasteson and E. Wessler, *Biochim. Biophys. Acta*, 1971, **252**, 13.

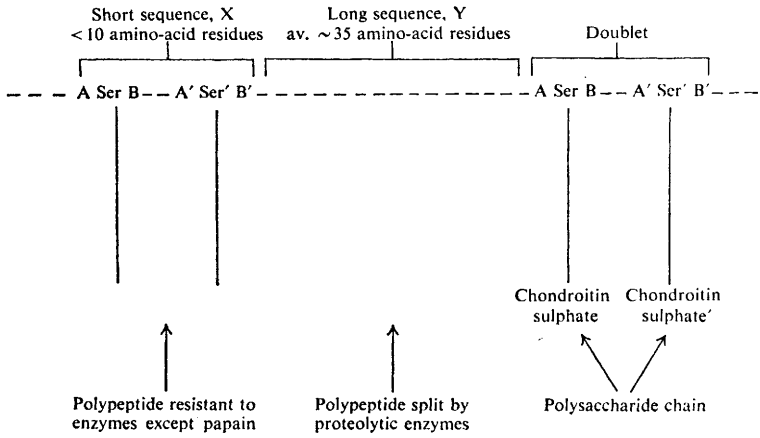
and characterized from normal human urine was shown to contain D-xylose, D-galactose, 2-amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-glucose, and D-glucuronic acid, as well as five amino-acids that included serine.[911] The molar ratios of the neutral, basic, and acidic carbohydrate components were shown to be 10 : 1 : 1 and the similarity of the glycopeptide to that of the linkage region of proteoglycans was noted.

The distribution of proteoglycans and the glycopeptides derived from them in macromolecular complexes of baboon (*Cyanocephalus papio-papio*) corneal stroma has been studied.[786]

Alkaline borohydride treatment of the chondroitin sulphate and heparan sulphate of bovine brain indicated that the polysaccharide chains are linked exclusively to L-serine.[912] Fractionation of the glycosaminoglycans of bovine corneal stroma on cetylpyridinium chloride–cellulose columns revealed the presence of keratan sulphate, as a major component, and chondroitin 4- and 6-sulphates.[913] The same technique has been used to separate the glycosaminoglycans of bovine retina into hyaluronic acid and three species of chondroitin sulphate.[914] Two of the latter species comprised 75% of the total chondroitin sulphate and were found to be undersulphated with sulphate : 2-amino-2-deoxy-D-galactose molar ratios of 1 : 10. Analysis of the two chondroitin sulphates indicated the involvement of D-xylose, D-galactose, L-serine, and L-threonine in the glycopeptide linkage regions.[915] From the molar ratios of 2-amino-2-deoxy-D-galactose to D-xylose and of 2-amino-2-deoxy-D-galactose to hydroxy-amino-acids, it was calculated that the molecules contain 19—22 disaccharide units per chain. This corresponds to a molecular weight ($\bar{M}_w$) of 8200—9500 (found: 9200) and it was concluded that the chondroitin sulphate chains are shorter than those found in other tissues.

Fragments consisting mainly of two polysaccharide chains joined by a short polypeptide bridge (doublets) have been prepared from chondroitin sulphate–proteins of bovine, sturgeon, elasmobranch, and lamprey connective tissues after proteolysis.[916] Consideration of their physicochemical characteristics led to the proposal of a structure for chondroitin sulphate–proteins (Scheme 8) containing a single polypeptide chain of 2000 amino-acid residues consisting of short (X) and long (Y) repeating sequences; the short sections carry two glycosidically-linked polysaccharide chains and constitute the doublet subunit. Bovine lung parenchyma and pleura have been shown to contain hyaluronic acid and all the sulphated glycosaminoglycans except keratan sulphate; dermatan sulphate is closely associated with the collagen fibres, whereas heparan sulphate is not associated with collagenous materials.[790]

[911] D. Basu, *Indian J. Biochem. Biophys.*, 1971, **8**, 133.
[912] R. U. Margolis, R. K. Margolis, and D. M. Atherton, *J. Neurochem.*, 1972, **19**, 2317.
[913] C. J. Handley and C. F. Phelps, *Biochem. J.*, 1972, **128**, 205.
[914] G. Bach and E. R. Berman, *Biochim. Biophys. Acta*, 1971, **252**, 453.
[915] G. Bach and E. R. Berman, *Biochim. Biophys. Acta*, 1971, **252**, 462.
[916] M. B. Matthews, *Biochem. J.*, 1971, **125**, 37.



Scheme 8

The dermatan sulphate of bovine liver has been separated into two fractions by electrophoresis on cellulose acetate.[917] Treatment of the more highly sulphated fraction with chondroitin sulphate lyase ABC yielded 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 4-sulphate, whereas treatment of the other fraction with the same enzyme yielded a product that was digested by chondro-6-sulphatase, but which differed from all known products of the action of chondroitin sulphate lyase ABC. Dermatan sulphates from bovine lung and rabbit skin were shown to contain polysaccharides that yielded 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 6-sulphate on treatment with chondroitin sulphate lyase ABC. Heparan sulphate from bovine liver differed from that from bovine lung in that, on fractionation by ion-exchange chromatography and gel filtration, it gave materials of different composition. Characterization of the oligosaccharides obtained from bovine liver heparin by the action of heparin lyase indicated that they all contain terminal, non-reducing, unsaturated uronic acid units and that 30% of the 2-amino-2-deoxy-D-glucose units of the heparin are disulphated.[918] At least 30% of the uronic acid units were found to be non-sulphated and, in agreement with other work, both glycosidic linkages were shown to be (1 → 4).

Purified bovine mucosal heparin, freed from other glycosaminoglycans and having relatively high proportions of linkage-region components, was reported to contain approximately three residues of 2-acetamido-2-deoxy-D-glucose per chain.[919] Degradation with nitrous acid indicated that these

[917] P. E. Gregoire, C. Dictus-Vermeulen, and J. P. Ameryckx, *Biochim. Biophys. Acta*, 1972, **279**, 102.
[918] A. Linker and P. Hovingh, *Biochemistry*, 1972, **11**, 563.
[919] J. A. Cifonelli and J. King, *Carbohydrate Res.*, 1972, **21**, 173.

units are distributed equally between the linkage region and interior structures; similar results were obtained for bovine lung and porcine mucosal heparins. A simplified structure (57), which was proposed on the basis of these results, supports the theory of a chemical relationship between heparins and heparan sulphates.

$(\text{Hex}p\text{UA-D-Glc}p\text{NS})_a\text{-}(\text{Hex}p\text{UA-D-Glc}p\text{NAc})_{0-2}\text{-}(\text{Hex}p\text{UA-D-Glc}p\text{NS})_b\text{-}(\text{Hex}p\text{UA-D-Glc}p\text{NAc})_{3-1}$-linkage glycopeptide

where a and b are integral numbers

(57)

The diffusional behaviour, as determined by steady-state isotopic flux measurements, of solutes of low molecular weight through gel membranes containing polyanionic material has been studied as a model for connective tissue.[920] The presence of solutions of bovine intervertebral disc sulphated proteoglycan and synovial fluid hyaluronic acid proteoglycan within a gelatin–agarose matrix had negligible effect on the diffusion of non-electrolytes, but markedly affected the behaviour of ionic species, suggesting that counterion fixation by the proteoglycans occurred. Donnan equilibria between the same proteoglycans and protein-free bovine nasal septum chondroitin sulphate and sodium ions have been measured by equilibrium dialysis, allowing estimates to be made of the degree of sodium-ion fixation by the polyanions.[921] Similar studies of carboxymethylcellulose and its sulphated derivative permitted the assessment of the relative importance of the carboxyl and sulphate ester groups in sodium-ion fixation.

Solubilization of 80% of the proteoglycans of bovine nasal cartilage has been achieved using lanthanum chloride, and the material was shown to be very similar to that obtained in like yield by extraction with higher concentrations of other salts.[922] A proteoglycan (containing 79% chondroitin sulphate and 5% keratan sulphate) from bovine nasal cartilage behaved as a single compound on density-gradient ultracentrifugation.[923] A molecular weight ($\bar{M}_n$) of 5.5×10^5 and a randomly coiled shape for the proteoglycan were deduced from light-scattering and viscosity measurements, and a diameter of 2000 Å was calculated for the equivalent sphere. In electron micrographs of monolayers of the proteoglycan, a long and a short form of the molecules could be discerned. The short form was shown to possess a central filament of 1700—1900 Å bearing 20—22 side-chains each 520—540 Å long, whereas the long chain possesses a central filament of 3200—3400 Å bearing 34 side-chains of the same dimensions as those in the short form. The data obtained for the short form are consistent with a protein moiety of 580 amino-acid residues and polysaccharide chains containing 40 disaccharide units. The uronic acid component of a glycoprotein from

[920] B. N. Preston and J. McK. Snowden, *Biopolymers*, 1972, **11**, 1627.
[921] B. N. Preston, J. McK. Snowden, and K. T. Houghton, *Biopolymers*, 1972, **11**, 1645.
[922] R. M. Mason and R. W. Mayes, *Biochem. J.*, 1972, **128**, 120P.
[923] P. Wellauer, T. Wyler, and E. Buddecke, *Z. physiol. Chem.*, 1972, **353**, 1043.

bovine nasal cartilage, containing neutral carbohydrate (2), 2-amino-2-deoxyhexose (3), and uronic acid (1%), appeared to be part of a glycosaminoglycan structure forming an integral part of the glycoprotein molecule.[924] A simple procedure to correct for the Johnston–Ogston effect, and which operates in the velocity sedimentation of two-component mixtures, has been applied to proteoglycan mixtures from bovine nasal cartilage.[925] The method permitted evaluation of the true concentration ratio of the components from sedimentation data obtained at two concentrations. Comparative studies employing ultracentrifugation and electrophoresis have been reported for PPL fractions from calf nasal septa and bovine embryonic rudiments. Since the proteoglycan was found in low-density fractions, a previous suggestion that the glycoprotein acts as a linking agent between proteoglycans was questioned.[926] Intact and Smith-degraded bovine nasal cartilage and porcine laryngeal cartilage proteoglycans have been shown to cross-react with their antisera, demonstrating that the antigenic determinant is common to all three molecular types.[927] The data indicated that neither the polysaccharide chains nor the carbohydrate units of the glycopeptide linkages contribute to the expression of the common determinant.

Electrophoresis of bovine nasal septa chondroitin sulphate before and after partial degradation with hyaluronidase revealed charge heterogeneity of the degraded, but not of the intact, polymer.[928] Fractionation of the enzyme-treated material gave two monodisperse subfractions (molecular weights 8600 and 4800), which differed considerably in their sulphate : disaccharide molar ratios. Amino-acid and neutral carbohydrate analyses of the subfractions showed that the less-sulphated material contained the glycopeptide linkage region, and it was concluded that chondroitin sulphate contains relatively less sulphate in the vicinity of the glycopeptide linkage than in the peripheral portions of the polysaccharide chain. The protein–keratan sulphate core isolated after limited digestion of bovine nasal cartilage proteoglycan with chondroitin sulphate lyase AC was shown to contain keratan sulphate (38%) and 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid-D-galactosyl-D-galactosyl-D-xylose (18%), whereas limited digestion with chondroitin sulphate lyase ABC left a residual disaccharide on the chondroitin sulphate chains of the core preparation.[929] Analysis of the products from degradation of the proteoglycan in monomeric and aggregate forms by chondroitin sulphate lyase AC indicated that non-covalent interactions, which are essential for the aggregation of proteoglycans, do not require the presence of a chondroitin sulphate portion in

[924] M. Janado and J. Dunstone, *J. Biochem.* (*Japan*), 1972, **71**, 635.
[925] M. Janado, L. W. Nichol, and J. R. Dunstone, *J. Biochem.* (*Japan*), 1972, **71**, 257.
[926] T. A. Mashburn and P. Hoffman, *J. Biol. Chem.*, 1971, **246**, 6497.
[927] E. Baxter and H. Muir, *Biochim. Biophys. Acta*, 1972, **279**, 276.
[928] Å. Wasteson and U. Lindahl, *Biochem. J.*, 1971, **125**, 903.
[929] V. C. Hascall, R. L. Riolo, J. Hayward, and C. C. Reynolds, *J. Biol. Chem.*, 1972, **247**, 4521.

the macromolecules. Chemical and physical analyses of subfractions of these products indicated that the core macromolecules are polydisperse (molecular weight 3.5—5.5 × 10^5) and that different chain lengths of keratan sulphate are attached to a protein of molecular weight 2 × 10^5.[930] Examination of the chains liberated by proteolysis showed them to be polydisperse and to have a molecular weight ($\bar{M}_w$) of 1.1 × 10^4. The data obtained are consistent with the hypothesis that papain-treated keratan sulphate consists of two polysaccharide chains connected through a peptide bridge.

Greater amounts of proteoglycans were extractable from transforming–ossifying regions than from resting regions of calf scapula cartilage.[931] The analytical composition of proteoglycans from the two zones was found to be identical, but different types of interaction seemed to exist among the components. Calcium-binding glycoprotein was isolated from the cartilage [931, 932] and it was suggested that this glycoprotein behaves as a proteoglycan-linking agent.

Various combinations of Pronase, pepsin, papain, and trypsin have been used to liberate glycosaminoglycans from porcine and rat brain tissues.[933] The highest yields resulted from the use of papain, and fractions containing hyaluronic acid and heparan sulphate were identified on fractionation of the extracts by ion-exchange chromatography.

A dermatan sulphate proteoglycan, representing approximately 30% of the dermatan sulphate in porcine skin, has been isolated and purified.[934] The proteoglycan was polydisperse; the major portion had $\bar{M}_w$ 2.9 × 10^6 and the constituent polysaccharide chains had $\bar{M}_w$ 1.5 × 10^4. All the constituent chains were shown to be bound to the protein by alkali-labile linkages. Oligosaccharides having the structures 3-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, 3-*O*-β-D galactopyranosyl-D-galactose, 4-*O*-β-D-galactopyranosyl-D-xylose, and *O*-β-D-galactopyranosyl-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-xylose were isolated after partial acid hydrolysis. It was established that the complete glycopeptide linkage involves the structure (58), which is identical to that found in chondroitin 4- and 6-sulphates– and heparin–protein.[935]

-β-D-Glc*p*UA-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Xyl*p*-*O*-Ser

(58)

The proteoglycans of porcine and rat cartilage have been extracted and the PPL fractions have been subfractionated.[936] The proteoglycans extracted

[930] V. C. Hascall and R. L. Riolo, *J. Biol. Chem.*, 1972, **247**, 4529.
[931] F. Vittur, M. C. Pugliarello, and B. de Bernard, *Biochim. Biophys. Acta*, 1972, **257**, 389.
[932] F. Vittur, M. C. Pugliarello, and B. de Bernard, *Biochem. Biophys. Res. Comm.*, 1972, **48**, 143.
[933] H. Srinivas, R. Leela, and P. B. N. Rao, *Indian J. Biochem. Biophys.*, 1972, **9**, 83.
[934] B. Öbrink, *Biochim. Biophys. Acta*, 1972, **264**, 354.
[935] E. L. Stern, B. Lindahl, and L. Rodén, *J. Biol. Chem.*, 1971, **246**, 5707.
[936] R. D. Campo, R. J. Bielen, and J. Hetherington, *Biochim. Biophys. Acta*, 1972, **261**, 136.

from porcine costal cartilage by dissociative means have been fractionated on QAE-Sephadex and it has been shown that the macromolecules contain varying proportions of chondroitin 4-sulphate and keratan sulphate.[937] Somewhat quantized molecular weights were found to arise from varying degrees of substitution of polysaccharide on the protein core. The stoicheiometry of polysaccharide-chain substitution was determined using the sulphite reaction; the average molecular weight (1.4×10^4) calculated by this method agreed well with that obtained from sedimentation equilibrium studies of the purified chondroitin sulphate chains. By addition of [^{35}S]sulphite after β-elimination of the polysaccharide chains, the sites of attachment in the polypeptide backbone were specifically and quantitatively labelled by conversion into [^{35}S]cysteic acid within the structure.[938] The molecular weight of the recovered polysaccharide chains correlated well with that calculated from the ratio of 2-amino-2-deoxy-D-galactose to cysteic acid. A detailed study of the molecular weights of several preparations of porcine rib cartilage proteoglycan has been reported.[939] Weight-average molecular weights were found to range upwards from 3.7×10^4 and to display considerable heterogeneity, whereas the chondroitin 4-sulphate chains were homogeneous ($\bar{M}_w$ 1.3×10^4). The heterogeneity was considered most likely to arise from variations in the polypeptide core due to different levels of saccharide substitution or catheptic proteolysis. Evaluation of the data also suggested that aggregation may occur.

The electrophoresis of proteoglycans and glycosaminoglycans on large-pore polyacrylamide gels containing agarose as the support medium has been described.[940] The average pore diameter was sufficient to allow penetration of all but the largest proteoglycans of porcine articular cartilage, making it possible to distinguish aggregated from disaggregated molecules. Since the mobilities of the molecules are inversely related to their hydrodynamic size, the relative sizes of the proteoglycans could be assessed. The method was used to separate single and double chains of chondroitin sulphate and was also applied to a pathological condition of pigs in which changes in the sizes of proteoglycans were evident. The extraction of proteoglycans from porcine foetal cartilage has been described and changes in the composition of the proteoglycans during prenatal life, development, and old age were studied in detail.[941] The results obtained could not be explained solely by changes in the proportion of link-glycoprotein in the proteoglycans.

Addition of small amounts of hyaluronic acid to disaggregated porcine laryngeal cartilage proteoglycans produced a large increase in their hydrodynamic size, as judged from gel filtration.[942] Chromatography showed

[937] J. Hranisavljević, D. L. Simpson, and E. A. Davidson, *Biochemistry*, 1972, **11**, 2983.
[938] D. L. Simpson and E. A. Davidson, *Biochemistry*, 1972, **11**, 1856.
[939] C. B. Woodward, J. Hranisavljević, and E. A. Davidson, *Biochemistry*, 1972, **11**, 1168.
[940] C. A. McDevitt and H. Muir, *Analyt. Biochem.*, 1971, **44**, 612.
[941] Z. Šimůnek and H. Muir, *Biochem. J.*, 1972, **126**, 515.
[942] T. E. Hardingham and H. Muir, *Biochim. Biophys. Acta*, 1972, **279**, 401.

that this was not an equilibrium phenomenon, since no interaction occurred with other polyanions. Maximal interaction was shown to occur at a hyaluronic acid concentration of 1% and the large increase in viscosity, which accompanied the interaction, could be eliminated by the addition of guanidine hydrochloride. From the optical properties of the proteoglycans and hyaluronic acid, and from estimates of their molecular weights, it was calculated that 10—30 proteoglycan molecules became associated with each hyaluronic acid chain. The reversible aggregation of porcine laryngeal cartilage proteoglycans has been studied; gel filtration located the aggregating component as a fraction containing $>50\%$ carbohydrate and accounting for $<1.5\%$ of the proteoglycan starting material.[943]

The qualitative and quantitative distributions of glycosaminoglycans in the mucosa of proximal and distal portions of the duodenum, ileum, and colon, and in the stomach of dogs have been reported.[944] Chondroitin sulphate was found to predominate in all tissues, but the presence of hyaluronic acid and heparin was also demonstrated.

The glycosaminoglycans of articular cartilage from femoral heads of normal dogs have been analysed and their compositions compared with those of dogs with degenerative joint disease.[945] The decreased amounts of chondroitin sulphate and keratan sulphate found in the abnormal tissue are considered to support the concept that a proteinase and a glycosidase may be of importance in the pathogenesis of degenerative joint disease.

Extraction of rabbit skin with ionic solutions yielded hyaluronic acid and chondroitin 4-sulphate, the analyses of which were reported.[946] It has been shown that all the uronic acid of an aqueous salt extract of rabbit skin is precipitable by cetylpyridinium chloride.[798] Purification and characterization of the glycosaminoglycans of rabbit kidney showed that the sulphated and non-sulphated varieties are present in a 3 : 1 ratio; the major glycosaminoglycans occurring are dermatan sulphate and heparan sulphate (molecular weight of predominant species 1.4×10^4).[947] The proteoglycans[948] and glycosaminoglycans[949, 950] of rabbit ear cartilage have been studied and minor constituent proteoglycans have been isolated as urea complexes.[951] These complexes were found to contain chondroitin sulphate as the constituent glycosaminoglycan, while physicochemical studies indicated that three of the complexes possess a 1 : 2 : 3 relationship in terms of their Stokes' radii and moelcular weights (maximum 1.89×10^4).

[943] C. P. Tsiganos, T. E. Hardingham, and H. Muir, *Biochem. J.*, 1972, **128**, 121P.
[944] C. E. Kupchella and F. R. Steggerda, *Trans. New York Acad. Sci.*, 1972, **34**, 351.
[945] G. Lust and W. Pronsky, *Clinica Chim. Acta*, 1972, **39**, 281.
[946] G. Prodi, A. M. Ferreri, S. Grilli, and P. Rocchi, *Ital. J. Biochem.*, 1971, **20**, 16.
[947] D. van Praag, A. L. Stone, A. J. Richter, and S. J. Farber, *Biochim. Biophys. Acta*, 1972, **273**, 149.
[948] A. Serafini-Fracassini and W. H. Stimson, *F.E.B.S. Letters*, 1972, **26**, 336.
[949] B. Rokosova and J. P. Bentley, *Biochim. Biophys. Acta*, 1972, **264**, 98.
[950] J. G. Derge and E. A. Davidson, *Biochem. J.*, 1972, **126**, 217.
[951] A. Serafini-Fracassini and W. H. Stimson, *F.E.B.S. Letters*, 1972, **15**, 245.

The glycosaminoglycans of rodent kidneys have been investigated.[952] Heparin extracted from mouse mastocytoma tissue under mild conditions has been purified by precipitation with cetylpyridinium chloride and by treatment with hyaluronidase.[953] Gel filtration separated the product into three fractions (average molecular weights 6—7 × 10^4, 2.6 × 10^4, and 0.9 × 10^4, respectively), the chromatographic behaviour of which was unaffected by treatment with either papain or alkali. Component analyses indicated that the two fractions of lower molecular weight consist largely of single polysaccharide chains lacking the carbohydrate–protein linkage region. Of the total glycosaminoglycan, 10% was digested by chondroitin sulphate lyase ABC to 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 4-sulphate.[954]

The principal glycosaminoglycan of rat brain has been shown to be chondroitin 4-sulphate;[955, 956] ion-exchange chromatography of the heparan sulphate present yielded two fractions differing in their total sulphate contents, but possessing a common molar ratio of sulphamido-groups : 2-amino-2-deoxyhexose units.[956] Degradation with nitrous acid indicated that, in contrast to the structure proposed for heparan sulphate from bovine lung, the rat brain material contains a smaller proportion of uronosyl-2-deoxy-2-sulphamidohexose disaccharide repeating units and consists mostly of *N*-acetylated segments having a relatively high content of sulphate ester. The amounts of glycosaminoglycans present in the prostate, salivary glands, and seminal vesicles of rat have been reported.[957] Hyaluronic acid, chondroitin 4- and 6-sulphates, dermatan sulphate, heparin, and heparan sulphate have been identified in the salivary glands and uterus of female rats.[958]

The levels of glycosaminoglycans in rat lung, kidney, liver, and blood [959] and the amounts of glycosaminoglycuronans in the three major zones of the kidney [960] have been reported. Heparan sulphate was shown to be the major glycosaminoglycan of rat kidney, although hyaluronic acid, dermatan sulphate, and chondroitin 4- and 6-sulphates are also present.[961] Glycosaminoglycans isolated from the kidneys of ascites-implanted rats were not significantly different from those of normal rats. The susceptibility of the glycosaminoglycans of a Golgi apparatus-rich

[952] L. F. Nikiforovskaia, V. V. Vinogradov, V. G. Rozin, and L. I. Batenko, *Doklady Akad. Nauk S.S.S.R.*, 1971, **200**, 986.
[953] S. Ögren and U. Lindahl, *Biochem. J.*, 1971, **125**, 1119.
[954] T. Helting, S. Ögren, U. Lindahl, H. Pertoft, and T. Laurent, *Biochem. J.*, 1972, **126**, 587.
[955] R. U. Margolis and R. K. Margolis, *Biochim. Biophys. Acta*, 1972, **264**, 426.
[956] R. U. Margolis and D. M. Atherton, *Biochim. Biophys. Acta*, 1972, **273**, 368.
[957] J. A. Kofoed, A. B. Houssay, A. A. Tocci, H. M. Curbelo, and C. H. Gamper, *J. Endocrinol.*, 1971, **51**, 465.
[958] J. A. Kofoed, A. B. Houssay, A. A. Tocci, and H. M. Curbelo, *Acta Endocrinol.*, 1972, **69**, 87.
[959] E. Lyubovich and T. D'yakova, *Voprosy Med. Khim.*, 1970, **16**, 613.
[960] J. M. Bowness and D. N. Barry, *Canad. J. Biochem.*, 1972, **50**, 610.
[961] N. Seno, K. Ariizumi, S. Nagase, and K. Anno, *J. Biochem.* (*Japan*), 1972, **72**, 479.

fraction of rat liver to mild alkali, hyaluronidase, and heparinase has been investigated.[962] Calcium transport in rat liver was blocked by the hexammine cobaltichloride cation, which formed a complex with the glycosaminoglycans.[963]

Various combinations of pepsin, pronase, trypsin, and papain have been tested in the isolation of glycosaminoglycans from rat intestine by proteolytic digestion and treatment with alkali.[964] Maximal extraction was achieved using pronase, whereas extraction of the tissue with alkali prior to proteolytic digestion was found to reduce the glycoprotein content of the extract. Hyaluronic acid, chondroitin sulphate, heparin, and heparan sulphate were detected in the extracts. When the tissue was labelled with [^{35}S]sulphate, radioactivity was found to be associated with the hyaluronic acid fractions, suggesting the presence of other sulphated glycosaminoglycan complexes.

Hyaluronic acid has been found in the peritoneal fluid of rats after injection with phytohaemagglutinin, and a hyaluronic acid–protein complex was isolated using cetylpyridinium chloride.[730] The effects of *X*-rays on the serum and ascitic fluid glycosaminoglycans of rats with yoshida hepatoma have been studied.[965]

The glycosaminoglycans of rat incisor dental pulp have been isolated and, using a cetylpyridinium chloride–cellulose column, were identified as hyaluronic acid, chondroitin 4- and 6-sulphates, and keratan sulphate.[966] A molecular-weight range of 4—7 $\times$ 10^4 was reported for the chondroitin sulphates and variations in the sulphate contents of the polysaccharides were indicated. The same four glycosaminoglycans were shown to be present in neonatal rat epiphysial cartilage.[845]

The precipitation of whale cartilage chondroitin sulphate from aqueous solution with ethanol showed that the polysaccharide is not readily precipitable in the absence of salts.[967] Little difference was found in the precipitating power of various anions, but divalent cations possessed greater precipitating powers than monovalent cations and also exhibited different effects. A linear relationship was found between the logarithm of the chondroitin sulphate or salt concentrations and the amount of ethanol required to cause precipitation. It was also shown that a decrease in temperature facilitated precipitation; above 0 °C, the ethanol concentration needed for precipitation is linearly related to the temperature and inversely proportional to the molecular weight of the material precipitated. A glycosaminoglycan–protein complex from whale nasal cartilage exhibiting polydispersity on cetylpyridinium chloride–cellulose and ion-exchange

[962] R. R. Wagner and M. A. Cynkin, *J. Biol. Chem.*, 1971, **246**, 143.
[963] B. A. Tashmukhamedov, A. I. Gagelgans, K. Mamatkulov, and E. M. Makhmudova, *F.E.B.S. Letters*, 1972, **28**, 239.
[964] H. Srinivas, R. Leela, and P. B. M. Rao, *Indian J. Biochem. Biophys.*, 1972, **9**, 78.
[965] M. T. Illeni and M. R. Gelsomino, *Boll. Soc. ital. Biol. sper.*, 1971, **47**, 249.
[966] A. Linde, *Biochim. Biophys. Acta*, 1972, **279**, 446.
[967] A. Nakashima, *Bull. Jap. Soc. Sci. Fish.*, 1972, **38**, 148.

columns was identified as keratan sulphate.[968] Component analyses revealed the presence of small amounts of mannose, fucose, and 2-amino-2-deoxygalactose as well as galactose, 2-amino-2-deoxyglucose, and sialic acid, which are the main components. Although the molar ratio of hexose : 2-amino-2-deoxyhexose was unity for all fractions, the molar ratios of sulphate and sialic acid : 2-amino-2-deoxyhexose differed widely among the fractions (0.18—0.53 : 1.00 and 1.19—0.23 : 1.00, respectively), with higher sialic acid contents corresponding to lower sulphate contents and lower molecular weights.

Two heparan sulphate fractions have been isolated and separated from chicken skin; component and i.r. analyses and their electrophoretic behaviour indicated that the two fractions are identical apart from their sulphate contents.[969] Electrophoresis in composite gels of polyacrylamide and agarose has been used to separate hyaluronic acid from other glycosaminoglycans and, along with staining of the gel, this technique has been applied to the identification of hyaluronic acid in cultures of Rous sarcoma cells of chick embryos.[970]

Crude preparations of glycosaminoglycans from the gills of carp (*Cyprinus carpio*) have been fractionated by precipitation with cetylpyridinium chloride into a major chondroitin sulphate component and either heparin or heparan sulphate.[971] Varying degrees of sulphation were found in the chondroitin sulphate subfractions, and anticoagulant activity was demonstrated in most of the glycosaminoglycuronan fractions. A novel type of dermatan polysulphate has been isolated from the skin of hagfish (*Eptatretus burgeri* and *Paramyxine atami*).[972] Digestion of the polysaccharide with chondroitin sulphate lyase ABC yielded a trisulphated disaccharide, which was characterized as 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid 2- or 3-sulphate)-D-galactose 4,6-disulphate, in addition to 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid 2- or 3-sulphate)-D-galactose 4-sulphate, 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 4,6-disulphate, and 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 4- and 6-sulphates. A PPL fraction has been obtained from squid (*Ommastrephes sloani pacificus*) skin by extraction with water and ultracentrifugation of the extract.[973] Component analyses and enzymic digestion showed that the purified PPL is a chondroitin–protein complex that is virtually free from ester sulphate. Chondroitin sulphate and heparin from sea urchin (*Anthocidaris crassispina* and *Hemicentrotus pulcherrimus*) embryos inhibited the enzymic activity of RNA-polymerase.[974] It has been found that the oviducts

[968] H. Nakagawa and K. Satake, *Bull. Jap. Soc. Sci. Fish.*, 1971, **37**, 919.
[969] K. Kondo-Otsuka, N. Seno, and K. Anno, *Biochim. Biophys. Acta*, 1972, **279**, 260.
[970] J. P. Bader, D. A. Ray, and T. L. Steck, *Biochim. Biophys. Acta*, 1972, **264**, 73.
[971] L. Wasserman, A. Ber, and D. Allalouf, *Comp. Biochem. Physiol.*, 1972, **42B**, 669.
[972] N. Seno, F. Akiyama, and K. Anno, *Biochim. Biophys. Acta*, 1972, **264**, 229.
[973] N. Isobe and N. Seno, *Biochim. Biophys. Acta*, 1971, **252**, 612.
[974] Y. Aoki and H. Koshihara, *Biochim. Biophys. Acta*, 1972, **272**, 33.

and uterus glands of *Pimpla turionellae*, an endoparasite of various lepidopterous pupae, contain glycosaminoglycans that are used to coat the egg of the parasite before deposition.[975]

Clinical Conditions.—The glycosaminoglycans of brain tissue from a case of amaurotic idiocy have been investigated[893] and glycosaminoglycans, which appeared to be neither chondroitin sulphate nor heparin, have been detected in urine samples from a case of aspartylglucosaminuria.[976] The glycosaminoglycan content of atherosclerotic intima and media of the aorta has proved to be normal,[782] and sodium chondroitin sulphate has been investigated as an anti-atherosclerotic agent.[977] It has been reported that the level of serum glycosaminoglycans increases with the progress of serum bilharziasis[978] and that brain tissues from cases of galactosemia and infantile neurolipidosis contain less than normal amounts of glycosaminoglycans.[646]

The glycosaminoglycans of fibroblasts from cases of Hurler syndrome have been detected by strip scanning the electrophoretograms of materials from cells cultured in the presence of radioactive precursors.[890] A method described for the measurement of urinary glycosaminoglycans, which involved their adsorption on ECTEOLA-cellulose, has been applied in cases of Hunter, Marfan, Morquio, and Sanfilippo syndromes, and gave results comparable to those obtained with other techniques.[907] The periodate–Schiff reaction, modified to measure dermatan sulphate only, has been applied to the determination of this glycosaminoglycan in urine from patients with Hunter, Hurler, and Maroteaux–Lamy syndromes.[867] Urine from children with a marfanoid syndrome reacted positively in the cetyltrimethylammonium bromide screening test, although the glycosaminoglycan levels were found to be within normal limits.[909] Hyaluronic acid has been recognized in the pleural fluids of some cases of mesothelioma.[979] Methodology for the identification of urinary glycosaminoglycans in cases of infantile mucopolysaccharidoses has been reported[980] and chondroitin 4-sulphate has been shown to predominate in the large amounts of urinary glycosaminoglycans secreted in a new variety of mucopolysaccharidosis.[981, 982]

The results of animal tests on the effects of fluoride ingestion on the biosynthesis of glycosaminoglycans have been related to bone growth in cases of osteofluorosis, *etc.*[983]

[975] E. Führer, *Naturwiss.*, 1972, **59**, 167.
[976] J. Palo and H. Savolainen, *Clinica Chim. Acta*, 1972, **36**, 431.
[977] T. Kobayakawa, *Yakugaku Zasshi*, 1972, **92**, 919.
[978] E. Z. Khafagy, H. G. Osman, E. H. Raziky, and F. Y. Shalaby, *Clinica Chim. Acta*, 1972, **40**, 371.
[979] R. Havez, P. Degand, A. Boersma, and C. Richet, *Clinica Chim. Acta*, 1971, **33**, 443.
[980] R. Humbel, Y. Badonnel, C. Marchal, M. Vidailhet, and M. Fall, *Compt. rend. Soc. Biol.*, 1970, **164**, 1096.
[981] C. R. Thompson, *Ann. Internal Med.*, 1971, **75**, 424.
[982] J. W. Spranger, W. Schorter, and F. Freitag, *Helv. Paed. Acta*, 1971, **26**, 387.
[983] L. R. Hac, *Proc. Soc. Exp. Biol. Med.*, 1972, **139**, 827.

The urinary output of both sulphated and non-sulphated glycosaminoglycans by groups of patients with psoriasis and other dermatoses has been found to be elevated above normal.[908] The glycosaminoglycans of pleural plaques and lesions of progressive massive fibrosis have been identified and the analyses suggested a similarity between the pathological processes and calcification.[984] The concentrations of hyaluronic acid and chondroitin sulphate in synovial fluids from cases of rheumatoid arthritis have been determined, but no correlation was found between these concentrations and the grade of local articular change.[905] From studies of the ability of cations to extract lysozyme from cartilage, it has been proposed that the enzyme occurs in human hyaline cartilage as a counter-ion to glycosaminoglycans and that cartilage degradation in cases of rheumatoid arthritis leads to the release of lysozyme through liberation of glycosaminoglycans.[899] Glycosaminoglycan storage has been examined in a patient with Sandhoff's disease,[985] and serum and urinary glycosaminoglycans have been determined in cases of Sanfilippo syndrome.[986]

Biosynthesis and Metabolism.—The biosynthesis of hyaluronic acid and sulphated glycosaminoglycans has been briefly reviewed [660] and the biosynthesis of glycosaminoglycans from glycopyranosyl esters of nucleoside pyrophosphates ('sugar nucleotides') has been reviewed in detail.[659] The name 'somatomedin' has been proposed for the growth hormone-dependent factor that stimulates the incorporation of sulphate into chondroitin sulphate.[987] A liquid scintillation-counting medium, which accepts large amounts of water and sodium chloride, has been used in the measurement of radioactively labelled glycosaminoglycans.[988] The detection of ^{35}S- and ^{14}C-labelled glycosaminoglycans on cellulose acetate membranes by dissolution of the membranes before counting has been reported to be inaccurate.[890] The best results were obtained by strip scanning of the membranes with a radiometer.

Catabolism of sulphated glycosaminoglycans by normal human fibroblasts in culture was shown to be inhibited progressively as the pH of the growth medium was increased from 6.8 to 8.0.[989] The capacity of the cells to degrade glycosaminoglycans was rapidly restored on lowering the pH, but such pH dependence was not observed in the cells from patients with genetic impairment of glycosaminoglycan degradation (*e.g.*, cases of Hunter and Hurler syndromes). The high correlation between the concentrations of hyaluronic acid and chondroitin sulphate in synovial fluids

[984] F. S. Wusteman, C. Gold, and J. C. Wagner, *Amer. Rev. Respirat. Diseases*, 1972, **106**, 116.
[985] D. A. Applegarth and G. Bozoian, *Clinica Chim. Acta*, 1972, **39**, 269.
[986] L. B. Ricci, M. R. Gelsomino, and L. Raimondi, *Boll. Soc. ital. Biol. sper.*, 1971, **47**, 250.
[987] W. H. Daughaday, K. Hall, M. S. Raben, W. D. Salmon, J. L. Van den Brande, and J. J. Van Wyk, *Nature*, 1972, **235**, 107.
[988] J. F. Kennedy, *Experientia*, 1969, **25**, 1120.
[989] S. O. Lie, V. A. McKusick, and E. F. Neufeld, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2361.

from normals and patients with rheumatoid arthritis indicated a common origin for the two glycosaminoglycans, *viz.* the synovial lining cells.[905]

The acid-soluble nucleotides and intermediates in the synthesis of glycosaminoglycans in bovine corneal stroma have been identified and, from incorporation studies with uniformly labelled D-[^{14}C]glucose, the rates of biosynthesis and turnover of the glycosaminoglycans have been calculated.[913] The concentrations of sugar nucleotides in bovine corneal epithelium, ovine nasal septum cartilage, and neonatal rat skin have been related to the spectrum of glycosaminoglycans found in each tissue; the production and utilization of UDP-D-glucuronic acid in these tissues have been studied.[990]

In a study of the biosynthesis of dermatan sulphate, the transfer of 2-acetamido-2-deoxy-D-galactose from UDP-2-acetamido-2-deoxy-D-galactose to an exogenous L-iduronosyl acceptor was catalysed by an enzyme preparation from porcine intestinal mucosa.[991] Chondroitin sulphate lyase AC was shown to have no effect on incorporated 2-acetamido-2-deoxy-D-[^{3}H]galactose, whereas chondroitin sulphate lyase ABC liberated all the radioactivity from the macromolecules. After incorporation of [^{35}S]sulphate, the proteoglycans of porcine and rat cartilage were separated into several PPL fractions; analysis of the specific activities directly and subsequent to mixing with identical fractions obtained from non-radioactive cartilage lent support to the theory that the soluble proteoglycans exist *in situ* as aggregates.[936] The metabolic behaviour of non-extractable proteoglycans was found to differ from that of soluble forms. From studies of the kinetics of incorporation of [^{35}S]sulphate into porcine laryngeal cartilage *in vitro*, it has been found that, whereas radioactivity appeared in the chondroitin sulphate within 2 minutes, there was a delay of 35 minutes before it appeared in the extractable proteoglycan.[992] It was also found that the smallest proteoglycan molecules possessed the highest specific radioactivity and that the specific activity increased as the chondroitin sulphate content of the molecules decreased. Such metabolic heterogeneities were considered to arise either from a partial separation of different types of proteoglycans or from differences in the rates of degradation of molecules of different sizes and compositions due to the nature and specificities of the normal degradative enzymes. The results also suggested that molecules of all sizes are formed at the same time.

Variations in the concentrations of proteoglycans of low molecular weight and of their rate of uptake of [^{35}S]sulphate *in vivo* have been determined during active glycosaminoglycan biosynthesis in rabbit ear cartilage.[948] The chondroitin sulphate released by papain from rabbit ear cartilage, after incubation with uniformly labelled D-[^{14}C]glucose, has been found to be metabolically heterogeneous, the molecules of higher molecular weight

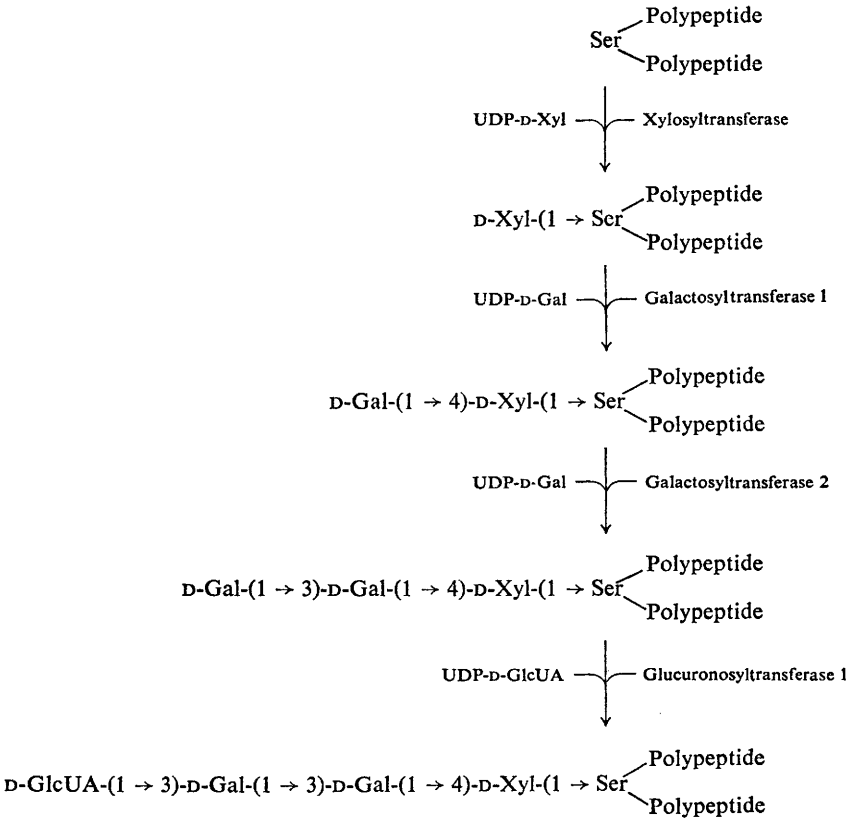
[990] P. A. Gainey and C. F. Phelps, *Biochem. J.*, 1972, **128**, 215.
[991] A. Malmström and L.-Å. Fransson, *F.E.B.S. Letters*, 1971, **16**, 105.
[992] T. E. Hardingham and H. Muir, *Biochem. J.*, 1972, **126**, 791.

exhibiting a higher rate of precursor uptake.[949] No transfer of radioactivity between different types of chondroitin sulphates occurred. Membrane-bound polysaccharides, obtained from ear cartilage of rabbits recovering from papain injection, have been examined for glycosyl acceptor properties and, although significant transfer of D-glucuronic acid from UDP-D-glucuronic acid could be demonstrated, the molecular size of the acceptor appeared to have no effect.[950] All the fragments examined contained stoicheiometric amounts of sulphate ester, suggesting a close relationship between extension and sulphation of the chondroitin chains. The enzyme activities involved in the incorporation of D-glucuronic acid into endogenous chondroitin sulphate and of 2-acetamido-2-deoxy-D-galactose into exogenous chondroitin 6-sulphate hexasaccharide by subcellular fractions from rabbit bone-marrow cells have been assayed.[993] The findings were consistent with the synthesis of glycosaminoglycans on microsomal or Golgi membranes for subsequent incorporation into cytoplasmic granules produced in the Golgi apparatus of the granulocyte.

The biosynthesis of glycosaminoglycans in mouse Furth mastocytoma tissue has been studied and the presence of a UDP-2-acetamido-2-deoxy-D-galactose-chondroitin 6-sulphate hexasaccharide:2-acetamido-2-deoxy-D-galactosyltransferase in microsomal particles from the mast cells was demonstrated.[954] Incubation of a microsomal fraction from a heparin-producing mouse mastocytoma with UDP-D-[^{14}C]glucuronic acid and unlabelled UDP-2-acetamido-2-deoxy-D-glucose resulted in the incorporation of radioactivity into endogenous polysaccharide.[994] When adenosine 3′-phosphate 5′-sulphatophosphate (PAPS) was included in the incubate, the product polysaccharide contained L-[^{14}C]iduronic acid as well as D-[^{14}C]-glucuronic acid. Pulse-chase experiments showed that D-[^{14}C]glucuronic acid was incorporated into the polymer during the pulse period (in the absence of PAPS) and was subsequently converted into L-[^{14}C]iduronic acid during the chase period (in the presence of PAPS). It was concluded that L-iduronic acid residues are formed by epimerization of D-glucuronic acid residues at the polymer level. Labelling *in vivo* of mouse mastocytoma tissue with [^{35}S]sulphate showed initial labelling of large heparin molecules, followed by a progressive shift of radioactivity to forms of lower molecular weight. Since incubation with a fraction of the mastocytoma was shown to degrade [^{35}S]heparin, but not [^{14}C]chondroitin sulphate, it was suggested that the heparin molecules are degraded by a specific, endogenous enzyme.[953] The biosynthesis of D-glucuronic acid residues, which occur in several glycosaminoglycans as a link between a serine-bound, neutral trisaccharide and the main polysaccharide chain, has been investigated in the heparin-synthesizing mastocytoma, and an enzyme that catalysed the transfer of D-glucuronic acid from UDP-D-

[993] I. Olsson, *Experimental Cell Res.*, 1972, **70**, 173.
[994] U. Lindahl, G. Bäckström, A. Malmström, and L.-Å. Fransson, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 985.

glucuronic acid to 3-*O*-β-D-galactopyranosyl-D-galactose was isolated and purified.[995] In conjunction with earlier studies, the results permitted a complete formulation of the mechanism of biosynthesis of the heparin–polypeptide linkage region (Scheme 9).



Scheme 9

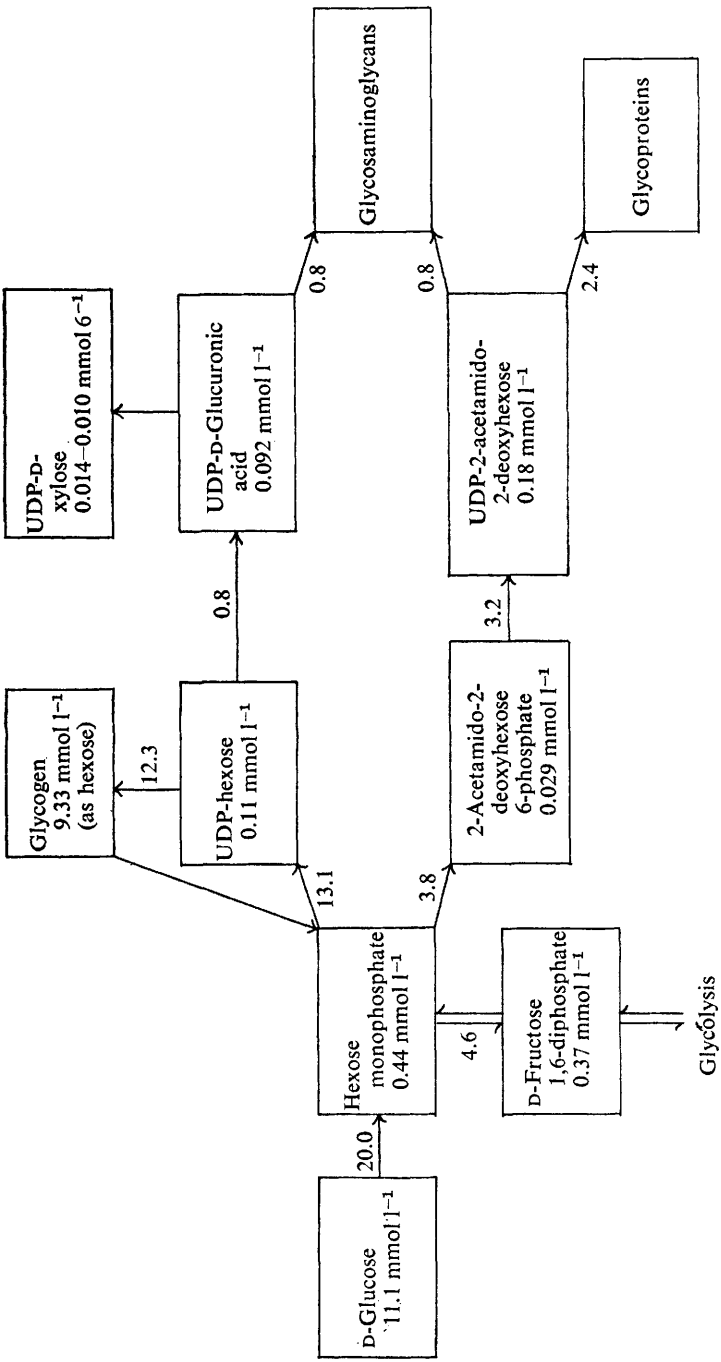
The turnover rates of chondroitin sulphate and heparan sulphate in rat brain have been measured ($t_{\frac{1}{2}}$ = 7 and 3 days, respectively). The biphasic disappearance of radioactivity from the [^{35}S]sulphate-labelled glycosaminoglycans ($t_{\frac{1}{2}}$ = 2.5 and 14 days) and other data indicated that the two components represent pools of sulphated molecules having different metabolic activities.[955] The effects of testosterone on the biosynthesis of glycosaminoglycans in the prostate, salivary glands, and seminal vesicles of rats,[957] and the effects of oestrogens on their biosynthesis in the salivary glands and uterus of female rats [958] have been investigated. Hydrocortisone

[995] T. Helting, *J. Biol. Chem.*, 1972, **247**, 4327.

had no effect upon the biosynthesis of glycosaminoglycans in rat kidney,[960] whereas a deficiency of vitamin A was shown to have different effects upon the production of glycosaminoglycans in the lung, liver, kidney, and blood.[959] The incorporation of glycosyl groups into endogenous acceptors in a Golgi apparatus-rich fraction of rat liver [962] and the metabolism of glycosaminoglycans in alloxan diabetic rats [996] have been studied. Injection of rats with phytohaemagglutinin appeared to induce the biosynthesis of hyaluronic acid in the peritoneal fluid.[730] The acid-soluble nucleotides and intermediates of glycosaminoglycan biosynthesis of neonatal rat epiphysial cartilage have been identified and their concentrations in the tissues have been determined (Scheme 10).[845] The rates of synthesis of UDP-D-glucuronic acid and UDP-2-acetamido-2-deoxy-D-glucose from D-glucose were measured and turnover times of 70 and 120 h were calculated for chondroitin sulphate and hyaluronic acid, respectively. The relative rates of synthesis of the sulphated glycosaminoglycans were calculated from the rates of incorporation of [^{35}S]sulphate and were found to be in good agreement with those obtained from D-[^{14}C]glucose labelling. It has been reported that the biosynthesis of glycosaminoglycans in rat bones can be either increased or decreased by the presence of fluoride ion according to the amount administered.[983]

The renewal of glycosaminoglycans in tissues of normal and vitamin A deficient chicks has been studied by means of incorporation of [^{35}S]sulphate.[997] Cytochalasin B has been shown to depress the incorporation of 2-amino-2-deoxy-D-[1-^{14}C]glucose into the glycosaminoglycans of various chick-embryo cells.[998] The uptake of 2-amino-2-deoxy-D-[6-^{3}H]glucose by the hyaluronic acid of Rous sarcoma cells during culture has been assessed.[970] Low concentrations of hyaluronate were found to block the formation of colonies and cartilage nodules in stationary cell cultures from embryonic chick somites and limb buds.[999] This phenomenon *in vitro* was correlated with the sequence of hyaluronic acid production and with its enzymic degradation during chondrogenesis in embryonic and regenerating tissues *in vivo*. From a study of the replacement of proteoglycans in embryonic chick cartilage in culture after treatment with hyaluronidase, it has been concluded that newly formed molecules contain fewer chains of chondroitin sulphate. This was considered to be due either to an increased synthesis of proteoglycans with low chondroitin sulphate contents or to a failure of the system to glycosylate the protein moiety to the usual extent.[1000] The intact protein core obtained from bovine nasal cartilage by Smith degradation acted as a substrate for D-xylosyl transfer from UDP-D-[^{14}C]xylose in a chick embryo cartilage

[996] K. Malathy and P. A. Kurup, *Indian J. Biochem. Biophys.*, 1971, **8**, 147.
[997] A. A. Dusheiko, M. M. Velyky, and M. A. Blazhevich, *Ukrain. biokhim. Zhur.*, 1971, **43**, 295.
[998] J. M. Sanger and H. Holzer, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 253.
[999] B. T. Toole, G. Jackson, and J. Gross, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 1384.
[1000] T. E. Hardingham, S. Fitton-Jackson, and H. Muir, *Biochem. J.*, 1972, **129**, 101.



Rates are expressed as nmol min^{-1} (g wet wt. of tissue)$^{-1}$. Concentrations relate to cell water.

Scheme 10

system.[1001, 1002] Characterization of the D-xylosylated molecule showed that D-xylose had been incorporated into the protein by an alkali-labile linkage from which it could be released as xylitol on treatment with alkaline borohydride; exhaustive proteolysis yielded xylosylserine.[1002] A number of compounds of low molecular weight were tested as acceptors for D-xylose, but only serylglycylglycine had any acceptor activity and this was much smaller than that exhibited by the Smith-degraded material. Furthermore, intact proteoglycan and the products obtained from it by digestion with either hyaluronidase or chondroitin sulphate lyase were found to be inactive as acceptors. It was concluded that D-xylose had been transferred to the hydroxy-groups of specific serine residues in the protein chain. The effects of puromycin, ribonuclease, and chloramphenicol on the incorporation of D-[6-^{14}C]glucose, L-[^{14}C]lysine, and [^{35}S]sulphate into the chondroitin sulphate–protein of chick embryonic cartilage have been studied, and the results suggested that the carbohydrate and protein moieties are synthesized at different relative rates.[1003]

A study of the macromolecular assembly of chondroitin in a microsomal preparation from chick epiphysial cartilage showed that endogenous chondroitin sulphate acts as a primer for the addition of relatively short, non-sulphated polysaccharide chains to form large molecules with an extensive sulphated portion and a small non-sulphated portion.[1004] A small primer was found to act as an acceptor for the polymerization of long, non-sulphated chains to form large, non-sulphated chondroitin species. Although the incorporation of carbohydrate was time-dependent, there was no detectable accumulation of short-chain molecules at the earliest times examined. It was concluded that the mechanism of polysaccharide-chain formation involves rapid assembly of the major part of the chain with subsequent additions taking place at a slower rate. The specific activity of xylosyltransferase in chick epiphyseal cartilage has been shown to be much greater than that of chick brain, liver, and intestine.[1005] The ability of the enzyme to transfer D-xylose from UDP-D-xylose to Smith-degraded bovine chondroitin sulphate proteoglycan was studied and utilized to differentiate the acceptor potentials of exogenous and endogenous molecules. Dibutyryladenosine 3′,5′-cyclic phosphate and theophylline affected the incorporation of [^{35}S]sulphate into various glycosaminoglycans of 3T3 and SV3T3 fibroblasts. A comparison of the rate of incorporation with the rate of loss of previously labelled glycosaminoglycans from the cells indicated that the apparent greater rate of biosynthesis effected by the additives is a true effect and is not due to a decrease

[1001] J. R. Baker, L. Rodén, and S. Yamagata, *Biochem. J.*, 1971, **125**, 93P.
[1002] J. R. Baker, L. Rodén, and A. C. Stoolmiller, *J. Biol. Chem.*, 1972, **247**, 3838.
[1003] P. L. Jeffrey and K. G. Rienits, *Austral. J. Biol. Sci.*, 1972, **25**, 115.
[1004] M E. Richmond, S. DeLuca, and J. E. Silbert, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 263.
[1005] A. C. Stoolmiller, A. L. Horwitz, and A. Dorfman, *J. Biol. Chem.*, 1972, **247**, 3525.

in the rate of degradation.[1006] SV40-Transformation of the fibroblasts resulted in a marked decrease in the rate of synthesis of glycosaminoglycans.

Miscellaneous Mammalian Bone, Cell, and Tissue Glycoproteins

The glycoproteins of various tissues of the vertebrate eye [777] and the subject of histocompatibility antigens [1007] have been reviewed. The polysaccharide and oligosaccharide moieties of blood-vessel glycoproteins have been reviewed and their possible functions discussed.[858]

The hexose, fucose, 2-amino-2-deoxyhexose, and *N*-acetylneuraminic acid contents of the glycoproteins from brain grey matter of cases of subacute sclerosing leukoencephalitis, infantile neurolipidosis, and galactosemia were shown to differ from those of normals.[646] The glycopeptides of brain grey matter have been investigated in cases of infantile amaurotic idiocy.[893] The occurrence of 2-acetamido-1-(4-L-aspartamido)-1,2-dideoxy-β-D-glucose has been demonstrated in the brain and liver as well as in the urine of patients with aspartylglycosaminuria.[1008] The same sources were also shown to contain a component which yielded 2-acetamido-1-(4-L-aspartamido)-1,2-dideoxy-β-D-glucose, 2-amino-2-deoxyhexose, uronic acid, and *N*-acetylneuraminic acid on hydrolysis.

Differences between pairs have been observed in the electrophoretic characteristics of glycoproteins from various tissues of human, bovine, and ovine twins.[1009] Methods for the isolation of histocompatibility antigens (major transplantation antigens) from human, porcine, mouse, and rat cells have been reported.[1010] The antigens were found to contain carbohydrate (10%), but it was thought that the carbohydrate moieties do not carry the specific immunological determinants, although their presence may be necessary for the expression of specificity. Atherosclerotic intima and media of human aorta have been shown to contain increased amounts of glycoproteins.[782]

Human mucosal biopsies have been found to contain galactose, mannose, fucose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, and sialic acid in bound form.[1011] Gel filtration of the soluble products from digestion of human gastric mucosa with pronase yielded a neutral glycopeptide fraction (molecular weight $> 3 \times 10^5$) containing galactose, fucose, 2-amino-2-deoxygalactose, and 2-amino-2-deoxyglucose (molar ratio 5 : 3 : 2 : 4).[1012] In contradiction to previous reports, no sulphate was found, but it was demonstrated that 75% of the threonine and serine

[1006] J. F. Goggins, G. S. Johnson, and I. Pastan, *J. Biol. Chem.*, 1972, **247**, 5759.
[1007] D. L. Mann and J. L. Fahey, *Ann. Rev. Microbiol.*, 1971, **35**, 679.
[1008] J. Palo and H. Savolainen, *J. Chromatog.*, 1972, **65**, 447.
[1009] S. R. Srinivasan, B. Radhakrishnamurthy, P. S. Pargaonkar, and G. S. Berenson, *Nature*, 1971, **229**, 58.
[1010] D. A. L. Davies, *Z. physiol. Chem.*, 1971, **352**, 27.
[1011] W. Barton, P. Brown, and J. R. Clamp, *Clinica Chim. Acta*, 1972, **36**, 262.
[1012] L. Hough and J. V. S. Jones, *Carbohydrate Res.*, 1972, **23**, 1.

residues of the polypeptide moiety are involved in *O*-glycosidic linkages to oligosaccharide chains of 2-acetamido-2-deoxygalactose residues of variable length. Structural studies indicated that all the monosaccharide residues are substituted at O-6 and, in most cases, at O-3 also.

Carcinoembryonic antigen from the human digestive system has been shown to contain galactose, mannose, fucose, 2-acetamido-2-deoxyglucose, and sialic acid (molar ratio 8 : 7 : 2 : 13 : 3), and this composition has been compared with that of human blood-group substances.[1013] Oligosaccharide fragments containing 2-acetamido-2-deoxyglucose retained, to a small extent, the immunological activity of the parent glycoprotein and it was considered that a heterosaccharide moiety containing a high proportion of 2-acetamido-2-deoxyglucose residues may feature to a large extent in the immunological site of the glycoprotein. The carcinoembryonic antigen was found to have a common antigenic determinant with a glycoprotein of smaller size extracted from normal adult tissues, suggesting that only a part of the antigen molecule carries onco-foetal specificity.[1014]

A glycoprotein (molecular weight 4.36×10^4) containing galactose (6.5), mannose (5.3), fucose (1.5), 2-amino-2-deoxyglucose (12.1), and sialic acid (10.0%) from human liver has been identified as α_1-acid glycoprotein.[1015] Fractionation of glycolipoprotein material with discrete allo-antigenic activity derived from human spleen cells yielded materials of high, intermediate, and low molecular weight, which were shown to contain carbohydrate (35, 16, and 29%, respectively) consisting of xylose, galactose, glucose, mannose, fucose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, and sialic acid.[1016] These monosaccharides have also been reported as components of purified HL-A substance from human spleen-cell membranes, and the involvement of carbohydrate in the immunochemical determinant area of the glycoprotein was investigated.[1017] Structural studies of the major glycoprotein in preparations exhibiting carcinoembryonic antigen activity from human colon have been reported.[1018] Saline-insoluble, non-collagenous glycoproteins of the human intervertebral disc have been shown to contain significant amounts of fucose and mannose;[783] similar carbohydrate contents have been demonstrated for non-collagenous glycoproteins of human and rat costal cartilage.[784]

Structural glycoproteins of macromolecular complexes of baboon (*Cyanocephalus papio-papio*) corneal stroma have been studied.[786] Glycoproteins isolated and purified from calf and sheep brains were shown to contain galactose (2.8), glucose (4.0), mannose (1.6), fucose (1.0), 2-amino-2-deoxygalactose (3.5), 2-amino-2-deoxyglucose (2.0), and sialic acid

[1013] C. Banjo, P. Gold, S. O. Freedman, and J. Krupey, *Nature New Biol.*, 1972, **238**, 183.
[1014] J. P. Mach and G. Pusztaszeri, *Immunochem.*, 1972, **9**, 1031.
[1015] L. C. Patrito and A. Martin, *Z. physiol. Chem.*, 1971, **352**, 89.
[1016] J. R. Clamp, K. W. Hughes, and J. C. McPherson, *Immunochemistry*, 1971, **8**, 874.
[1017] A. R. Sanderson, P. Cresswell, and K. I. Welsh, *Nature New Biol.*, 1971, **230**, 8.
[1018] W. D. Terry, P. A. Henkart, J. E. Coligan, and C. W. Todd, *J. Exp. Med.*, 1972, **136**, 200.

(2.4%); attention was drawn to the high glucose contents.[1019] Glycopeptides released from bovine brain by digestion with pronase were shown to contain *O*-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose (*N*-acetyl-lactosamine) and *O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose.[1020] A further glycopeptide fraction was found to contain uronic acid. The sialoglycoproteinaceous contents of calf brain have been related to the activity of endogenous sialidase.[1021] A series of glycoproteins isolated from bovine retina were found to contain hexose, fucose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and sialic acid.[914] From structural studies, it was concluded that sialic acid and L-fucose residues occupy terminal positions and D-galactose residues sub-terminal ones.[915]

A disease of Angus cattle, previously known as pseudolipidosis, has been shown to be an inherited lysosomal storage disease in which a glycopeptide material containing mannose and 2-amino-2-deoxyglucose is accumulated.[1022] Valvular glycoprotein fractions from bovine hearts have been investigated for their immunological specificity.[1023, 1024] Fractionation of the soluble glycoprotein components of *S*-carboxymethylated bovine renal glomerular basement membrane gave a number of fractions, the heteropolysaccharide moieties consisting of galactose, mannose, fucose, 2-amino-2-deoxyhexose, and sialic acid, which occurred at the rate of 1—4 moieties per 1000 amino-acid residues.[791] A soluble glycolipoprotein extracted from bovine liver mitochondria has been purified to homogeneity on polyacrylamide gel electrophoresis.[1025] The glycolipoprotein (protein molecular weight 4.2×10^4) was found to contain 5% carbohydrate consisting of xylose, glucose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and sialic acid; it was shown to possess two types of calcium-binding sites. Two glycoprotein fractions have been isolated from bovine Achilles tendon by extraction with solutions of sodium and magnesium chlorides; although both fractions gave several bands on isoelectric focusing, the heterogeneity disappeared on interaction with tropocollagen.[792, 793]

Purified preparations of the glycoprotein component of resting and transforming–ossifying regions of calf scapula cartilage have been analysed and the glycoprotein from the latter region exhibited calcium-binding properties.[931] Further characterization of this glycoprotein (molecular

[1019] A. van Nieuwamerongen, D. H. van den Eijnden, J. Heijlman, and P. A. Roukema, *J. Neurochem.*, 1972, **19**, 2195.
[1020] R. L. Katzman, *J. Biol. Chem.*, 1972, **247**, 3744.
[1021] J. Heijlman and P. A. Roukema, *J. Neurochem.*, 1972, **19**, 2567.
[1022] J. D. Hocking, R. D. Jolly, and R. D. Batt, *Biochem. J.*, 1972, **128**, 69.
[1023] E. Kasp-Grochowska, D. Kingston, and L. E. Glynn, *Ann. Rheumatic Diseases*, 1972, **31**, 282.
[1024] E. Kasp-Grochowska, D. Kingston, and L. E. Glynn, *Ann. Rheumatic Diseases*, 1972, **31**, 290.
[1025] G. Sottocasa, G. Sandri, E. Panfili, B. de Bernard, P. Gazzotti, F. D. Vasington, and E. Carafoli, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 808.

weight 2×10^5) showed that it possesses two classes of calcium-binding sites (having different dissociation constants) and that maximum binding occurs at pH 8.3.[932] It was suggested that the glycoprotein acts as a linking agent for proteoglycans. A glycoprotein (molecular weight 4.5×10^4) containing neutral sugars (2), 2-amino-2-deoxyhexose (3), and uronic acid (1%) has been found to be associated with the glycosaminoglycan structure of bovine nasal cartilage.[924] Minor components of the glycoprotein preparation were detected by polyacrylamide gel electrophoresis and were considered to arise from the major component by rearrangements involving disulphide bonds.

Purified ovine submaxillary glycoprotein (molecular weight 8×10^5) was found to contain 50% carbohydrate consisting of *ca.* 800 α-*N*-acetylneuraminosyl-(2 → 6)-2-acetamido-2-deoxygalactose units linked glycosidically to the serine and threonine residues of the polypeptide chain(s).[1026] It was established that the carbohydrate moieties contribute to the antigenic determinants of the molecule and that the precipitin reaction was inhibited by glycopeptides derived from it by the action of pronase. Digestion with neuraminidase and 2-acetamidodeoxygalactosidase removed all the carbohydrate, leaving the protein moiety intact.

Extracts of porcine and rat brain tissues containing glycosaminoglycans were also found to contain galactose, glucose, arabinose, and fucose due to the presence of glycoproteins.[933]

A glycoprotein from porcine brain microtubules was shown to contain galactose (1.28), mannose (1.05), fucose (0.61), 2-acetamido-2-deoxygalactose (1.21), 2-acetamido-2-deoxyglucose (1.66), and sialic acid (0.93 moles per mole of dimer); treatment with alkaline borohydride indicated the involvement of 40% of the 2-acetamido-2-deoxygalactose residues in *O*-glycosidic linkages to serine and/or threonine residues.[1027] The stability to alkali of a portion of the linkages between carbohydrate and protein indicated the presence of two types of oligosaccharide moieties, which were calculated to consist of seven monosaccharide residues per mole of glycoprotein dimer (molecular weight 1.1×10^5).

A glycopeptide (molecular weight 3200) has been purified to homogeneity from the posterior lobes of porcine pituitaries and was shown to contain 55% carbohydrate consisting of galactose (1), mannose (3), fucose (1), and 2-amino-2-deoxyglucose (4 units per mole).[1028] The carbohydrate was found to be linked to a seventeen-unit amino-acid chain through a single asparagine residue.[1029] The glycopeptide exhibited none of the known physiological activities of the hypophysis. Sialoglycoproteins of the subcellular membranes and cortisole of porcine submaxillary gland have been studied and the bound acylneuraminic acids consisted of

[1026] A. Gottschalk, H. Schauer, and G. Uhlenbruck, *Z. physiol. Chem.*, 1971, **352**, 117.
[1027] R. K. Margolis, R. U. Margolis, and M. L. Shelanski, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 432.
[1028] D. A. Holwerda, *European J. Biochem.*, 1972, **28**, 334.
[1029] D. A. Holwerda, *European J. Biochem.*, 1972, **28**, 340.

N-acetylneuraminic acid (10%) and *N*-glycollylneuraminic acid (90%).[1030] From the biosynthetic viewpoint, it was concluded that the *N*-acylneuraminic acid units are transferred to the growing glycoprotein chains during the time that they are attached to subcellular membranes and that the complete glycoprotein is subsequently released from the membranes and stored in the cytosole.

A procedure has been devised for the isolation of glycoprotein from the intimal region of porcine aorta; a 4.86S glycoprotein so isolated was found to be homogenous by several criteria.[1031] The glycoprotein (molecular weight 7.1×10^4) was shown to contain galactose (3), glucose (3), mannose (2), fucose (1), 2-acetamido-2-deoxyhexose (4), and sialic acid (1 unit(s) per mole), and attention was drawn to the equimolar contents of galactose and glucose. The occurrence of sulphated glycoproteins in porcine gastric mucosal membrane has been investigated[881] and optimum conditions have been reported for the incorporation of radioactive precursors into porcine gastric mucosal scrapings *in vitro*.[1032] Of the uniformly labelled D-[^{14}C]glucose incorporated, 22% was found to be water-soluble, whereas the remainder was soluble in other solvents. Pronase was the most successful proteolytic enzyme for solubilization of the latter fraction and the glycopeptides so obtained contained hexose, fucose, and 2-amino-2-deoxyhexose. Sulphation of a glycopeptide derived from porcine duodenal mucosa yielded a sulphated glycopeptide of high molecular weight exhibiting antipeptic activity.[1033, 1034] A glycoprotein fraction was identified in the fractionation of proteoglycans from porcine costal cartilage on QAE-Sephadex.[937]

Purified pepsinogen from canine fundic mucosa was found to be physicochemically homogeneous.[1035] The pre-enzyme molecule (molecular weight 4×10^4) was found to contain neutral carbohydrate (4), 2-amino-2-deoxyhexose (8 moles per mole), and one phosphate residue. The biosynthesis of cell-coat glycoprotein of canine and ferret lung, kidney, and macrophage cells has been investigated.[1036]

Treatment of glycopeptides from rabbit and rat brain with alkaline borohydride indicated that the terminal, reducing 2-acetamido-2-deoxy-D-galactose residues are linked *O*-glycosidically to the hydroxy-groups of L-serine and L-threonine residues.[912] Partial acid hydrolysis of the alkali-resistant glycopeptides yielded 2-acetamido-1-(β-L-aspartamido)-1,2-dideoxy-β-D-glucose. L-[^{3}H]Fucose has been incorporated into the glycoproteins of rabbit retinal ganglion cells, and the rapid transport of the label along the optic pathway to the nerve terminals of the lateral geniculate

[1030] R. Schauer and M. Wember, *Z. physiol. Chem.*, 1970, **351**, 1353.
[1031] P. V. Wagh and B. I. Roberts, *Biochemistry*, 1972, **11**, 4222.
[1032] D. Snary and A. Allen, *Biochem. J.*, 1972, **127**, 577.
[1033] A. Lietti, R. Niada, and G. Prino, *Boll. Soc. ital. Biol. sper.*, 1971, **47**, 493.
[1034] G. Prino, A. Lietti, and S. Paglialunga, *Arzneim.-Forsch.*, 1971, **21**, 918.
[1035] J. P. Marciniszyn and B. Kassell, *J. Biol. Chem.*, 1971, **246**, 6560.
[1036] G. Poste and L. W. Greenham, *Cytobios*, 1971, **3**, 5.

body *etc.* was investigated.[1037] The glycoproteins appeared to be largely associated with membranes, and molecular weights of 4×10^4 were estimated for their subunits from electrophoresis in the presence of sodium dodecylsulphate. Sialoglycoproteins of normal rabbit tissues and of tissues at early stages of myocardial infarction have been studied.[1038] Glycoproteins containing hexose, fucose, 2-amino-2-deoxyhexose, and sialic acid were isolated in addition to glycosaminoglycans from rabbit skin.[946] One of the glycoproteins was found to contain up to 70% glucose. An ethanol-precipitable material obtained from rabbit skin after removal of glycosaminoglycans gave, on ion-exchange chromatography and gel filtration, four glycoprotein fractions each containing galactose, mannose, glucose, fucose, 2-amino-2-deoxyglucose, and sialic acid.[798] Two glycoproteins containing the same carbohydrates, with the exception of glucose, were obtained from non-precipitable material in extracts from the same tissue. Glycocalyx-bound glycoprotein of isolated brush borders of rabbit small intestine has been reported to contain glucose and 2-amino-2-deoxygalactose; its biosynthesis has been studied.[1039] An investigation of the biosynthesis of the sialic acid of rabbit bone marrow revealed that the neuraminic acid units of the glycoproteins present can be labelled using 2-amino-2-deoxy-D-[1-^{14}C]glucose as precursor.[1040]

The apparent molecular weights of the major glycoproteins from plasma membranes and endoplasmic reticulum of hamster kidney fibroblasts, and the extent of incorporation of 2-amino-2-deoxy-D-[^{14}C]glucose and L-[^{3}H]-fucose into these glycoproteins during biosynthesis, have been assessed by polyacrylamide gel electrophoresis.[1041] Gel-filtration patterns of L-[^{3}H or ^{14}C]fucose-labelled carbohydrates derived from the surface glycoproteins of virus-transformed hamster cells may apparently be altered to resemble those derived from normal cells by treatment with neuraminidase.[1042] The results indicated that consistent differences between control and transformed cells may depend upon a sialyltransferase, which is found in greater amounts in the latter and in dividing cells than in non-dividing cells.

Using the incorporation of L-[^{3}H]fucose as an indicator, it was found that the biosynthesis of glycoproteins in scrapie-affected mouse brain is increased above normal.[1043] The relationship of microsomal glucosaminyltransferases to the biosynthesis of glycoproteins in the liver and spleen of mice infected with myxovirus has been studied.[1044] An alkali-soluble fraction of mouse liver plasma membranes contained only one major glycoprotein that possessed comparatively low amounts of 2-amino-2-

[1037] J.-O. Karlsson and J. Sjöstrand, *J. Neurochem.*, 1971, **18**, 2209.
[1038] Z. G. Orlova and E. I. Shnyak, *Ukrain. biokhim. Zhur.*, 1971, **43**, 197.
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[1042] L. Warren, J. P. Fuhrer, and C. A. Buck, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 1838.
[1043] A. J. Suckling, G. C. Millson, and G. D. Hunter, *Biochem. J.*, 1972, **128**, 128P.
[1044] A. Defrene and P. Louisot, *Ann. Inst. Pasteur*, 1972, **122**, 529.

deoxyhexose and sialic acid.[1045] Extraction of the alkali-insoluble residue with aqueous pyridine resulted in the selective extraction of glycoproteins that were richer in the carbohydrate components. An inverse relationship was found between the molecular weight and the sialic acid content of the fractions obtained by gel filtration of the pyridine-soluble glycoproteins in the presence of sodium dodecylsulphate.

In an examination of the release of macromolecules from BALB/c mouse cell lines, it was found that, on treatment with edta or ethylenebis(oxyethylenenitrilo)tetra-acetic acid, 3T3- and SV40-transformed cells slowly liberated glycoproteins.[1046] Three classes of glycoproteins were associated with the cells. The biosynthesis of glycoproteins by mitochondria of a contact-inhibited mouse 3T3 fibroblast has been studied by incorporation experiments with UDP-D-[^{14}C]glucose and GDP-D-[^{14}C]mannose; decreased biosynthesis was found in oncogenic virus SV40-transformed derivatives of the cells.[1047] Although the carbohydrate contents of glycoproteins and glycopeptides of the two cell lines, as examined by long-term incorporation of radioactive 2-amino-2-deoxy-D-glucose, were demonstrably different, the glycoproteins and glycopeptides of membrane fractions were indistinguishable by gel filtration and polyacrylamide gel electrophoresis.[1048] Several transformation mechanisms were suggested for the biosynthetic differences.

Membrane-located murine histocompatibility-2 allo-antigens, labelled with radioactive monosaccharides during cell culture, have been solubilized and purified from three types of tumours maintained in mice.[1049] An antibody–antigen binding reaction was utilized as the final purification step. It was established that galactose, mannose, fucose, 2-amino-2-deoxyglucose, and sialic acid are components of the glycoproteins of all histocompatibility strains examined, and close similarities were demonstrated between the glycopeptides (molecular weights 3300) derived from them by digestion with pronase. Digestion of the murine histocompatibility-2 alloantigen glycoproteins from cultured mouse mast-cell tumour cells with papain gave a major glycopeptide (molecular weight 3300) containing fucose, 2-amino-2-deoxyglucose, *N*-acetylneuraminic acid, and *N*-glycollylneuraminic acid.[1050] Strain A mouse ascites tumour cells, on treatment with neuraminidase, showed decreased tumour-forming ability, but were able to regenerate sialoglycoproteins at the cell surface during culture.[1051] The incorporation of 2-amino-2-deoxy-D-[^{3}H]glucose into the membrane glycoproteins of treated and untreated cells proceeded at similar rates; it

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[1046] L. A. Culp and P. H. Black, *Biochemistry*, 1972, **11**, 2161.
[1047] M. W. Myers and H. B. Bosmann, *F.E.B.S. Letters*, 1972, **26**, 294.
[1048] H. Sakiyama and B. W. Burge, *Biochemistry*, 1972, **11**, 1366.
[1049] T. Muramatsu and S. G. Nathenson, *Biochemistry*, 1970, **9**, 4875.
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[1051] R. C. Hughes, B. Sanford, and R. W. Jeanloz, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 942.

was concluded that surface sialoglycoproteins are synthesized *de novo* during culture of treated cells and that, in non-dividing cells, synthesis is accompanied by turnover of the membrane glycoproteins.

The soluble glycoproteins of rat brain have been shown to be heterogeneous, four species being identified by column electrophoresis.[1052] Each of the species contained hexose, fucose, 2-amino-2-deoxyhexose, and *N*-acetylneuraminic acid, but they contained less fucose and exhibited greater electrophoretic mobilities than the membrane-bound glycoproteins. One of two glycoproteins isolated from rat liver was found to correspond with one of the four brain species. The major component of the glycoproteins isolated from myelin fractions of rat brain has been identified as a glycoprotein (molecular weight 1.1×10^5) by incorporation studies with L-[^{3}H and ^{14}C]fucose,[1053] and the incorporation of L-[^{3}H]fucose into rat brain glycoproteins has also been studied in conjunction with the α-fucosidase activity of the tissue.[1054] From a study of the biosynthesis of the glycoproteins of rat brain at a subcellular level, it was concluded that D-galactosyltransferase and D-mannosyltransferase activities are located in the synaptic vesicles, whereas 2-acetamido-2-deoxy-D-glucosyltransferase activity is located in all neurone membranes.[1055, 1056] Sulphated glycoproteins of rat brain labelled *in vivo* with [^{35}S]sulphate underwent biphasic disappearance ($t_{\frac{1}{2}}$ = 2.5 and 14 days), suggesting that they represent pools of sulphated glycoproteins having different metabolic activities.[955]

Glycopeptides prepared by digestion of rat brain microsomal fractions with pronase have been fractionated by affinity chromatography on cross-linked concanavalin A; their competition against concanavalin A in stimulating neurite growth was investigated.[1057] Rat brain microsomal UDP-2-acetamido-2-deoxy-D-galactose:protein 2-acetamido-2-deoxy-D-galactosyltransferase has been characterized and its ability to catalyse the transfer of 2-acetamido-2-deoxy-D-galactose from its nucleotide derivative to endogenous and exogenous acceptors has been studied.[1058] The occurrence of common glycoproteins in the synaptic vesicles and the synaptosomal plasma membranes from rat brain has been investigated by polyacrylamide gel electrophoresis of soluble extracts.[1059] The effects of testosterone on glycoprotein biosynthesis in rat prostate, salivary glands, and seminal vesicles have been reported.[957] The effect of oestrogens upon the biosynthesis of glycoproteins of rat salivary glands and uterus was to decrease their rate of formation.[958]

[1052] C. DiBenedetta, I. Chang, and E. G. Brunngraber, *Ital. J. Biochem.*, 1971, **20**, 49.
[1053] R. H. Quarles, J. L. Everly, and R. O. Brady, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 491.
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[1059] W. C. Breckenridge and I. G. Morgan, *F.E.B.S. Letters*, 1972, **22**, 253.

Rat parathyroid glands maintained in organ culture incorporated radioactive 2-amino-2-deoxy-D-glucose into macromolecules, which were secreted into the medium.[1060] The secreted material was resolved into three fractions by gel filtration and the radioactive content of two of them was inversely related to the concentration of Ca^{2+} ion in the culture medium. The smallest, labelled glycoprotein migrated close to rat parathyroid hormone, but was separable from it.

The stimulatory effect of cytidine 5′-diphosphocholine on 2-acetamido-2-deoxyglucosyltransferase activity was marked in rough microsomes, but was absent in Golgi-rich membranes and sera of rats.[1061] Glycoproteins released from rat liver, kidney brush border, and erythrocyte ghost membranes on treatment with sodium dodecylsulphate, alkali, and reducing agents have been examined by polyacrylamide gel electrophoresis.[1062] Although each membrane has its own glycoprotein subunit pattern, liver and kidney gave rise to a number of subunits, four of which from each tissue corresponded (molecular weights 2.50×10^5, 1.95×10^5, 1.30×10^5, and 0.96×10^5). All the subunits examined were found to contain sialic acid. The effects of cyclophosphamide on the carbohydrate contents of glycoproteins of rat glomerular basement membrane have been reported.[1063] A nephrotoxic glycoprotein isolated from rat glomerular basement membrane was found to resemble a basement membrane glycoprotein antigen from the urine of nephritic rabbits.[1064]

The kinetics of isotope incorporation into rat kidney *in vivo* have been studied.[1065] From a characterization of a number of subcellular fractions, it was concluded that mannose and 2-acetamido-2-deoxyglucose are incorporated into lysosomal glycoprotein in the rough endoplasmic reticulum, whereas *N*-acetylneuraminic acid is incorporated into the smooth endoplasmic reticulum and/or the Golgi apparatus. Newly synthesized glycoproteins are then rapidly transferred to lysosomal dense bodies.

A chromatographic procedure has been described for the separation of serum albumin-type molecules from extracts of rat liver labelled with L-[1-^{14}C]leucine and, on the basis of this, a technique for the assay of the glycoprotein in liver was established.[1066] Two macromolecular fractions isolated from rat hepatic membranes were shown to contain sialic acid and neutral sugars.[1067] The incorporation of glycosyl groups into endogenous acceptors has been studied in a Golgi apparatus-rich fraction of rat liver;[962] the differential incorporation of D-[1-^{14}C]galactose and D-[2-^{14}C]mannose

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[1061] S. Mookerjea, *Canad. J. Biochem.*, 1972, **50**, 1082.
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[1066] J. D. Judah and M. R. Nicholls, *Biochem. J.*, 1971, **123**, 643.
[1067] F. R. Simon, O. O. Blumenfeld, and I. M. Arias, *Biochim. Biophys. Acta*, 1970, **219**, 349.

suggested that the D-mannose pathway in the biosynthesis differs from that of other sugars.[1068] From studies of the incorporation of 2-amino-2-deoxy-D-[^{3}H]glucose into the Golgi complex, it was concluded that newly synthesized glycoprotein moves from the Golgi membrane to the soluble phase and thereafter to the serum.[1069] Cytidine 5′-diphosphocholine has been shown to play a role in the transfer of D-galactose[1070] and 2-acetamido-2-deoxy-D-glucose[1071] from the corresponding nucleotide derivatives to rat liver microsomal glycoprotein. A rat liver mitochondrial glycoprotein has been located in the inter-membrane space and a technique was described for its isolation and purification to homogeneity.[1072] The effects of L-canavanine on the biosynthesis of glycoproteins in rat liver and intraneural mitochondria have been reported.[1073]

Extracts of rat intestine containing glycosaminoglycans were also found to contain arabinose, galactose, glucose, mannose, xylose, and fucose due to the presence of glycoproteins.[964] A 14S glycoprotein has been obtained in physicochemically homogeneous form (molecular weight 5.6×10^5) from a soluble extract of trypsin-digested rat small intestinal mucosa.[1074] The glycoprotein was shown to contain galactose plus mannose (7.8), fucose (2.6), 2-amino-2-deoxygalactose plus 2-amino-2-deoxyglucose (7.7), and sialic acid (3.3%), and could be labelled *in vivo* using D-[1-^{14}C]galactose, D-[1-^{14}C]mannose, or 2-amino-2-deoxy-D-[1-^{14}C]glucose. The biosynthesis of the acid-precipitable glycoprotein in rat intestinal slices has been investigated by means of the incorporation of 2-amino-2-deoxy-D-[1-^{14}C]-glucose.[1075] Cycloheximide and sodium salicylate were shown to have an effect on incorporation of the sugar.[1076] The decreased incorporation caused by sodium salicylate appeared to be specific for the biosynthesis of the oligosaccharide moiety, since inhibition primarily affected acetylation of 2-amino-2-deoxy-D-glucose. A fucosyltransferase which transferred L-fucose from GDP-L-fucose to oligosaccharide or glycoprotein acceptors has been isolated from rat small intestinal mucosa and the enzyme was found to attach L-fucose by an α-(1 $\rightarrow$ 2)-linkage to terminal β-D-galactosyl residues.[768]

The biosynthesis of glycoproteins in neuroendocrine tissue has been studied by incubating rat pineal glands with L-[^{3}H]fucose; essentially all of the labelled trichloroacetic-acid-insoluble material consisted of glycoproteins (molecular weights 3—20 $\times$ 10^4 in sodium dodecylsulphate),

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[1069] J. M. Sturgess, M. Mitranic, and M. A. Moscarello, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1270.
[1070] S. Mookerjea, D. E. C. Cole, and A. Chow, *F.E.B.S. Letters*, 1972, **23**, 257.
[1071] S. Mookerjea, D. E. C. Cole, A. Chow, and P. Letts, *Canad. J. Biochem.*, 1972, **50**, 1094.
[1072] G. L. Sottocasa, G. Sandri, E. Panfili, and B. de Bernard, *F.E.B.S. Letters*, 1971, **17**, 100.
[1073] R. A. Winston and H. B. Bosmann, *Chem. Biol. Interactions*, 1971, **3**, 131.
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[1075] B. E. Lukie and G. G. Forstner, *Biochim. Biophys. Acta*, 1972, **261**, 353.
[1076] B. E. Lukie and G. G. Forstner, *Biochim. Biophys. Acta*, 1972, **273**, 380.

polyacrylamide gel electrophoresis of which showed a heterogeneous pattern.[1077] The labelled glycoproteins secreted into the medium were shown to possess somewhat smaller molecular weights ($3—7 \times 10^4$) and biosynthetic differences were recognized between the two types of glycoproteins. Glycopeptides prepared by digestion of membranous glycoproteins of microsomes of a rat ascites hepatoma with pronase have been separated into neutral and acidic fractions.[1078] The former fraction was nearly homogeneous and its composition was similar to that of the corresponding glycopeptides from rat liver microsomes, the only carbohydrate present being mannose and 2-amino-2-deoxyglucose. The acidic fraction was heterogeneous on ion-exchange chromatography and each peak contained galactose, mannose, fucose, 2-amino-2-deoxyglucose, and sialic acid in amounts different from those of the corresponding glycopeptides from rat liver microsomes. A glycoprotein containing 2-amino-2-deoxyglucose has been separated from a glycosaminoglycan of rat incisor pulp.[966] Intermediates in the biosynthesis of rat epiphysial cartilage glycoproteins have been investigated.[845]

The elution profiles on gel filtration of glycopeptides derived from various normal cells by tryptic and pronasic digestion differed from those obtained for murine sarcoma, Rous sarcoma, and polyoma virus-transformations of the cells.[1079] Examination of the major glycoprotein obtained from HeLa cell plasma membrane ghosts by polyacrylamide gel electrophoresis in sodium dodecylsulphate showed it to possess a molecular weight of $4.0—4.5 \times 10^4$.[1080] The metabolism of the membranes during the cell cycle was studied following incorporation of L-[^{3}H]fucose.[1081] A glycoprotein fraction obtained from the surface of TA3 mammary adenocarcinoma ascites cells by short-term incubation with trypsin contained galactose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, and sialic acid.[1082] The two components of the fraction were shown to possess molecular weights within the range $0.9—3.0 \times 10^5$.

Hormonal Glycoproteins

The proceedings of the Second International Symposium on Protein and Polypeptide Hormones have been published and included sections on the isolation, purification, chemical aspects, and immunological aspects of the hormonal glycoproteins.[1083, 1084]

[1077] I. T. Lott, R. H. Quarles, and D. C. Klein, *Biochim. Biophys. Acta*, 1972, **264**, 144.
[1078] I. Funakoshi and I. Yamashina, *J. Biochem. (Japan)*, 1972, **72**, 2459.
[1079] C. A. Buck, M. C. Glick, and L. Warren, *Science*, 1971, **172**, 169.
[1080] P. H. Atkinson and D. F. Summers, *J. Biol. Chem.*, 1971, **246**, 5162.
[1081] M. Nowakowski, P. H. Atkinson, and D. F. Summers, *Biochim. Biophys. Acta*, 1972, **266**, 154.
[1082] J. F. Codington, B. H. Sanford, and R. W. Jeanloz, *Biochemistry*, 1972, **11**, 2559.
[1083] 'Structure–Activity Relationships of Protein and Polypeptide Hormones', Part 1, ed. M. Margoulies and F. C. Greenwood, International Congress Series, vol. 241, Excerpta Medica, Amsterdam, 1971.
[1084] 'Structure–Activity Relationships of Protein and Polypeptide Hormones', Part 2, ed. M. Margoulies and F. C. Greenwood, International Congress Series, vol. 241, Excerpta Medica, Amsterdam, 1972.

The extraction, chemistry, and immunoassay of pituitary, plasma, and urinary gonadotrophins have been reviewed.[1085] From a review of the relationship of the primary, secondary, and tertiary structures of gonadotrophins and thyroid-stimulating hormone to their biological and immunological activities, it was concluded that the β-subunits of the hormones are hormone-specific, and that the protein, but not the carbohydrate, moieties of the α-subunits are common.[1086] Recent studies of the dissociation of glycoprotein hormones and their subunits have been reviewed.[1087]

Methods and problems in determining the carbohydrate compositions and the structures of glycoprotein hormones have been discussed.[1088]

Polyacrylamide gel isoelectric focusing studies of human follicle-stimulating and luteinizing hormones and of human chorionic gonadotrophin have shown that highly purified preparations previously reported may be heterogeneous.[1089] An improved method for the purification of human pituitary follicle-stimulating hormone has been reported, the production of highly active material depending upon the sequence of the ion-exchange and gel-filtration steps.[1090] The most potent material (8765 i.u. mg^{-1}) was stable to freeze drying. The Stokes' radius of the iodinated hormone was estimated to be 32.6 Å by gel filtration and a minor component (Stokes' radius 23 Å) was recognized in the penultimate stage of the purification process. The freeze-dried material was suitable as a standard for use in the radioimmunoassay of follicle-stimulating hormone. A number of preparations of human follicle-stimulating hormone have been examined by polyacrylamide gel electrophoresis and the molecular size of the urinary hormone was found to remain constant throughout life, although varying according to the pH at which it was measured ($2.3—3.1 \times 10^4$ at pH 10.2).[1091] A molecular weight of 5.6×10^4 was estimated for human pituitary hormone.

Human follicle-stimulating and luteinizing hormones and human chorionic gonadotrophin bind strongly to insolubilized concanavalin A, displacement being achieved with methyl α-D-glucopyranoside.[731] Subunits of the chorionic gonadotrophin behaved similarly to the intact hormones, but asialo- and asialo-agalacto-chorionic gonadotrophin were less easily dissociated from the matrix. These findings have been applied to the isolation and purification of chorionic gonadotrophin from human pregnancy plasma and urine.

Methylation analysis of human pituitary follicle-stimulating hormone has demonstrated that the D-galactose, mannose, fucose, and 2-amino-2-deoxyglucose units exist in the pyranose forms. The 2-amino-2-deoxy-

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[1090] W. R. Butt, S. S. Lynch, and J. F. Kennedy, in ref. 1084, p. 355.
[1091] R. D. Maffezzoli, G. N. Kaplan, and A. Chrambach, *J. Clin. Endocrinol. Metabol.*, 1972, **34**, 375.

glucose units were found to be *N*-acetylated and the fucopyranose units to occupy terminal, non-reducing positions.[1092] Whereas the D-galactopyranose units are linked in the 1- and 2-positions, the 2-acetamido-2-deoxyglucopyranose units are 1,6-substituted and the mannopyranose units were shown to exist in three forms — as terminal, non-reducing residues, as 1,6-substituted residues, and as 1,3,4-linked branch points. The structural assignments were compared with other data previously obtained for the carbohydrate moieties of follicle-stimulating hormone. The structural integrities of human follicle-stimulating and luteinizing hormones and of human chorionic gonadotrophin have been examined after enzymic iodination.[1093]

Fragmentation of human pituitary follicle-stimulating hormone with aqueous sodium dodecylsulphate yielded two types of fragments differing in their molecular weights.[1094] The larger of these exhibited biological activity *in vivo*, whereas the smaller exhibited biological activity *in vitro*. The two species were immunologically active, and evidence for recombination on removal of the sodium dodecylsulphate was presented. The biological (*in vivo* and *in vitro*) and immunological activities of human pituitary follicle-stimulating hormone on modification with a number of reagents have been determined.[1095]

The importance of the sialic acid residues of human follicle-stimulating hormone and human chorionic gonadotrophin has been demonstrated by the finding that the desialylized molecules are rapidly removed from the circulation of rats and may be recovered from the liver.[1096] Human plasma and urinary levels of follicle-stimulating hormone have been measured in cases of oligospermia.[1097]

Immunological studies of ovine and porcine follicle-stimulating hormones permitted the identification of the human chorionic gonadotrophin-augmenting principle.[1098] The follicle-stimulating hormone and luteinizing hormone contents of the pituitaries of sheep have been investigated.[1099] A highly purified preparation of ovine follicle-stimulating hormone (157 units NIH-FSH-S1 mg^{-1}) was shown to contain hexose (8.9), fucose (1.2), 2-amino-2-deoxygalactose (1.1), 2-amino-2-deoxyglucose (7.1), and sialic acid (6.0%); the high activity was attributed to the relatively high sialic acid content.[1100] Follicle-stimulating, luteinizing, and thyroid-stimulating

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[1094] J. F. Kennedy, W. R. Butt, V. Robinson, and M. Ryle, in ref. 1084, p. 348.
[1095] J. F. Kennedy, S. Ramanvongse, W. R. Butt, W. Robinson, M. Ryle, and A. Shirely, in ref. 1084, p. 351.
[1096] A. G. Morell, G. Gregoriadis, I. H. Scheinberg, J. Hickman, and G. Ashwell, *J. Biol. Chem.*, 1971, **246**, 1461.
[1097] J. M. Leonard, R. B. Leach, M. Couture, and C. A. Paulsen, *J. Clin. Endocrinol. Metabol.*, 1972, **34**, 209.
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[1099] R. B. Land, D. B. Crighton, and G. E. Lamming, *J. Reprod. Fert.*, 1972, **30**, 313.
[1100] H. J. Grimek, L. C. Nuti, and W. H. McShan, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 879.

hormones have been partially purified from canine pituitaries.[1101] Changes in the levels of pituitary follicle-stimulating and luteinizing hormones and luteinizing hormone from plasma of rats have been observed during treatment with pregnant mare's serum.[1102]

The immunologically active components of human luteinizing hormone and chorionic gonadotrophin from normals and cases of choriocarcinoma have been fractionated by isoelectric focusing.[1103] Differences in the p*I*'s of the two hormones were explored with a view to establishing analytical techniques for them in the presence of one another. Estimates of the molecular weights of the two hormones from normal men and pre- and post-menopausal women and cases of choriocarcinoma have been reported.[1104] Three species of luteinizing hormone (2.34, 2.24, and 2.70S, having 9, 8, and 4 units NIH-LH-S1 mg^{-1}, respectively) have been isolated from human pituitaries by isoelectric focusing and were shown to contain neutral carbohydrate (13.3, 13.2, and 6.9%) and sialic acid (0.5, 1.3, and 1.8%, respectively).[1105] Minor differences in the physicochemical properties of the three forms were attributed to differences in their carbohydrate moieties. Incubation of the 2.34S form yielded the α- and β-subunits, which were separable by ion-exchange chromatography, but which could be recombined to regenerate biological activity. It was found that the α-subunit of the hormone and those of human follicle-stimulating hormone and chorionic gonadotrophin could be substituted for each other in combination with the β-subunit of each hormone, suggesting that the β-subunits are hormone-specific and that the α-subunits may be homologous.

The purification of equine luteinizing hormone has been reported and the hormone was shown to contain hexose (8.3), fucose (0.6), 2-amino-2-deoxygalactose (3.1), 2-amino-2-deoxyglucose (4.5), and sialic acid (7.9%).[1106] Hybrid luteinizing hormones have been prepared by combination of the β-subunit of bovine luteinizing hormone with the α-subunits of either bovine thyroid-stimulating hormone or human chorionic gonadotrophin and by combination of the β-subunit of human chorionic gonadotrophin with the α-subunits of either bovine luteinizing hormone or bovine thyroid-stimulating hormone.[1107] The biological potencies of the hybrid molecules were measured. Both α- and β-subunits from bovine and porcine luteinizing hormones have been separated by gel filtration in the presence of guanidine hydrochloride, one symmetrical peak being attributable to each

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[1103] R. D. Meffezzoli, G. N. Kaplan, and A. Chrambach, *J. Clin. Endocrinol. Metabol.*, 1972, **34**, 361.
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[1107] A. F. Parlow, K. Kovacic, and R. C. Bhalla, *J. Clin. Endocrinol. Metabol.*, 1972, **35**, 319.

subunit.[1108] Reduction of the bovine subunits did not alter the chromatographic pattern, but the porcine α- and β-subunits gave two and three peaks, respectively, on reduction, suggesting that these subunits possess multiple chains.

An insolubilized form of anti-luteinizing hormone immunoglobulin IgG has been successfully employed for a one-step isolation of luteinizing hormone from ovine pituitary extracts.[1109] The immunoadsorbent has also been applied to the specific removal of luteinizing hormone from sera; after recovery, the hormone exhibited identical properties to preparations obtained by other methods. Investigations of the primary structure of ovine luteinizing hormone have revealed that carbohydrate moieties are attached to asparagines 56 and 82 of the α-subunit protein chain [1110] and to asparagine 12 of the β-subunit chain.[1111] Luteinizing hormone insolubilized by attachment to Sepharose has been used to investigate the interaction of the hormone with cell surfaces.[1112]

The intrinsic viscosity, extinction coefficient, p*I*, acid–base titration curve, and c.d. and u.v. spectra of human chorionic gonadotrophin and its asialo-derivative have been studied and it was concluded that the two molecules have similar gross conformations and are nearly spherical.[1113] The effects of removing the terminal sialic acid and subterminal galactose residues of human chorionic gonadotrophin have confirmed the importance of the terminal carbohydrate residues in metabolic disposal of the hormone *in vivo* and have emphasized their lack of involvement in binding to tissue receptor sites.[1114] From the results of carbohydrate analyses of crude human urinary extracts, it was concluded that sialic acid has no role in the chorionic gonadotrophin inhibitor molecule.[1115] Human chorionic gonadotrophin synthesized by choriocarcinoma cells in culture has been separated into two fractions (p*I*'s 3.0 and 4.5) by isoelectric focusing.[1116] The ratios of the biological and immunological activities of the fractions were different. The lactogenic action of human chorionic gonadotrophin in rats [1117] and the uptake of the labelled hormone by rat brain [1118] have been investigated.

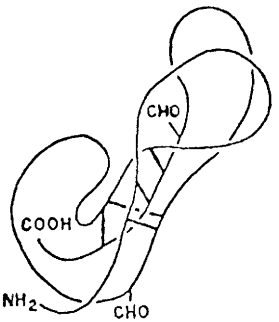
Human chorionic gonadotrophin insolubilized by attachment to Sepharose has been used to separate specific and non-specific anti-follicle-stimulating

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[1109] D. Gospodarowicz, *J. Biol. Chem.*, 1972, **247**, 6491.
[1110] W. Liu, H. S. Nahm, C. M. Sweeney, W. M. Lamkin, H. N. Baker, and D. N. Ward, *J. Biol. Chem.*, 1972, **247**, 4351.
[1111] W. Liu, H. S. Nahm, C. M. Sweeney, G. N. Holcomb, and D. N. Ward, *J. Biol. Chem.*, 1972, **247**, 4365.
[1112] R. C. L. Selinger and M. Civen, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 793.
[1113] K. F. Mori and T. R. Hollands, *J. Biol. Chem.*, 1971, **246**, 7223.
[1114] T. Tsuruhara, H. L. Dufau, J. Hickman, and K. T. Catt, *Endocrinology*, 1972, **91**, 296.
[1115] M. Ota, K. S. Hsieh, and K. Obara, *Endocrinol. Jap.*, 1970, **17**, 165.
[1116] J. M. Hammond, W. E. Bridson, A. Chrambach, and P. O. Kohler, *J. Clin. Endocrinol. Metabol.*, 1972, **34**, 185.
[1117] N. J. Kuhn, *Biochem. J.*, 1972, **129**, 495.
[1118] T. Yaginuma, *Acta Endocrinol.*, 1972, **71**, 245.

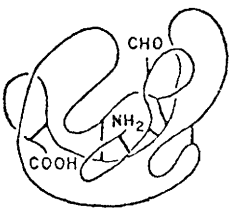
hormone antibodies.[1119] Evidence was presented for the existence of two populations of the antibodies, one being specific for follicle-stimulating hormone and the other binding both follicle-stimulating and luteinizing hormones. Human chorionic gonadotrophin coupled to peroxidase has been used in an enzyme-based immunoassay procedure.[1120]

Pregnant mare serum gonadotrophin has been purified to homogeneity by chromatography on SE-Sephadex and gel filtration, and it was found to contain hexose (24), 2-amino-2-deoxyhexose (15), and sialic acid (12%).[1121] The gonadotrophin was stable in urea and guanidine solutions, but was inactivated by treatment with neuraminidase. Antiserum to the molecule was specific and did not cross-react with human or ovine follicle-stimulating or luteinizing hormones.

The compositions, physicochemical properties, and immunological and biological potencies of the subunits of pituitary thyroid-stimulating hormone have been compared with those of other glycoprotein hormones. The tertiary structures of the subunits have been represented diagramatically: α-subunits (59), β-subunits of thyroid-stimulating hormone (60), and luteinizing hormone and, possibly, human chorionic gonadotrophin (61); the locations of the carbohydrate moieties are shown as —CHO.[1122] Four homogeneous, bovine pituitary glycoproteins with both thyrotropic and exophthalmogenic activities have been isolated and a fraction obtained from limited proteolysis with pepsin was shown to contain galactose (2.0—2.5), mannose (6.1—7.0), fucose (1.0—1.1), 2-amino-2-deoxygalactose (4.1—4.2), and 2-amino-2-deoxyglucose (6.5—6.8 moles per mole).[1123] This glycopeptide (molecular weight 2.1×10^4), which appeared to be composed of two subunits (1.4 and 0.6×10^4), contained terminal, non-reducing galactosyl units and possessed exophthalmogenic, but not thyrotropic, activity.

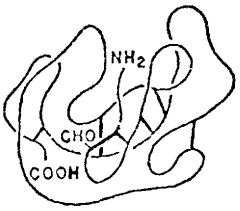


(59)



(60)

[1119] N. Sato and C. M. Cargille, *Endocrinology*, 1970, **90**, 302.
[1120] B. K. Van Weeman and A. H. W. M. Schuurs, *F.E.B.S. Letters*, 1971, **15**, 232.
[1121] D. Schams and H. Papkoff, *Biochim. Biophys. Acta*, 1972, **263**, 139.
[1122] J. G. Pierce, *Endocrinology*, 1971, **89**, 1331.
[1123] L. D. Kohn and R. J. Winand, *J. Biol. Chem.*, 1971, **246**, 6570.



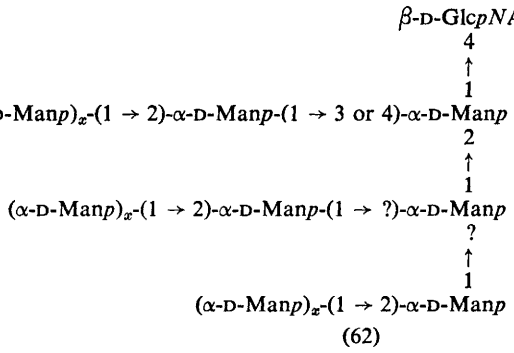
(61)

Isoelectric focusing of a crude extract of erythropoietin, a hormonal glycoprotein from ovine plasma, yielded four biologically active peaks.[1124] The large shift in p*I* that occurred on desialization and the demonstration of partially desialylized species suggested a high sialic acid content for the hormone. Whereas all activity *in vivo* was lost on desialization, oxidation of the modified hormone with galactose oxidase completely restored the activity.

Thyroglobulins

Thyroglobulins have been extracted from the thyroid glands of human, pig, monkey, rat, whale, chicken, and *Xenopus laevis*, with purification by ion-exchange chromatography.[1125] The sedimentation coefficients, electrophoretic mobilities, isoelectric points, and immunological properties of the molecules were similar in many respects, but species differences were observed. Type 'A' carbohydrate moieties of human and calf thyroglobulins have been shown to contain mannose (average 8—9) and 2-acetamido-2-deoxyglucose (2 residues per moiety); whereas 7 or 8 moieties of this type occur in the molecule from humans, only 5 or 6 could be demonstrated in the bovine molecule.[1126] In both cases, the mannose contents ranged from 5 to 11 residues per moiety in the various species separated. Type 'B' moiety of the molecule from humans consisted of galactose (2 or 3), mannose (3), fucose (1), 2-acetamido-2-deoxyglucose (4), and sialic acid (1 residue(s) per moiety) — a composition different from that previously reported for the calf molecule; the two molecules were found to contain 22 and 13 or 14 type 'B' moieties, respectively. The 2-acetamido-2-deoxygalactose residues of thyroglobulin were shown to occur in the 'C' type moieties only. Glycosylamine linkages to asparagine residues were identified for the 'A' and 'B' types, and *O*-glycosidic linkages to serine and threonine residues for the 'C' type. Structural data have been obtained for the type 'A' moiety[1127] and, although the smallest moiety [see (62)] contained only five mannose residues, the additional mannose residues of the

[1124] W. A. Lukowsky and R. H. Painter, *Canad. J. Biochem.*, 1972, **50**, 909.
[1125] T. Hoshino and N. Ui, *Endocrinol. Jap.*, 1970, **17**, 521.
[1126] T. Arima, M. J. Spiro, and R. G. Spiro, *J. Biol. Chem.*, 1972, **247**, 1825.
[1127] T. Arima and R. G. Spiro, *J. Biol. Chem.*, 1972, **247**, 1836.



(62)

larger moieties were found to be attached to the terminal residues of the smaller moiety by α-(1 → 2)-linkages.

Comparison of the physicochemical properties and subunit formation phenomena of native and desialylized human thyroglobulin indicated that no appreciable gross conformational changes took place on removal of the sialic acid residues, but the contribution of these residues to the acidic properties of the molecule was demonstrated.[1128] The association of thyroglobulins from normal and goitrous human and bovine thyroids with a derivative of lysozyme has been reported.[1129]

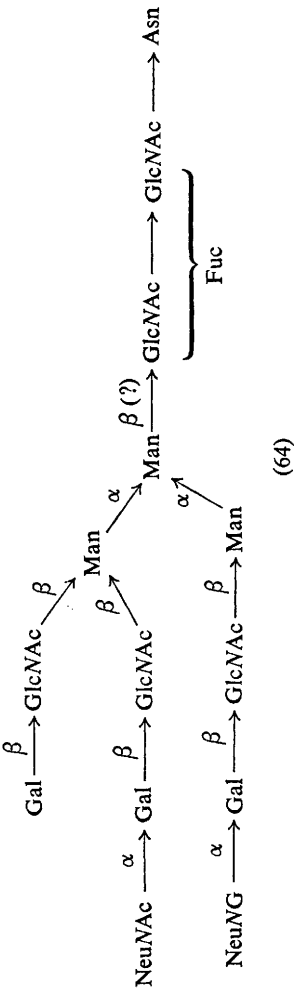
Purified bovine and ovine 19S thyroglobulins were dissociated into 12S subunits after treatment with sodium tetraphenylborate or sodium dodecylsulphate.[1130] Investigation of the properties of calf thyroidal UDP-D-glucose 4-epimerase indicated that UDP-D-galactose is a donor for the biosynthesis of the carbohydrate chains of the thyroglobulin molecule.[1131] Enzymes involved in the metabolism of bovine thyroglobulin have been studied.[1132] Since desialization of bovine thyroglobulin caused its prompt removal from the circulation of rats after injection, it was concluded that the sialic acid units are important for the maintenance of the levels in the blood-stream.[1096]

Studies of two types of glycopeptides isolated from porcine thyroglobulin have led to the identification of the structures of the carbohydrate moieties as (63) and (64).[718] The importance of the galactosyl residues of (64) in binding the glycopeptide to phytohaemagglutinins was shown by the finding that treatment with β-galactosidase abolished the haemagglutinating-inhibitory reaction. The various chains of porcine thyroglobulin

$$(\text{Man})_3 \longrightarrow (\text{Glc}N\text{Ac})_2 \longrightarrow \text{Asn}$$

(63)

[1128] O. Tarutani and S. Shulman, *Biochim. Biophys. Acta*, 1971, **229**, 642.
[1129] A. B. Rawitch and G. Weber, *J. Biol. Chem.*, 1972, **247**, 680.
[1130] J. Mauchamp, *Biochim. Biophys. Acta*, 1971, **251**, 281.
[1131] P. Rodriguez, O. Bello, and K. Gaede, *Arch. Biochem. Biophys.*, 1971, **143**, 579.
[1132] J. L. Trujillo, W. J. Horng, and J. C. Gan, *Biochim. Biophys. Acta*, 1971, **252**, 443.


Gal
β
GlcNAc
β
Man
α
β (?)
Man
GlcNAc
GlcNAc
Asn
NeuNAc
α
Gal
β
GlcNAc
β
NeuNG
α
Gal
β
GlcNAc
β
Man
α
Fuc

(64)

have been studied.[1133] Blockage *in vivo* of the iodination of porcine thyroglobulin caused a significant decrease in the sialic acid content of the molecule, suggesting that differences in the sialic acid contents of preparations of thyroglobulin reflect changes in the regulatory effect of thyrotropin-stimulating hormone.[1134] The relationship between the maturation of the thyroglobulin molecule and its sialic acid content was discussed.

At low temperatures, the 19S thyroglobulin of guinea-pig gave rise to an alternative 15S conformer, and this transition was reversible.[1135] Components related to 19S thyroglobulin of rat thyroids have been isolated and characterized.[1136] Incorporation of radioactive components demonstrated that D-mannose units are present in the fully assembled 19S and 12S species as well as in the slower-sedimenting 6S and 7S subunits, whereas D-galactose is incorporated only after formation of the otherwise fully assembled molecule.

Milk Glycoproteins

The isolation, analysis, and structures of oligosaccharides from human milk and their relationship to the blood-group substances have been reviewed.[746, 747]

The stabilization of milk glycoproteins by sodium carboxymethylcellulose has been studied.[1137] Under standard conditions, samples of carboxymethylcellulose having a heterogeneous distribution of ether groups were most effective in stabilizing α_S-casein.

Structural investigations have been carried out on five oligosaccharides isolated from human milk by gel filtration, and partial structures (65)—(69) were proposed[1138] and commented upon.[1139] A new hexasaccharide (lacto-N-*neo*hexaose) (70) has been isolated from human milk.[1140, 1141] Various glycosidases were employed in the structural studies and a number of fucosyl and sialyl derivatives of the hexasaccharide were also partially characterized.

Gal-(1 → 3)-[Neu*N*Ac-(2 → 4)]-Glc*N*Ac-(1 → 3)-Gal-(1 → 4)-Glc

(65)

Gal-(1 → 3)-[Neu*N*Ac-(2 → 4)]-Glc*N*Ac-(1 → 3)-[Fuc-(1 → 2)-Gal(1 → 3)-Glc*N*Ac-(1 → 6)]-Gal-(1 → 4)-Glc

(66)

[1133] T. Hayashi, K. Iwai, and N. Ui, *Biochim. Biophys. Acta*, 1971, **251**, 208.
[1134] O. Tarutani, *Endocrinol. Jap.*, 1971, **18**, 403.
[1135] A. B. Schneider, H. Bornet, and H. Edelhoch, *J. Biol. Chem.*, 1971, **246**, 2835.
[1136] G. Vecchio, M. S. Carlomagno, and E. Consiglio, *J. Biol. Chem.*, 1971, **246**, 6676.
[1137] Y. Asano and Y. Ishida, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1018.
[1138] R. T. C. Huang, *Z. physiol. Chem.*, 1971, **352**, 1645.
[1139] H. Wiegandt, *Z. physiol. Chem.*, 1971, **352**, 1653.
[1140] A. Kobata and V. Ginsburg, *J. Biol. Chem.*, 1972, **247**, 1525.
[1141] A. Kobata and V. Ginsburg, *Arch. Biochem. Biophys.*, 1972, **150**, 273.

Gal-(1 → 3)-[Neu*N*Ac-(2 → 4)]-Glc*N*Ac-(1 → 3)-[Gal-(1 → 3)-Glc*N*Ac-(1 → 6)]-Gal-(1 → 4)-Glc

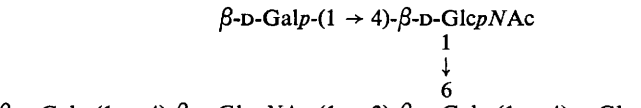
(67)

Neu*N*Ac-(2 → 3)-Gal-(1 → 4)-Glc

(68)

Neu*N*Ac-(2 → 6)-Gal-(1 → 4)-Glc

(69)



(70)

Chemical changes in casein during roasting have been investigated.[1142] Gel-chromatographic and ultracentrifugal techniques have been employed to demonstrate structural differences in the soluble casein complexes obtained by several pathways.[1143] In solutions containing whole acid casein or purified α_{S_1}- and κ-casein fractions, a unit weight ratio complex with a Stokes' radius of 114 Å and a molecular weight of 7×10^5 was formed; the complex redissociated at low concentrations. These results and those of studies on α_{S_1}-, β-, and κ-caseins support a theoretical model for the natural casein micelle built up from a 100 Å-complex containing all the casein fractions.

The secondary structures (particularly the β-structures) of bovine α_S-casein and chemically modified versions thereof have been studied by i.r. and o.r.d. measurements in a number of solvent systems.[1144] Functional-group analysis of bovine α_{S_1}-, β-, and κ-caseins has been related to the mode of micelle formation.[1145] The effect of removing β-casein on bovine casein micelles suggested that hydrophobic interactions are involved in maintaining β-casein within the micelle core.[1146] Similarities have been recognized between the products of proteolytic decomposition of β-casein and those of temperature-sensitive and R-caseins.[1147]

Raman spectra have been obtained for aqueous solutions of β-lactoglobulin and it appears that the molecule exists predominantly in a random-coil form.[1148] The biosynthesis of β-lactoglobulin by the mammary gland of sheep has been studied *in vitro*.[1149] Polyacrylamide gel electrophoresis and

[1142] M. Fujimaki, H. Kato, and F. Hayase, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 416.
[1143] L. Pepper, *Biochim. Biophys. Acta*, 1972, **278**, 147.
[1144] K. Shimazaki, S. Sugai, R. Niki, and S. Arima, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1995.
[1145] S. H. Ashoor, N. F. Olson, and T. Richardson, *J. Agric. Food Chem.*, 1971, **19**, 930.
[1146] W. K. Downey and R. D. Kearney, *Biochem. J.*, 1971, **125**, 96P.
[1147] S. Kaminogawa and K. Yamauchi, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 255.
[1148] A. M. Bellocq, R. C. Lord, and R. Mendelsohn, *Biochim. Biophys. Acta*, 1972, **257**, 280.
[1149] P. Gaye, N. Viennot, and R. Denamur, *Biochim. Biophys. Acta*, 1972, **262**, 371.

gel filtration in the presence of sodium dodecylsulphate have been used to study the glycoproteins associated with the milk-fat globule membrane of bovine milk, and their molecular weights were estimated.[1150] β-Galactosidase has been used to determine the milk glycoprotein contents of foods.[1151]

Serum, Plasma, and Blood Cellular Element Glycoproteins, and Immunoglobulins

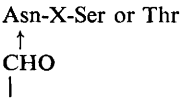
Serum and Plasma Glycoproteins.—Chromatographic patterns of sialic acid derivatives liberated from human serum glycoproteins by different neuraminidases have been reported.[1152] Serum glycoprotein levels in cases of serum bilharziasis have been determined,[978] and insolubilized forms of concanavalin A and bovine serum albumin have been used for affinity chromatography of serum glycoproteins and various proteinaceous materials, respectively.[691] Serum glycoproteins have been studied in cattle during growth and pregnancy.[1153] Incorporation studies have been carried out to determine the biosynthetic routes to rat serum glycoproteins.[1068, 1069] It was shown that incorporation of D-[2-^{3}H]mannose gives rise to labelled and bound galactose, mannose, fucose, 2-amino-2-deoxyhexose, and sialic acid.[1154] Maximum incorporation into each of the monosaccharide units was attained at different times each within two hours (maximum incorporations 11, 14, 16, 11, and 84% of total incorporation, respectively).

The serum concentrations of α_1-acid glycoprotein (orosomucoid), albumin, α_2-HS-glycoprotein, α_2-glycoprotein, α_2-macroglobulin, and transferrin of normal children and adults have been determined.[1155] Measurements have also been made of the serum concentrations of α_1-acid glycoprotein, α_2-HS-glycoprotein, β_2-glycoprotein 1, haptoglobulin, hemopexin, α_2-macroglobulin, and transferrin in normals, pathological controls, and cases of malignancy.[1156] Minor structural differences have been observed in the genetic variants of human α_1-acid glycoprotein.[1157]

Hydroxylapatite has been used for the chromatography of α_1-acid glycoprotein, albumin, and other components of human serum and plasma;[1158] polyacrylamide gel electrophoresis has been similarly applied to human plasma.[1159] Human plasma α_1-acid glycoprotein has been separated by isoelectric focusing into five species (p*I*'s 3.0, 3.2, 3.4, 3.5, 3.6), the first three species constituting 90% of the total glycoprotein and containing neutral carbohydrate (14), 2-amino-2-deoxyhexose (13), and

[1150] D. Kobylka and K. L. Carraway, *Biochim. Biophys. Acta*, 1972, **288**, 282.
[1151] R. K. Bahl, *Analyst*, 1971, **96**, 814.
[1152] R. Calio and N. Caliò-Villani, *Giorn. Microbiol.*, 1970, **18**, 95.
[1153] H. Walkowiak and B. Skubiszewski, *Bull. Acad. polon. Sci., Sér. Sci. biol.*, 1970, **18**, 533.
[1154] M. Mitranic and M. A. Moscarello, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 74.
[1155] B. Weeke and P. A. Krasilnikoff, *Acta Med. Scand.*, 1972, **192**, 149.
[1156] S. Snyder and G. Ashwell, *Clinica Chim. Acta*, 1971, **34**, 449.
[1157] R. Nimberg, T. Motoyama, and K. Schmid, *J. Biol. Chem.*, 1971, **246**, 5817.
[1158] G. Vidal and J. P. Ruel, *J. Chromatog.*, 1971, **60**, 424.
[1159] J. Barbosa, R. P. Doe, and U. S. Seal, *Clinica Chim. Acta*, 1972, **37**, 105.

sialic acid (12.5—11.3%).[1160] The binding of progesterone to the glycoproteins was investigated. The properties of α_1-acid glycoprotein from ascitic and pleural fluids have been studied.[1161] A homogeneous glycoprotein (molecular weight 4.36×10^4) from human liver, containing galactose (6.5), mannose (5.3), 2-amino-2-deoxyglucose (12.1), fucose (1.5), and sialic acid (10.0%), was identified as α_1-acid glycoprotein.[1015] Sequence studies of cyanogen bromide fragments from α_1-acid glycoprotein have located the linkage positions of the carbohydrate moieties [1162–1164] and have shown that carbohydrate is linked to aspartyl residues in the sequence (71).[1164]



(71)

Cross-reactions have been observed between human α_1-acid glycoprotein, its tryptophan-poor counterpart, and Zn-α_2-glycoprotein.[1165] The effect of modifying the *N*-acetylneuraminic acid residues of human α_1-acid glycoprotein on its binding to influenza virus and susceptibility to neuraminidase has been investigated.[1166] It has been shown that the sialic acid residues of human α_1-acid glycoprotein, haptoglobulin, lactoferrin, α_2-macroglobulin, and bovine fetuin, but not those of human transferrin, are essential for the survival of the glycoproteins in circulation.[1096] The binding of asialo human α_1-acid glycoprotein, transferrin, and bovine fetuin and of asialo-agalacto-α_1-acid glycoprotein to plasma membranes of rat liver has been investigated.[665]

α_1-Acid glycoprotein and α_2-macroglobulin from rat serum have been shown to possess molecular weights of 4.3×10^4 and 8.0×10^5 and p*I*'s of 2.95 and 4.60, respectively, and to contain neutral carbohydrate (14.4, 9.3), 2-amino-2-deoxyhexose (9.5, 4.0), and sialic acid (10.2 and 2.6%, respectively).[1167] α_1-Acid glycoprotein, albumin, and α_2-macroglobulin contents in sera from rats suffering from induced inflammation have been reported and biosynthetic studies with radioactive precursors have indicated that the rates of synthesis of α_1-acid glycoprotein and α_2-macroglobulin are greater than normal.[1168]

[1160] M. F. Ryan and U. Westphal, *J. Biol. Chem.*, 1972, **247**, 4050.
[1161] M. Hamazaki, M. Kurokawa, and K. Hotta, *Seikagaku*, 1972, **44**, 255.
[1162] J. Emura, T. Ikenaka, J. H. Collins, and K. Schmid, *J. Biol. Chem.*, 1971, **246**, 7821.
[1163] T. Ikenaka, M. Ishiguro, J. Emura, H. Kaufmann, S. Isemura, W. Bauer, and K. Schmid, *Biochemistry*, 1972, **11**, 3817.
[1164] K. Kitamura and I. Yamashina, *J. Biochem.* (*Japan*), 1972, **71**, 65.
[1165] K. Bauer, *Physiol. Chem. Phys.*, 1970, **2**, 587.
[1166] M. Suttajit and R. J. Winzler, *J. Biol. Chem.*, 1971, **246**, 3398.
[1167] J. C. Jamieson, A. D. Friesen, F. E. Ashton, and B. Chou, *Canad. J. Biochem.*, 1972, **50**, 856.
[1168] J. C. Jamieson, F. E. Ashton, A. D. Friesen, and B. Chou, *Canad. J. Biochem.*, 1972, **50**, 871.

The Bromocresol Green method for the determination of human serum albumin has been modified to simplify the technique and to increase its sensitivity.[1169] The SMA 12/60 analyser has been adapted to assay human serum albumin by the Bromocresol Green method, and the normal range of albumin levels has been determined.[1170] Albumin levels have been determined in normal human pancreatic juice and have been compared with the corresponding serum levels.[1171] The albumin content of human cerebrospinal fluid has been estimated immunologically.[1172] Insolubilized anti-human serum albumin may be used as an immunosorbent in the purification of human serum albumin and bovine serum glycoprotein,[639] and for the measurement of fragments derived from human serum albumin.[1173] Gel filtration in combination with reversed osmosis has been used for the large-scale preparation of human serum albumin, thereby avoiding freeze-drying.[1174]

The plurality of human serum albumin has been demonstrated by gel filtration and ion-exchange chromatography, three major and two minor fractions being obtained.[1175] Hydrogen–deuterium exchanges have revealed conformational changes in human serum albumin,[1176] and the reactions of this molecule[1177] and the c.d. spectra of its bilirubin complexes[1178, 1179] have been studied. Zinc[1180] and copper(II)–L-histidine[1181] complexes of human serum albumin have also been investigated and the preparation of a water-insoluble derivative of human serum albumin has been described.[1182]

Serum albumin from bovine and rat sources has been isolated by precipitation of all other macromolecules with polyethylene glycol; the albumin subsequently obtained by gel filtration was homogeneous.[1183] Bovine plasma albumin has been purified by precipitation from acid solution as the glutamate salt, elevation of the pH subsequently yielding the free albumin.[1184] Species differences were apparent in the glycoprotein distributions of bovine, porcine, and avian sera following electrophoretic separation of the albumin, α_1-, α_2-, α_3-, β-, and γ-globulin fractions.[1185] Typical monosaccharides were found in each of the glycoprotein fractions.

[1169] I. G. McPherson and D. W. Everhard, *Clinica Chim. Acta*, 1972, **37**, 117.
[1170] J. O. Westgard and M. A. Poquette, *Clinical Chem.*, 1972, **18**, 647.
[1171] F. Clemente, T. Ribeiro, C. Figarella, and H. Sarles, *Clinica Chim. Acta*, 1971, **33**, 317.
[1172] B. Delpech and E. Lichtblau, *Clinica Chim. Acta*, 1972, **37**, 15.
[1173] M. J. Escribano, F. Bellon, and C. Lapresle, *Immunochemistry*, 1972, **9**, 111.
[1174] H. Friedli and P. Kistler, *Chimia (Switz.)*, 1972, **26**, 25.
[1175] P. K. Shrivastava, H. Goch, and K. Zakrewski, *Biochim. Biophys. Acta*, 1972, **271**, 310.
[1176] A. Hvidt and K. Wallevik, *J. Biol. Chem.*, 1972, **247**, 1530.
[1177] J. J. Arrotti and J. E. Garvin, *Biochim. Biophys. Acta*, 1972, **255**, 79.
[1178] G. Blauer, D. Harmatz, and J. Snir, *Biochim. Biophys. Acta*, 1972, **278**, 68.
[1179] G. Blauer and D. Harmatz, *Biochim. Biophys. Acta*, 1972, **278**, 89.
[1180] E. L. Giroux and R. I. Henkin, *Biochim. Biophys. Acta*, 1972, **273**, 64.
[1181] S. Lau and B. Sarkar, *J. Biol. Chem.*, 1971, **246**, 5938.
[1182] T. Seki, T. A. Jenssen, Y. Leuin, and E. G. Erdos, *Nature*, 1970, **225**, 864.
[1183] D. Gambal, *Biochim. Biophys. Acta*, 1971, **251**, 54.
[1184] A. Hansen, *Acta Pathol. Microbiol. Scand.*, 1972, **80B**, 19.
[1185] M. Channon and P. A. Anastassiadis, *Comp. Biochem. Physiol.*, 1971, **38B**, 53.

The electrophoretic mobilities of bovine serum albumin and human transferrin in polyacrylamide gels have been studied,[1186] but it was shown that bovine serum albumin polymers do not behave in accordance with their molecular weights in such systems.[1187] The molecules appeared to be of greater sizes and the results were discussed in terms of the use of albumin as a standard marker. The behaviour of bovine plasma albumin on isoelectric focusing has been studied[1188] and pressure-jump kinetic studies have been carried out on the conformational changes occurring in bovine serum albumin in neutral solutions.[1189] Laser Raman spectroscopy of bovine serum albumin revealed a degree of ordered structure,[1148] and the dimerization of bovine plasma albumin at low pH has been investigated.[1190]

The uptake of labelled bovine serum albumin by rat brain[1118] and the interactions of bovine serum albumin with tetracycline,[1191] human erythrocyte glycoprotein,[1192] and methyl orange[1193] have been studied. Bovine serum albumin has been used in the microencapsulation of an enzyme[1194] and insolubilized bovine serum albumin has been employed as an immunoadsorbent for the homologous antibody.[1195–1197] A polysaccharide conjugate of bovine serum albumin has been used for the isolation of antidextran immunoglobulin,[1198] and other insoluble derivatives of bovine serum albumin have been reported.[734]

The separation of intracellular serum albumin from rat liver has been described and, on the basis of this work, an isotope-dilution technique was devised for quantitation of the glycoprotein.[1066] The gel-electrophoretic behaviour[1199] and biosynthesis[1200, 1201] of rat liver albumin have been studied. The preparation and properties of the 4.6S serum albumin (molecular weight 6.7×10^4) from the bullfrog (*Rana catesbeiana*) have been reported,[1202] and the heterogeneity and polymorphism of serum

[1186] C. J. O. R. Morris and P. Morris, *Biochem. J.*, 1971, **124**, 517.
[1187] R. J. Barton, *Biochem. J.*, 1972, **129**, 983.
[1188] L. J. Kaplan and J. F. Foster, *Biochemistry*, 1971, **10**, 630.
[1189] D. E. Goldsack and P. M. Waern, *Canad. J. Biochem.*, 1971, **49**, 1267.
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[1197] N. Weliky and H. H. Weetall, *Immunochemistry*, 1972, **9**, 967.
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[1200] L. Kelman, S. J. Saunders, S. Wicht, L. Frith, A. Corrigall, R. E. Kirsch, and J. Terblanche, *Biochem. J.*, 1972, **129**, 805.
[1201] T. Peters and J. C. Peters, *J. Biol. Chem.*, 1972, **247**, 3858.
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albumin in fishes have been studied.[1203] Serum albumin and α_1-, α_2-, and β_1-globulins of blood from monotremes and marsupials have been investigated.[1204]

The preparation of C_{1q} (molecular weight 3.93—4.10 × 10^5), a subcomponent of human complement exhibiting affinity for various immunoglobulins, has been described and the glycoprotein was shown to contain galactose (3.1), glucose (3.4), 2-amino-2-deoxyglucose (0.8), and neuraminic acid (0.5%).[1205] The molecule was dissociated into subunits on treatment with sodium dodecylsulphate and the glycopeptide linkage appeared to involve a glucosylgalactosylhydroxylysine unit as in collagen. The isolation of C_{1q} together with two α-globulins has also been described.[1206]

Factor VIII (antihaemophilic globulin) has been purified from human plasma after digestion with chymotrypsin to reduce the fibrogenic viscosity.[1207] The glycoprotein was found to contain galactose, mannose plus fucose (total 2), 2-amino-2-deoxyhexose (2.8), and sialic acid (1%); the major subunit has a molecular weight of 2.4 × 10^5. Bovine factor VIII has been purified from plasma by a process involving precipitation with concanavalin A and, although the product was homogeneous, aggregation was evident.[1208] The glycoprotein (molecular weight 1.1 × 10^6) was shown to contain hexose (11), 2-amino-2-deoxyhexose (7), and sialic acid (2%). Various fetuin molecules, modified by specific removal of carbohydrate units, acted as acceptors for the transfer of carbohydrates from their nucleotide derivatives by glycosyltransferases.[680]

A method has been reported for the estimation of human fibrinogen,[1209] and the electrophoretic heterogeneity of human fibrinogen has been attributed to variations in the amounts of carbohydrate bound to the protein chain.[1210] The abnormality of human fibrinogen 'Metz' has been located in the α(A)-chain.[1211] Whereas fibrinogen 'St Louis' possesses the physicochemical characteristics of the normal molecule, the carbohydrate contents of the two molecules were found to differ slightly (hexose, 1.87, 1.67; 2-amino-2-deoxyhexose, 0.8, 0.8; and sialic acid, 0.5 and 0.5%, respectively).[1212] The properties of the abnormal molecule were also compared with those of fibrinogen 'Zurich'. The primary structure of human fibrinogen has been investigated [1213] and it has been shown that the carbohydrate

[1203] V. I. Lukianenko, A. V. Popov, and E. A. Mishin, *Doklady Akad. Nauk S.S.S.R.*, 1971, **201**, 737.
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[1210] P. J. Gaffney, *Nature New Biol.*, 1971, **230**, 54.
[1211] J. Soria, C. Soria, and C. Boulard, *Biochimie*, 1972, **54**, 415.
[1212] L. A. Sherman, A. Sherman, L. W. Gaston, M. E. Kaplan, and A. R. Spivack, *J. Clin. Invest.*, 1972, **51**, 590.
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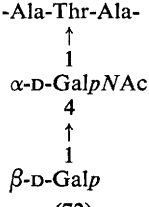
moieties are attached to the β(B)- and γ-chains.[1214] The majority of the carbohydrate was conserved in fragments of high molecular weight during lysis of the molecule with plasmin. The reactions of fibrinogen with thrombin[1215] and plasmin[1216] have been investigated and the α(A)-, β(B)-, and γ-chains have been separated from a *S*-carboxymethylated molecule from human fibrinogen.[1217] The metabolism of fibrinogen in rabbits[1218] and rats[1219] has been studied.

Changes in the levels of serum α_1- and α_2-glycoproteins have been observed in cases of malaria,[1220] and human foetal α-globulin from hepatoma serum has been characterized.[1221] A 3.8S α_2-glycoprotein from human serum was separated into four bands (p*I*'s 5.6—6.2) by isoelectric focusing and it remained heterogeneous following treatment with neuraminidase.[1222] The molecule (average molecular weight 3.85×10^4) was found to be composed of two identical subunits and to contain galactose (3.2), mannose (3.2), 2-acetamido-2-deoxyhexose (4.4), and *N*-acetylneuraminic acid (3.5%). The α_2-glycoprotein bound heparin. α_2H-Globulin (molecular weight 6×10^5), a macroglobulin of hepatic origin found in the sera of patients with malignant diseases, was shown to form dimers and tetramers, and to contain neutral carbohydrate.[1223] The monomeric and dimeric forms were also found in normal liver and spleen. An 8S α_3-glycoprotein isolated as a side-fraction from the large-scale preparation of human serum albumin and human immunoglobulin IgG gave several bands on isoelectric focusing in the pH range 4.2—4.6.[1224] The glycoprotein (molecular weight 2.2×10^5) contained glucose (5.5), mannose (5.5), fucose (0.4), 2-acetamido-2-deoxyhexose (10.8), and *N*-acetylneuraminic acid (9.2%), and its behaviour in dissociating media was studied. Genetic polymorphism has been demonstrated in human glycine-rich β-glycoprotein and it was concluded that the molecule is a tetramer composed of two types of subunits.[1225]

Glycoproteins of seal (*Leptonychotes wedelii*) serum have been examined and the levels were found to be similar to those of other mammalian species.[1226] The structures of a series of serum glycoproteins from the Antarctic fish *Trematomas borchgrevinki* have been studied in detail.[1227]

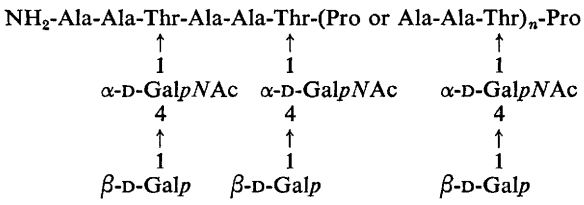
[1214] P. J. Gaffney, *Biochim. Biophys. Acta*, 1972, **263**, 453.
[1215] R. Flengsrud, B. Østerud, and H. Prydz, *Biochem. J.*, 1972, **129**, 83.
[1216] D. Mills and S. Karpatkin, *Biochim. Biophys. Acta*, 1972, **271**, 163.
[1217] A. Henschen and P. Edman, *Biochim. Biophys. Acta*, 1972, **263**, 351.
[1218] L. A. Sherman, *J. Lab. Clinical Med.*, 1972, **79**, 710.
[1219] I. Nadelhaft and F. Lamy, *J. Lab. Clinical Med.*, 1972, **79**, 724.
[1220] S. G. Murphy, A. S. Klainer, and D. F. Clyde, *J. Lab. Clinical Med.*, 1972, **79**, 55.
[1221] A. Adinolfi, M. Adinolfi, and S. Cohen, *Biochim. Biophys. Acta*, 1971, **251**, 197.
[1222] N. Heimburger, H. Haupt, T. Kranz, and S. Baudner, *Z. physiol. Chem.*, 1972, **353**, 1133.
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[1227] W. T. Shier, Y. Lin, and A. L. De Vries, *Biochim. Biophys. Acta*, 1972, **263**, 414.

The glycoproteins are present in a range of molecular weights and consist entirely of the repeating unit (72). These glycoproteins have been shown to



(72)

be partly responsible for the unusually low temperatures at which the sera of Antarctic fishes freeze; testing of a number of derivatives of the glycoproteins suggested that the activity resulted from an interaction with water or ice that involved many of their structural features. Two groups of glycopeptides isolated from serum of the same species were shown to possess similar structures (73), but to differ in molecular size (3500 and



(73)

2600).[1228] Aqueous solutions of these glycopeptides displayed the same thermal hysteresis as those of the analogous glycoproteins.

Isoelectric focusing of a serum fraction containing haptoglobulin, hemopexin, and transferrin separated the glycoproteins into three bands of p*I*'s 4.6—4.8, 5.6—5.8, and 5.6—6.3, respectively.[1229] Rat haptoglobin and α_2-macroglobulin have been labelled by incorporation of ^{75}Se *in vivo*.[1230] Studies on the carbohydrate moieties of haemocyanin from *Octopus vulgaris* have been reported.[1231] Purified Lp(a) lipoglycoprotein from human plasma can be dissociated into three components by electrophoresis and has been shown to contain neutral carbohydrate (10.8), 2-amino-2-deoxyhexose (8.4), and sialic acid (6.6%).[1232]

Serum levels of α-macroglobulin have been reported for women taking oral contraceptives.[1233] 18S α_2-Macroglobulin (molecular weight 7.25 ×

[1228] Y. Lin, J. G. Duman, and A. L. De Vries, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 87.
[1229] B. Bergrahm, *Scand. J. Clin. Lab. Invest.*, 1970, **25**, Suppl. 113, p. 30.
[1230] R. Engler, C. Lombart, and M. F. Jayle, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1483.
[1231] V. Albergoni and A. Cassini, *Boll. Soc. ital. Biol. sper.*, 1970, **46**, 936.
[1232] C. Ehnholm, H. Garoff, O. Renkonen, and K. Simons, *Biochemistry*, 1972, **11**, 3229.
[1233] R. K. Chandra, *J. Reprod. Fert.*, 1972, **28**, 463.

10^5) has been prepared from human serum and dissociation studies indicated that the molecule normally exists as a dimer.[1234] The interaction of the macroglobulin with collagenase has been studied using insolubilized anti-α_2-macroglobulin.[1235] Both trypsin and chymotrypsin have been reported to form a complex with rabbit α_2-macroglobulin.[1236]

Bovine prothrombin has been purified by a modified procedure and the subunits of the glycoprotein (molecular weight 7.2×10^4) have been examined [1237] as well as its ability to complex with calcium ions and phospholipids.[1238] The carbohydrate chains of bovine prothrombin have been found to contain galactose, mannose, 2-amino-2-deoxyglucose, and sialic acid (molar ratio 2—3 : 2 : 3 : 4—6) and it has been established that there are four chains in each molecule.[1239] Enzymic degradation of the carbohydrate moieties showed them to have the partial sequence (74),

Sialic acid———α-H and β-D-Gal*p*-β-D-Glc*pN*Ac-α-DMan*p*
Sialic acid———α-H and β-D-Gal*p*-β-D-Glc*pN*Ac-α-DMan*p*
} inner carbohydrate

whereas the inner carbohydrate, which contains mannose and 2-amino-2-deoxyhexose and is linked to asparagine residues, was resistant to the enzymes applied. Purified rat prothrombin was shown to contain hexose (11.1), 2-amino-2-deoxyhexose (2.1), and sialic acid (4.9%); analysis of the same molecule from dicoumarol-treated rats, in which prothrombic activity was reduced, suggested that the carbohydrate moieties are not involved in the manifestation of activity.[1240] A water-insoluble derivative of prothrombin has been used to study the conversion of prothrombin into thrombin.[1241]

Desialylized human plasma transcortin displayed heterogeneity on isoelectric focusing and all samples tested contained two cortisol-binding components.[1242]

Iron-free human transferrin has been separated into two isoelectrically different components (p*I*'s 5.4 and 5.8).[1243] The physicochemical characteristics of the apo-molecule have been compared with those of iron-transferrin [1244] and the latter molecule has also been studied by e.s.r. spectroscopy.[1245]

[1234] J. M. Jones, J. M. Creeth, and R. A. Kekwick, *Biochem. J.*, 1972, **127**, 187.
[1235] S. Abe and Y. Nagai, *Biochim. Biophys. Acta*, 1972, **278**, 125.
[1236] T. L. De Vonne, H. Mouray, G. Berthillier, and R. Got, *Biochim. Biophys. Acta*, 1972, **257**, 365.
[1237] K. G. Mann, C. M. Heldebrant, and D. N. Fass, *J. Biol. Chem.*, 1971, **246**, 6106.
[1238] R. K. Bull, S. Jevons, and P. G. Barton, *J. Biol. Chem.*, 1972, **247**, 2747.
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[1241] A. M. Engel and B. Alexander, *J. Biol. Chem.*, 1971, **246**, 1213.
[1242] H. Van Baelen, M. Beck, and P. DeMoor, *J. Biol. Chem.*, 1972, **247**, 2699.
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[1244] P. A. Charlwood, *Biochem. J.*, 1971, **125**, 1019.
[1245] E. M. Price and J. F. Gibson, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 646.

The structure of human transferrin has been likened to that of lactoferrin;[1246] dissociation studies have led to preliminary location of the carbohydrate moieties and to the conclusion that the molecule is a dimer.[1247] The binding of iron by the molecule has been studied.[1248] A colorimetric method has been described for the assay of human urinary transferrin and normal levels were reported.[1249] Transferrin has been isolated from male urine by an anti-transferrin immunosorbent procedure, and the physicochemical properties and carbohydrate contents of the molecule (molecular weight 8.2×10^4) were identical to those of the serum molecule, although the two differ in other respects.[1250] The physicochemical properties of horse spleen transferrin have been compared with those of the molecule from humans.[1251] Transferrin has been identified electrophoretically in serum from the bowfin (*Amia calva*).[1252]

The *O*-acetyl groups of Vi antigen have been demonstrated to be essential for the formation of specific Vi antigen–protein complexes.[681]

Blood Cellular Element Glycoproteins.—The distribution and function of the polysaccharides of healthy and diseased blood-vessel walls have been reviewed.[858]

A method has been described whereby glycoprotein antigens may be extracted from haemoglobin-free stroma of mammalian erythrocytes with hot aqueous ethanol.[773] Using this technique, infectious mononucleosis receptors have been isolated from human, equine, bovine, and ovine sources; each receptor was shown to contain hexose, fucose, 2-acetamido-2-deoxyhexose, and sialic acid. The electrophoretic behaviour of these antigens in sodium dodecylsulphate has been examined.[1253] Analysis of the glycoproteins of human erythrocyte membranes by polyacrylamide gel electrophoresis and gel filtration gave quite different molecular weight values (approximately 7 and 3×10^4, respectively).[1254] The carbohydrate compositions of human erythrocyte membrane glycoproteins have been studied[640] and the relationships of these glycoproteins to blood-group activities have been investigated.[752, 770] Human erythrocyte membrane glycoproteins have been labelled by periodate oxidation followed by reduction with sodium borotritide; the label was located in oligosaccharide moieties of the glycoproteins and predominantly in those units that were originally sialic acid.[1255] The effect of modifying the *N*-acetylneuraminic

[1246] P. Aisen and A. Leibman, *Biochim. Biophys. Acta*, 1972, **257**, 314.
[1247] A. Bezkorovainy and D. Grohlich, *Biochim. Biophys. Acta*, 1972, **263**, 645.
[1248] C. van der Heul, H. G. van Eijk, W. F. Wiltink, and B. Leijnse, *Clinica Chim. Acta*, 1972, **38**, 347.
[1249] P. Tavenier, C. E. Raaijmakers, H. G. van Eijk, and B. Leijnse, *Clinica Chim. Acta*, 1971, **32**, 63.
[1250] N. Frénoy, Y. Goussault, and R. Bourrillon, *Biochimie*, 1971, **53**, 1207.
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[1254] D. Kobylka, A. Khettry, B. C. Shin, and K. L. Carraway, *Arch. Biochem. Biophys.*, 1972, **148**, 475.
[1255] O. O. Blumenfeld, P. M. Gallop, and T. H. Liao, *Biochem. Biophys. Res. Comm.*, 1972, **48**, 242.

acid residues of human erythrocyte membrane stroma glycoproteins by periodate oxidation has been studied with respect to their virus-binding ability and susceptibility to neuraminidase.[1166] A sialoglycopeptide released from human erythrocytes by trypsin was found to interact with positively charged substances by way of a semipolar or ionic bond reaction, suggesting that it has cation-exchange properties.[1192] The structure of a glycopeptide (75) released from human erythrocyte membranes by sodium chloride was shown to involve an *S*-glycosidic bond.[1256]

Glc-Glc-Glc-Cys-Glu-(Gly,His,Ser,His,Asp,His)-Gly-Ala
(75)

Two glycoproteins, which were agglutinated by concanavalin A, have been purified from rat erythrocyte stroma.[720] In the presence of sodium dodecylsulphate, their molecular weights were estimated as 2×10^5 and 3×10^5, and they were shown to contain hexose (32 and 23%) and sialic acid (2 and 7%, respectively), but no 2-amino-2-deoxyhexose. Investigation of the subunits of glycoproteins from rat erythrocyte ghost membranes showed that they all contain sialic acid and that four of them possess the same molecular weights as subunits derived from other rat cell membranes.[1062]

Porcine lymphocyte plasma membrane glycoproteins have been solubilized with sodium deoxycholate and were then purified by affinity chromatography using insolubilized *Lens culinaris* phytohaemagglutinin [736] or insolubilized concanavalin A.[732, 733] In one case, the purified glycoprotein fraction was shown to contain neutral carbohydrate (6.8%) and *N*-acetylneuraminic acid (1.9%).[733] The biological activity of migration-inhibitory factor obtained from the lymphocytes of sensitized guinea-pigs was abolished by the action of neuraminidase, suggesting that the molecule is a glycoprotein.[1257]

Three size classes of glycopeptides have been obtained from low- and high-density human platelet plasma membranes.[902] It has been concluded from physicochemical and immunological investigations that fibrinogen purified from human blood platelets, whilst showing some of the properties of its plasma counterpart, is structurally different and smaller.[1258]

Immunoglobulins.—The structure, identification, turnover, and function of immunoglobulins as well as their involvement in clinical conditions have been reviewed.[1259] The primary structures of normal and abnormal immunoglobulins have also been reviewed [1260] and the genetic markers of human immunoglobulins have been discussed in detail.[1261] Proposals have

[1256] J. B. Weiss, C. J. Lote, and H. Bobinski, *Nature New Biol.*, 1971, **234**, 25.
[1257] H. G. Remold and J. R. David, *J. Immunol.*, 1971, **107**, 1090.
[1258] P. Ganguly, *J. Biol. Chem.*, 1972, **247**, 1809.
[1259] J. R. Hobbs, *Adv. Clin. Chem.*, 1971, **14**, 220.
[1260] J. Novotný, *Chem. listy*, 1972, **66**, 953.
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been made for the definition and for use of standards and international reference preparations of human serum immunoglobulins.[1262]

Disulphide-cross-linked antibodies can be used for the purification of immunoglobulins.[639] Immunoglobulins, and their respective heavy and light chains, from a number of species representing the Mammalia, Aves, Reptilia, Amphibia, Chondrichthyes, and Osteichthyes have been analysed for their carbohydrate compositions.[1263] Galactose, mannose, 2-amino-2-deoxyglucose, and sialic acid are present in all immunoglobulins, whereas fucose is found in all but those of two species. Most of the carbohydrate is associated with the H-chains, but since fucose and sialic acid are often found in average amounts of less than one residue per H-chain, it is considered that the moieties are heterogeneous. Immunoglobulins from mammals and birds were found to possess greater molar ratios of mannose : galactose than those from other species. A further report has detailed the electrophoretic mobilities and the hexose, fucose, 2-amino-2-deoxyhexose, and sialic acid contents of bovine, porcine, and avian immunoglobulins.[1185] *X*-Ray crystallographic studies have been reported for the Fab and Fc fragments of human immunoglobulins and have enabled an electron-density map for the Fab′ molecule to be constructed.[1264] The isoelectric focusing and electrophoretic properties of immunoglobulins separated from the whey of bovine colostrum have been investigated.[1265] Immunological methods for the detection of α-, κ-, and λ-chains of mammalian immunoglobulins have been reported.[1266] The immunoglobulins of monotremes and marsupials have been investigated and high levels were found in *Lasiorhinus* and *Tachyglossus* species.[1204] Insolubilized bovine[1195] and rabbit[1267] immunoglobulins have been used for antibody detection.

The accuracy of quantitative measurement of immunoglobulins IgA, IgG, and IgM by radial immunodiffusion has been investigated[1268] and an improved disc-electrophoretic method for the separation of immunoglobulin IgA from IgG and IgM was reported.[1269] Nephelometric methods for the determination of human serum immunoglobulins IgA, IgG, and IgM as antigen–antibody complexes have been reported and compared with other procedures for accuracy.[1270] The levels of these three immunoglobulins in sera of normal children and adults have been determined.[1155]

[1262] S. G. Anderson, D. R. Bangham, I. Batty, W. Becker, B. Cinader, M. van der Giessen, F. Hymes, D. Long, F. Peetom, K. Pondman, M. Raynaud, C. B. Reimer, D. S. Rowe, H. G. Schwick, and S. Vrancheva, *Ann. Inst. Pasteur*, 1971, **121**, 629.
[1263] R. T. Acton, W. Niedermeier, P. F. Niedermeier, L. W. Clem, G. A. Leslie, and J. C. Bennett, *J. Immunol.*, 1972, **109**, 371.
[1264] R. J. Poljak, L. M. Amzel, H. P. Avey, L. N. Becka, D. J. Goldstein, and R. L. Humphrey, *Cold Spring Harbor Symp. Quant. Biol.*, 1971, **36**, 421.
[1265] E. Uusi-Rauva, P. Moisio, and M. Antila, *Suomen Kem.*, 1970, **43**, *B*, 344.
[1266] E. Orlans and A. Feinstein, *Nature New Biol.*, 1971, **233**, 45.
[1267] V. T. Skvortsov, *Immunochemistry*, 1972, **9**, 366.
[1268] W. Grabner, D. Bergner, D. Sailer, and G. Berg, *Clinica Chim. Acta*, 1972, **39**, 59.
[1269] K. Felgenhauer, *Clinica Chim. Acta*, 1972, **39**, 177.
[1270] L. M. Killingsworth and J. Savory, *Clinical Chem.*, 1972, **18**, 335.

Immunoglobulin IgA can be purified from human serum by immunoadsorption onto insolubilized anti-human IgG light chain.[1271] The product, obtained in high yield, possesses both κ- and λ-light-chain determinants. J-Chains prepared from human serum IgA and human secretory IgA both contained carbohydrate (7.6%).[1272] Each J-chain was shown to contain galactose (2), mannose (3), fucose (1), 2-amino-2-deoxyglucose (3—4), and sialic acid (1 mole per molecular weight of 2.6×10^4). The rates of release of monosaccharides on methanolysis of glycopeptides derived from human IgA and IgM have been determined.[1273] Different classes of immunoglobulins IgA and IgG have been studied in connection with their effects on aggregation of blood platelets.[1274]

Immunoglobulin IgA (molecular weight 3.85×10^5) has been isolated from human colostrum; reduction and alkylation yielded two serum-type IgA molecules (molecular weight 1.59×10^5) and a molecule of secretory piece (7.55×10^4).[1275] Bound and free secretory pieces from colostrum were shown to be physicochemically and chemically identical and to contain galactose (3.1), mannose (4.0), fucose (2.3), 2-amino-2-deoxyhexose (4.4), and sialic acid (1.9%). Another report on the two secretory pieces also demonstrated their similarities in composition, molecular weight (7.1×10^4), and antigenic determinants.[1276] Human colostrum secretory IgA itself was shown to contain galactose (1.6), mannose (2.6), fucose (0.7), 2-amino-2-deoxygalactose (0.2), 2-amino-2-deoxyglucose (2.5), and sialic acid (1.1%).[1277] The carbohydrate is distributed unevenly among the polypeptide chains as follows: H-chain (63), L-chain (2.5), J-chain (6.5), and secretory component (28%). The carbohydrate compositions of the chains were also compared. Human colostrum IgA and guinea-pig serum IgM reacted with staphylococcal protein A.[1278] The levels of immunoglobulins IgA and IgG in normal human pancreatic juice have been compared with those in the corresponding sera.[1171]

Serum levels of immunoglobulins IgA, IgG, and IgM in cases of infectious diseases,[1279] rheumatoid arthritis,[1280,1281] and systemic hypertension,[1282] and of serum IgA, IgD, IgG, and IgM in malignancy[1156] have been measured and compared with normal values. Serum anti-immunoglobulins of IgA, IgG, and IgM types have been measured in patients (with rheumatoid arthritis or psoriatic arthritis) and normals with the aid of cross-linked

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[1282] F. Olsen, *Acta Pathol. Microbiol. Scand.*, 1972, **80A**, 257.

IgG.[1283] The development of a second monoclonal IgG in a case of late manifestation of myeloma has been investigated and IgA, IgD, IgE, and IgG levels were reported.[1284] The levels of serum and salivary IgA in leukaemia and paraproteinemia,[1285] and of serum and bronchial fluid IgA, IgG, and IgM in patients with chronic obstructive pulmonary disease[1286] have also been reported. Serum immunoglobulin IgA levels were found to be abnormal in a few cases on account of their association lactate dehydrogenase.[1287, 1288]

The physicochemical properties of the immunoglobulin produced in excess in a case of IgA κ-type multiple myeloma have been reported[1289] and chemical typing of myeloma $IgA_{1\&2}$ revealed differences between them.[1290, 1291] Several myeloma immunoglobulins IgA from humans have been shown to contain 89—96% of their total carbohydrate in the H-chains — galactose (5—6), mannose (4—5), fucose (0.3—0.6), 2-amino-2-deoxygalactose (2), 2-amino-2-deoxyglucose (4—5), and sialic acid (3—4 residues per H-chain).[1292] Preparations of the corresponding L-chains were characterized by the absence of 2-amino-2-deoxygalactose, as was a further H-chain. The L-chains prepared from dimeric immunoglobulins contained J-chains and, in one case, these chains contained 50% of the total carbohydrate. Water-insolubilized forms of myeloma immunoglobulins IgA, IgG, and IgM have been used for the purification of a variety of anti-immunoglobulins.[1293] Changes in the levels of immunoglobulins IgA, IgG, and IgM in cases of alcoholic cirrhosis, primary hepatocellular carcinoma, and various forms of hepatitis have been monitored and the findings may have diagnostic applications.[1294]

Serum levels of equine immunoglobulins IgA, IgB, IgG, and IgM have been reported.[1295] A method has been described for the preparation of immunoglobulin IgT, the major antitoxin of equine anti-diphtheria sera, and the 7S glycoprotein was shown to contain galactose (1.85), mannose (1.85), 2-amino-2-deoxyhexose (2.5), fucose (0.7), and sialic acid (0.3%). The β_2-immunoglobulin found in equine secretion fluid was considered to

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be IgA; it was shown to contain galactose (2), mannose (2), fucose (0.5), 2-amino-2-deoxyhexose (1.5), and sialic acid (0.35%), and was separable into 7S (80%) and 11S (20%) species.

Bovine secretory immunoglobulin IgA (molecular weight 4.1×10^5) was found to be composed of α-chains (6.1×10^4), L-chains (2.3×10^4), and free secretory component ($7.0—8.6 \times 10^4$, glycoprotein 'a').[1296] The α-chains are antigenically and physicochemically distinct from the H-chains of immunoglobulins IgG_1, IgG_2, and IgM, whereas the L-chains are identical to those of the other bovine immunoglobulins. The secretory IgA itself was found to be physicochemically similar to human, bovine, and rabbit serum IgA. Lachrymal and nasal bovine IgA's possess antigenic determinants additional to those of the colostral IgA. Dimeric IgG_1 is abundant in colostrum and is similar in size to the IgA. The isolation of an immunoglobulin from bovine milk with the immunological characteristics of secretory IgA has been described.[1297] The component (11.2S) and a 6.7S component were isolated from serum. An additional antigenic determinant was demonstrated on the secretory immunoglobulin similar to 'secretory piece' in other species. The presence of free 'secretory piece' was demonstrated in milk, colostrum, and intestinal secretions and the molecular-size characteristics of IgA in these secretions were determined. Contrary to the observations in other species, IgA is present only in low levels in bovine saliva and milk. The main immunoglobulin of bovine lachrymal fluid is IgA, but IgG_1 and IgM are also present.[1298] The levels of the latter two correlate with the corresponding serum levels and it was suggested that they are derived from the plasma.

Three homogeneous immunoglobulins have been isolated from the serum and urine of dogs with plasma-cell dyscrasias.[1299] One of the immunoglobulins was identified as a myeloma form of IgA and all were shown to contain hexose, fucose, 2-amino-2-deoxyglucose, and sialic acid.

The properties of rabbit secretory immunoglobulin IgA and IgG have been investigated.[1300]

The carbohydrate compositions of mouse myeloma immunoglobulins IgA, IgG, and IgM have been determined.[1301] A glycopeptide derived from the IgG by proteolysis contained galactose (1), mannose (6), fucose (2), and 2-acetamido-2-deoxyglucose (5 residues per mole); in some instances, the carbohydrate moieties carried neuraminic acid units. Normalization of the immunoglobulins was achieved by treatment with glycosidases, but the antibodies did not appear to react against oligosaccharide moieties. A mouse myeloma IgA, which bound nitrophenyl ligands, and its Fab′ fragment have been purified by affinity chromatography

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on an ε-*N*-dinitrophenyl-lysine derivative of Sepharose.[1302] Investigation of the addition of terminal monosaccharide units to a mouse myeloma immunoglobulin IgA by incorporation experiments with radioactive L-fucose, 2-amino-2-deoxy-D-glucose, and sialic acid showed that the L-fucose units are added at a later stage than the sialic acid units.[1303] A transplantable BALB/c mouse plasmacytoma (MOPC 406) has been shown to produce an immunoglobulin of the IgA class.[1304] The best hapten inhibitor of the antibody-combining site was methyl 2-acetamido-2-deoxy-β-D-mannopyranoside and this finding was used in elucidation of the antibody-combining sites. The myeloma immunoglobulin IgA of mouse BALB/c origin could be precipitated with antigenic materials in food and bedding (larchwood arabinogalactan, gum ghatti, hardwood extract) containing side-chains of β-(1 → 6)-linked D-galactopyranose units.[1305] Oligosaccharides of the type $\{O\text{-}\beta\text{-D-galactopyranosyl-}(1 \rightarrow 6)\}_n$-D-galactose were potent inhibitors of the precipitation with the arabinogalactan. One IgA was precipitated with antigenic material found in wheat. The surface immunoglobulins IgA, IgG, and IgM of mouse lymphocytes have been determined.[1306]

Antigenic inter-relationships among equine and canine immunoglobulins IgB, IgG, and IgT have been studied.[1307]

The effects of heat upon the reactivity and aggregation of human immunoglobulin IgD have been investigated.[1308] Increased levels of IgD in cases of Down's syndrome and mentally retarded subjects have been attributed to infection.[1309] Sedimentation coefficients, Stokes' radii, molecular weights, and frictional coefficient ratios have been estimated for Fc and Fab fragments of human myeloma IgD; the molecular conformation of the molecule was related to its proteolytic degradation.[1310]

Immunoglobulin IgE has been located on the surface membrane of human basophils,[1311] and mouse myeloma IgF, IgG, and IgH have been separated by isoelectric focusing.[1312]

Measurements of immunoglobulin IgG with the aid of an IgG–peroxidase conjugate and cross-linked IgG,[1313] and also with an IgG–alkaline phosphatase conjugate and insolubilized anti-IgG,[1314] have been described. Hydroxylapatite has proved useful in the separation of human immunoglobulin IgG from other serum and plasma components,[1158] and protein A

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from *Staphylococcus aureus*, insolubilized by attachment to Sepharose, has been used for the purification of human IgG.[1315] Factors affecting the crystallization of human serum immunoglobulin IgG have been investigated[1316] and crystallographic studies of this immunoglobulin[1316] and of human IgG_3[1317] have been reported. The major electrophoretic fractions of the products arising from digestion of human IgG with plasmin have been characterized in detail.[1318] It was found that heterogeneity did not arise from differences in the hexose and sialic acid contents of the fractions. The adsorption of human IgG by insolubilized bovine serum albumin has been investigated.[1196]

C.d. spectral studies of the constant-region halves of human immunoglobulin IgG H- and L-chains demonstrated the presence of β-structured regions in the C_κ, C_λ, Fc, and pF'c fragments.[1319] Extensive unfolding of the H-chains, but not the L-chains, occurred in acid. The effect of acid and heat treatments upon the i.r. spectra and *X*-ray diffraction patterns of human IgG H-chains and their β-pleated-sheet contents have been studied.[1320] According to the c.d. spectra, conformational differences between human IgG and IgM are more pronounced in their Fc regions, which carry class-specific groups, than in their Fab portions, which contain antigen-combining sites.[1321] Conformational studies of subfractions from the Fc region have been reported.[1322]

The interaction of L-chain fragments and H-chains of human immunoglobulin IgG has been studied[1323] and conformational effects resulting from modification of the IgG molecule have been reported.[1324] Removal of the hexose units by periodate oxidation caused total loss of platelet-stimulating activity, but hardly affected complement fixation.[1325] Levels of IgG in human cerebrospinal fluid have been measured.[1172]

Abnormally large human serum amylases appeared to be due to complexing of the enzyme molecules with immunoglobulins such as IgG.[1326] Serum levels of IgG have been related to the incidence of cryofibrinogenaemia in women taking oral contraceptives.[1233] Surface immunoglobulins of blood lymphocytes have been studied in cases of Hodgkin's disease.[1327]

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[1327] V. Grifoni, G. S. Del Giacco, P. E. Manconi, and S. Tognella, *Boll. Soc. ital. Biol. sper.*, 1972, **48**, 373.

α-NeuNAc-(2→6)-β-D-Gal*p*-(1→6)-β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-D-Man*p*-D-GlcNAc-D-GlcNAc-Asp

6 ? ↑ ↑ 1 1

α-D-Man*p* α-L-Fuc*p*

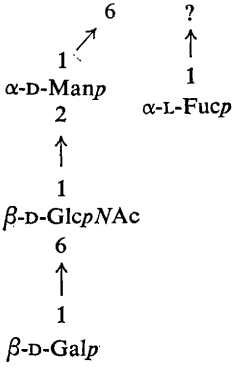
2 ↑ 1

β-D-GlcNAc

(76)

The plasma and serum from a patient with multiple myeloma were found to contain a crystallizable immunoglobulin IgG (molecular weight 1.582×10^5) of the κ-type, which was able to bind abnormally large amounts of heparin.[1328] Comparison of the structural features of human normal and myeloma IgG's light chains suggested the presence of two compact domains connected by a small switch region.[1329] *X*-Ray crystallography of human myeloma IgG_1 has led to an improved overall structure for the molecule.[1330] Glycopeptides obtained from serum immunoglobulins of patients with myeloma by digestion with pronase have been subjected to sequential hydrolyses with glycohydrolases; combination of the data obtained with those previously reported led to the structures (76) and (77)

β-D-Gal*p*-(1 → 6)-β-D-Glc*pN*Ac-(1 → 2)-α-D-Man*p*-(1 → 3)-D-Man*p*-D-Glc*N*Ac-Asp



(77)

for the carbohydrate portions of glycopeptides derived from IgG and IgM, respectively.[1331]

The basic immunoglobulin isolated from the serum of a patient with papular mucinosis appeared to be an immunoglobulin IgG_1 that is distinct from myeloma forms.[1332] The basicity of the immunoglobulin was much greater than that of normal IgG. An unusual IgG complex[1333] and a non-melomatous monoclonal IgG-L[1334] have been demonstrated in the serum and synovial fluid, respectively, of cases of rheumatoid arthritis. The subclasses of immunoglobulin IgG of cases of systemic lupus erythematosus and progressive systemic sclerosis have been studied.[1335] An

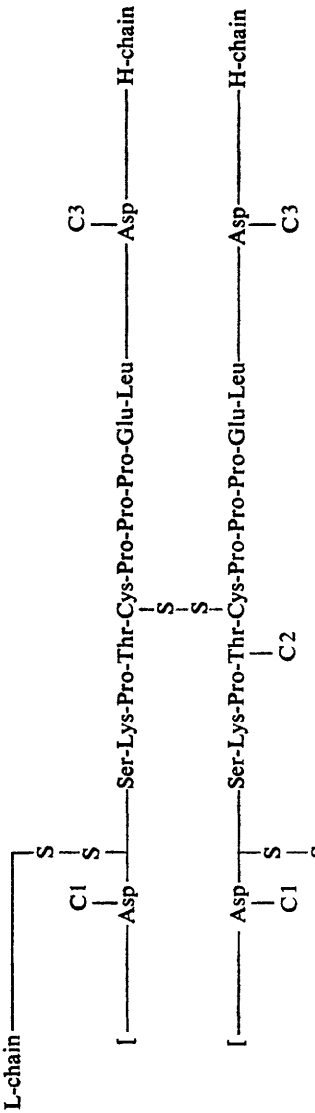
[1328] H. I. Glueck, M. R. MacKenzie, and C. J. Glueck, *J. Lab. Clinical Med.*, 1972, **79**, 731.
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[1334] Z. Hrnčíř, M. Tichý, and J. Vaňásek, *Clinica Chim. Acta*, 1972, **40**, 237.
[1335] P. H. Schur, M. Monroe, and N. Rothfield, *Arthritis Rheum.*, 1972, **15**, 174.

$F(ab')_2$-like immunoglobulin fragment found in the urine of patients with systemic lupus erythematosus resembled that obtained from IgG and similar serum and urinary components obtained from cases of burns.[1336]

Bovine serum IgG_2 interacted with human rheumatoid factor and the association was found to occur at a site in the hinge region of the immunoglobulin.[1337] This is in contrast to human and rabbit IgG, where the combining sites are located in the Fc region. The differences were related to differences in the carbohydrate moieties of the three immunoglobulins. Fractions physicochemically and immunologically identical to serum immunoglobulin IgG have been isolated from bovine lymph-node fractions by electrodecantation.[1338] Sheep IgG coupled to plastic tubes and rabbit IgG coupled to alkaline phosphatase have been used in a competitive solid-phase radioimmunoassay for IgG.[1339]

Carbohydrate analysis of fragments of rabbit immunoglobulin IgG indicated that three oligosaccharide moieties are located at separate sites on the H-chain and the corresponding glycopeptides were isolated.[1340] Oligosaccharide C1, containing galactose (2), mannose (1), and 2-amino-2-deoxyglucose (2 residues per mole), is linked to the amide group of asparagine in a peptide from the Fd fragment and is present in 15% of the H-chains. Oligosaccharide C2, containing galactose (1), 2-amino-2-deoxygalactose (1), and sialic acid (1—2 residues per mole), is linked glycosidically to the hydroxy-group of threonine in a peptide from the hinge region and is present in 40% of the H-chains. Oligosaccharide C3, containing galactose (2), mannose (5), fucose (1), 2-amino-2-deoxyglucose (5), and sialic acid (1 residue per mole), is linked to the amide group of asparagine in a peptide from the Fc fragment. It was possible to present a structure (78) showing the locations of all the oligosaccharide moieties in the immunoglobulin molecule. Two populations of IgG molecules have been isolated from pooled rabbit sera; one of these was devoid of 2-amino-2-deoxygalactose and the other contained one such residue per immunoglobulin molecule.[1341] The 2-amino-2-deoxygalactose residue was located in one of the four H-chains and the location of the carbohydrate moiety in a glycopeptide containing a disulphide bridge and derived from the IgG was also determined. An IgG rabbit antibody to group C Streptococcal polysaccharide has been isolated by affinity chromatography[1342] and the reactions of rabbit IgG and IgM with staphylococcal protein have been investigated.[1343] Rabbit IgG co-insolubilized with ovomucoid or bovine

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C1, C2, and C3 denote oligosaccharides referred to in the text

(78)

serum albumin has been used in the affinity chromatography of proteins.[691]

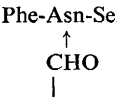
Immunoglobulins $IgG_{1\,\&\,2}$ and IgM have been detected in hedgehog serum.[1344] Treatment of radio-labelled mouse myeloma IgG and its glycopeptide with an endo-β-acetamidodeoxyglucosidase yielded an oligosaccharide, containing mannose and 2-amino-2-deoxyglucose, that yielded 2-amino-2-deoxyglucitol on reduction and hydrolysis.[1345] Incorporation of L-[^{3}H]fucose, D-[^{14}C]mannose, and 2-amino-2-deoxy-D-[^{3}H]glucose into the glycopeptide during culture was studied and it was concluded that two fractions obtained from the glycopeptide by enzymic hydrolysis contained trimannosyl-2-acetamido-2-deoxyglucose, and fucose, mannose, 2-acetamido-2-deoxyglucose, and peptide, respectively.

The kinetics of incorporation of labelled monosaccharides into secreted and intracellular mouse MOPC 21 IgG_1 and MOPC 46 κ-type L-chains have shown that the carbohydrate moieties can be labelled by specific monosaccharides and suggested that fucose residues are added while the glycoprotein is in the final stage of secretion from the plasma cell.[1346] L-Chains isolated from subcellular fractions of mice MOPC 46 myeloma cells and urine were shown to contain galactose (0.3—1.3, 4), mannose (4.0—4.2, 4), fucose (0.0—0.6, 2), 2-amino-2-deoxyglucose (3.0, 3), and sialic acid (?, 0—2%, respectively).[1347] From further studies, it was concluded that the single carbohydrate moiety attached to the L-chains is synthesized by acquisition of different monosaccharides in the rough and smooth membrane regions. Subcellular fractions from mouse plasma cell MOPC 21 and 26 tumours have been labelled with various radioactive monosaccharides; analyses of the labelled immunoglobulins not only supported the idea of the addition of different monosaccharides in different regions, but demonstrated the stages at which the additions take place.[1348] Incorporation of D- and L-[1-^{3}H]fucose into the IgG_1 myeloma immunoglobulin secreted by MOPC 21 was selective for the L-isomer and the H-chains carried 90% of the label as L-fucose.[1349] The synthesis and secretion of the glycoprotein were facilitated by the presence of D-galactose, D-mannose, and 2-amino-2-deoxy-D-glucose. Suppression of IgG biosynthesis in mouse myeloma and fibroblast cells has been studied.[1350]

Evidence has been reported for the synthesis of immunoglobulin IgG by rat spleen nuclei.[1351] The light[1352] and the heavy chains[1353] of

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[1352] J. C. Travis and B. G. Sanders, *Comp. Biochem. Physiol.*, 1972, **43B**, 627.
[1353] J. C. Travis and B. G. Sanders, *Comp. Biochem. Physiol.*, 1972, **43B**, 637.

immunoglobulins extracted from the plasma of fin-backed (*Balaenoptera physalus*), hump-backed (*Megaptera novaeangliae*), and Sei (*Physeter catoden*) whales have been studied. Glycopeptides (79) derived from the heavy chains were similar to those derived from human IgG and immunoglobulins of other species.



(79)

IgG and IgM immunoglobulins have been isolated from turtle (*Chelydra serpentina*) serum.[1354] The IgG (molecular weight 3.8×10^4) was shown to contain hexose (0.23), fucose (0.4), and 2-amino-2-deoxyhexose (0.05%), and the subunit structures of both immunoglobulins were compared with those of mammalian immunoglobulins. Immunoglobulins of high and low molecular weight have been isolated from the serum of the bowfin (*Amia calva*) after it had been immunized with *Brucella abortus*.[1252] The 13.6S and 6.3S components were shown to contain hexose (5.9, 4.8), fucose (0.7, 0.6), 2-amino-2-deoxyhexose (3.3, 2.6), and sialic acid (0.8, 1.1%, respectively), and the compositions were compared with those of human IgG and IgM.

The acquisition of carbohydrates in the disulphide-linked heavy and light chains of IgG of murine ADJPC5 myeloma has been studied.[1355] That some mannose and 2-amino-2-deoxyglucose units are acquired by precursor molecules was demonstrated by their presence in HH, HHL, and LHHL molecules, but galactose was observed only in LHHL molecules. The various stages of carbohydrate addition were found to occur in different microsome fractions. Insolubilized IgG has been prepared using a nylon support.[734]

The isolation of immunoglobulin IgM from human serum by a combination of gel filtration and electrochromatography has been described [1356] and the structure of the light chain has been investigated.[1357] The properties of IgM-L have been reported [1358] and the monomeric unit of the human immunoglobulin was found to contain galactose (11), mannose (35), fucose (6), 2-acetamido-2-deoxyglucose (27), and sialic acid (9 residues).[1359] Two types of oligosaccharide moieties were present in the immunoglobulin; one of these (A) contained all the foregoing monosaccharides, whereas

[1354] S. L. Chartrand, G. W. Litman, N. Lapointe, R. A. Good, and D. Frommel, *J. Immunol.*, 1971, **107**, 1.
[1355] E. W. Sutherland, D. H. Zimmerman, and M. Kern, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 167.
[1356] J. Salák and P. Roch, *J. Chromatog.*, 1972, **71**, 459.
[1357] J. D. Capra, J. M. Kehoe, R. C. Williams, T. Feizi, and H. G. Kunkel, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 40.
[1358] M. Tichý, Z. Hrnčíř, and Z. Hrkal, *Clinica Chim. Acta*, 1972, **36**, 363.
[1359] I. Johnson and J. R. Clamp, *Biochem. J.*, 1971, **123**, 739.

the other (B) contained D-mannose and 2-acetamido-2-deoxy-D-glucose only. A structure (80) was proposed for B and the IgM was shown to

α-D-Man-α-D-Man-α-D-Man-α-D-Man-α-D-Man-(1 → 3 or 4)-β-D-Glc*p*NAc-(1 → 3)-α-D-Man*p*-(1 → 3)-α-D-Man*p*-(1 → 3 or 4)-β-D-Glc*p*NAc → Asp

(80)

contain six type A and two type B oligosaccharide moieties. Human and murine thymus lymphocytes possess only IgM immunoglobulins on their surfaces, whereas murine splenic lymphocytes possess both IgG and IgM on their surfaces.[1360]

A fragment (molecular weight 2.0×10^4) containing 25% carbohydrate has been identified in $(Fc)_{5u}$ preparations of trypsin digests of human λ-type monoclonal IgM molecules.[1361] Heterogeneity has been observed in the M-components of macroglobulinaemia sera with anti-polysaccharide activity.[1362] The oligosaccharide moieties of Fab-like fragments, prepared by proteolysis of IgM immunoglobulins from patients with Waldenström's macroglobulinaemia, have been located.[1363] A moiety comprising galactose (2), mannose (3), fucose (1), 2-amino-2-deoxyglucose (2), and sialic acid (1 residue(s) per mole) was located near the *N*-terminus and a second containing galactose (2), mannose (6—7), and 2-amino-2-deoxyglucose (3 residues per mole) in the hinge region. The Fc portion of the molecule contains one or more oligosaccharides consisting collectively of galactose (1), mannose (10—11), fucose (1), 2-amino-2-deoxyglucose (5), and sialic acid (3 residues per H-chain). Five different glycopeptides have been isolated from an IgM of the human Waldenström type and structures (81)—(84) were proposed for four of them on the basis of extensive studies.[1364] Microheterogeneity was demonstrated in some cases, with more complex moieties sometimes lacking a full complement of sialic acid and fucose residues.

Heavy and light chains (molecular weights 6.18 and 2.28×10^4, respectively) have been prepared from bovine immunoglobulin IgM and the heavy chain was found to contain hexose (6.8), 2-amino-2-deoxyhexose (4.7), and sialic acid (1.9%).[1365] Similarities were observed between the arrangements of the various chains in this molecule and those of human IgM, and a general representation (85) of the IgM molecule was proposed on the basis of the data obtained.

Mouse anti-Type III pneumococcal polysaccharide immunoglobulin IgM has been studied[1366] and dextran conjugates have been used for the

[1360] J. J. Marchalonis, R. E. Cone, and J. L. Atwell, *J. Exp. Med.*, 1972, **135**, 956.
[1361] A. G. Plaut, N. Calvanico, and T. B. Tomasi, *J. Immunol.*, 1972, **108**, 289.
[1362] K. Hannestad and K. Sletten, *J. Biol. Chem.*, 1971, **246**, 6982.
[1363] W. Niedermeier, R. E. Schrohenloher, and M. Hurst, *J. Immunol.*, 1972, **108**, 346.
[1364] S. Hickman, R. Kornfeld, C. K. Osterland, and S. Kornfeld, *J. Biol. Chem.*, 1972, **247**, 2156.
[1365] D. Beale and N. Buttress, *Biochim. Biophys. Acta*, 1972, **257**, 372.
[1366] J. G. Howard, G. H. Christie, and B. M. Courtenay, *Proc. Roy. Soc.*, 1971, **B178**, 417.

α-D-Man*p*-(1 → 2)-α-D-Man*p*-(1 → 3)-D-Man*p*-D-Glc*N*Ac-D-Glc*N*Ac-peptide
6
↑
1
α-D-Man*p*
D-Man

(81)

α-Neu*N*?-(2 → 6)-β-D-Gal*p*-(1 → 6)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 3)-D-Man*p*-D-Glc*N*Ac-D-Glc*N*Ac-peptide
6
↑
1
L-Fuc
β-D-Gal*p*-(1 → 6)-β-D-Glc*p*NAc-(1 → 2)-α-DMan*p*

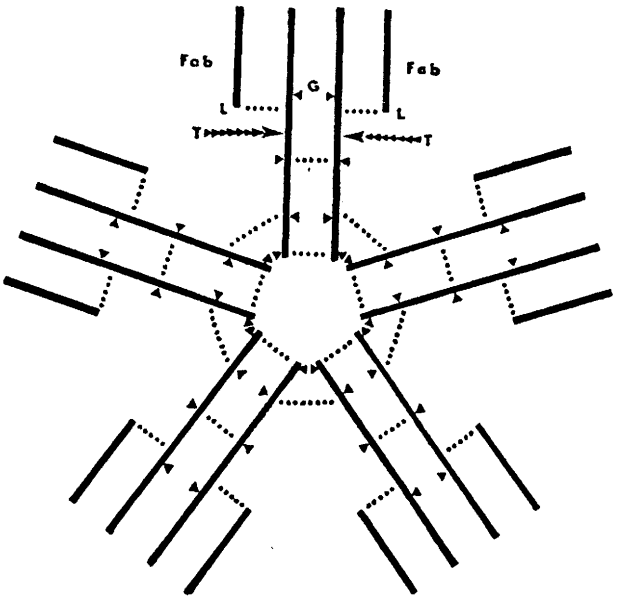
(82)

α-Neu*N*?-(2 → 6)-β-D-Gal*p*-(1 → 6)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 3)-D-Man*p*-D-Glc*N*Ac-D-Glc*N*Ac-peptide
6
↑
1
D-Man, L-Fuc
β-D-Gal*p*-(1 → 6)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*

(83)

α-Neu*N*?-(2 → 6)-β-D-Gal*p*-(1 → 6)-β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 3)-D-Man*p*-D-Glc*N*Ac-D-Glc*N*Ac-peptide
6
↑
1
L-Fuc
α-Neu*N*?-(2 → 6)-β-D-Gal*p*-(1 → 6)-β-D-Glc*p*NAc-(1 → 2)-α-DMan*p*

(84)



isolation of mouse MOPC-104E plasma tumour cell immunoglobulin IgM.[1198, 1367] 7S IgM subunits prepared from the cells contained galactose (0.5), glucose (2), mannose (33), fucose (2.5), 2-amino-2-deoxygalactose (2), and 2-amino-2-deoxyglucose (23 moles per mole), but no neuraminic acid.[1368] However, extracellular 19S IgM, either secreted by the plasma cells or present in the sera of mice, contained *N*-glycollylneuraminic acid in addition to these monosaccharides. The reversibility of formation of the 7S species from 19S IgM was investigated. Studies of the incorporation of D-[1-^{3}H]mannose, D-[1-^{3}H]galactose, and L-[1-^{3}H]fucose into mouse MOPC-104E cells suggested a stepwise addition of the carbohydrate residues to the immunoglobulin IgM.[1369] D-Mannose and 2-amino-2-deoxy-D-glucose residues were added at an early stage, whereas D-galactose and L-fucose residues were added at the time of leaving the cell. Free light chains secreted by the same cells contained only small amounts of carbohydrate.

IgY and traces of IgM have been detected in chick secretions.[1370] An immune macroglobulin synthesized by a turtle (*Pseudemus scripta*) against

[1367] V. K. Ghanta, N. M. Hamlin, T. G. Pretlow, and R. N. Hiramoto, *J. Immunol.*, 1972, **109**, 810.
[1368] F. Melchers, *Biochemistry*, 1972, **11**, 2204.
[1369] R. M. E. Parkhouse and F. Melchers, *Biochem. J.*, 1971, **125**, 235.
[1370] G. A. Leslie, H. R. Wilson, and L. W. Clem, *J. Immunol.*, 1971, **106**, 1441.

Salmonella typhosa 'H' antigen was shown to possess a molecular weight of 8.5×10^5 and to contain 6.7% carbohydrate comprising galactose (89), mannose (79), 2-amino-2-deoxyglucose (104), and sialic acid (11 moles per mole).[1371] Heavy and light chains (molecular weights 6.7 and 2.25×10^4, respectively) were derived from the molecule, which resembles mammalian immunoglobulin IgM.

A 14S serum immunoglobulin (molecular weight 6.5×10^5) has been isolated in homogeneous form from the gar (*Lepisosteus platyrhinicus*) and was found to contain 3.5% hexose.[1372] The glycoprotein was dissociable into equal amounts of two subunits (molecular weights 7.0 and 2.2×10^4) and it was concluded that it is a tetrameric form of IgM. An immune macroglobulin (molecular weight 6.6×10^5) has been purified from the paddlefish (*Polyodon spathula*); on dissociation, it yielded heavy and light chains (molecular weights 5.81 and 2.10×10^4, respectively), which possessed similar compositions to the H- and L-chains of IgM.[1373] An immunoglobulin (molecular weight 8.20×10^5) has been isolated from serum of the stingray (*Dasyatis americana*) and was shown to contain galactose (2.6), mannose (2.8), fucose (0.2), 2-amino-2-deoxyglucose (2.4), and sialic acid (1.3%).[1374] Reduction and alkylation yielded the subunits (molecular weights 7.0 and 2.3×10^4), and structural data for the macroglobulin were similar to those for mammalian IgM.

Following immunization with *Salmonella typhosa* H (STH) antigen, the serum of marine toad was found to contain 17.9S and 7.3S species (molecular weights 8.8 and 1.6×10^5) designated immune macroglobulin and low molecular weight antibody, respectively.[1375] The molecules contained galactose (103, 14), mannose (103, 9), fucose (5, 0), 2-amino-2-deoxyglucose (124, 10), and sialic acid (23, 3 residues per mole, respectively). A 7S globulin, which contained small amounts of carbohydrate and possessed anti-human blood-group H(O) specificity, was precipitable by 3-*O*-methyl-D-fucose, 3-*O*-methyl-D-galactose, and blood-group H(O)-specific macromolecules.[1376]

4.3S Free secretory component from human milk whey has been purified to homogeneity by immunoadsorbent techniques.[1377] The component was shown to contain galactose (8 or 9), mannose (8 or 9), fucose (5), 2-acetamido-2-deoxyglucose (11), and *N*-acetylneuraminic acid (6 residues per molecular weight of 6.6×10^4).

[1371] R. T. Acton, P. F. Weinheimer, E. Shelton, W. Niedermeier, and J. C. Bennett, *Immunochemistry*, 1972, **9**, 421.
[1372] C. M. Bradshaw, L. W. Clem, and M. M. Sigel, *J. Immunol.*, 1971, **106**, 1480.
[1373] R. T. Acton, P. F. Weinheimer, H. K. Dupree, T. R. Russell, M. Wolcott, E. E. Evans, R. E. Schrohenloher, and J. C. Bennett, *J. Biol. Chem.*, 1971, **246**, 6760.
[1374] W. H. Johnston, R. T. Acton, P. F. Weinheimer, W. Niedermeier, E. E. Evans, E. Shelton, and J. C. Bennett, *J. Immunol.*, 1971, **107**, 782.
[1375] R. T. Acton, E. E. Evans, P. F. Weinheimer, W. Niedermeier, and J. C. Bennett, *Biochemistry*, 1972, **11**, 2751.
[1376] G. F. Springer and P. R. Desai, *Biochemistry*, 1971, **10**, 3749.
[1377] P. J. J. Van Munster, G. B. A. Stoelinga, J. R. Clamp, J. J. T. Gerding, J. C. M. Reijnen, and M. Voss, *Immunology*, 1972, **23**, 249.

Salivary, Mucous, and other Mammalian Body-fluid Glycoproteins

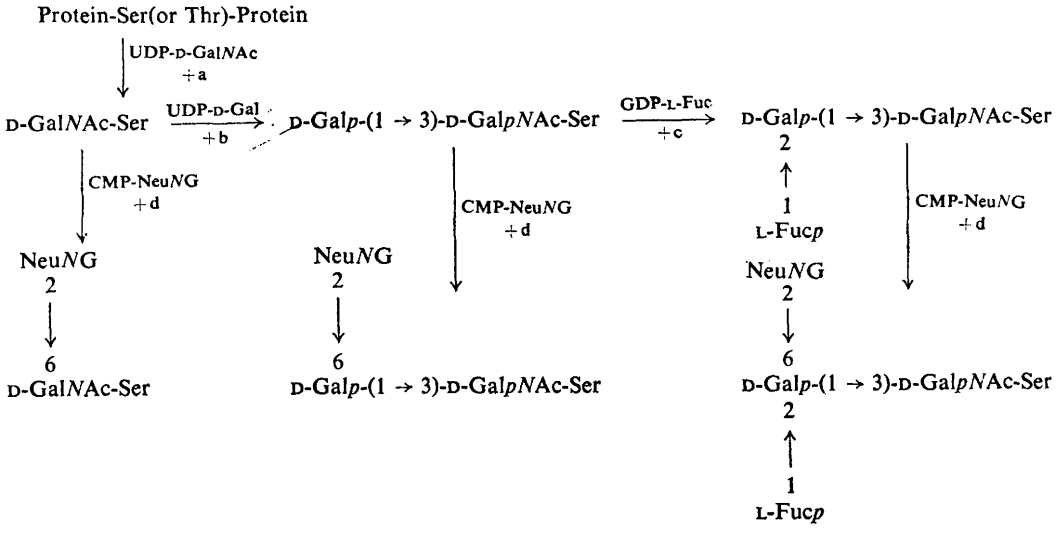
The use of methanolysis in determination of the carbohydrate components of human mucus specimens has been assessed.[1273] Soluble and insoluble mucus glycoproteins were all found to contain galactose, mannose, fucose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, and sialic acid.[1011]

Bovine submaxillary glycoprotein contains sialic acid residues, which were released at a slow rate under standard conditions of hydrolysis (0.05 M-H_2SO_4, 1 h, 80 °C).[1378] These residues were also resistant to neuraminidases and were considered to be *O*-acetylated neuraminic acid units. The 2-amino-2-deoxyhexose and sialic acid units of bovine submaxillary mucin were removed by the action of UDP-2-acetamido-2-deoxy-D-galactose:protein 2-acetamido-2-deoxy-D-galactosyltransferase of human serum.[1379] The glycoprotein has been studied as an inhibitor of proteolysis.[881]

Dilute periodate has been shown to oxidize selectively the *N*-acetylneuraminic acid units of ovine submaxillary mucin to the eight- and seven-carbon analogues; the action of neuraminidases on the modified glycoprotein has been studied.[1166] Incubation of ovine submaxillary mucin and the mucin of the Cowper's gland of boar with a factor from animal tissues caused up to 20% of the bound sialic acid residues to act as the free form in a Warren assay.[1380] The transfer of radioactive monosaccharides from nucleoside diphosphates to desialylized porcine submaxillary mucin by glycosyltransferases has been investigated.[680] The structures of oligosaccharides isolated from blood-group A^-H^--inactive mucins secreted by porcine submaxillary glands have been investigated in an attempt to explain the serological relationship of the mucin to H^+-active mucin.[767] The following oligosaccharides were identified after treatment with alkaline borohydride: *O*-α-L-fucopyranosyl-(1 → 2)-{*N*-glycollylneuraminyl-(2 → 6)}-*O*-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-D-galactitol, *N*-glycollylneuraminyl-(2 → 6)-*O*-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-D-galactitol, *N*-glycollylneuraminyl-(2 → 6)-2-acetamido-2-deoxy-D-galactitol, and the corresponding desialylized, neutral oligosaccharides. From the data obtained, it was deduced that two sialyltransferases are involved in the biosynthesis of the mucin, and a biosynthetic pathway (Scheme 11) was proposed. The biosynthesis of glycoproteins in the isoproterenol-stimulated salivary glands of mice has been studied.[1381]

Differences have been observed in the carbohydrate compositions of human biliary glycoproteins from normal and lithogenic specimens.[1382]

[1378] A. Neuberger and W. A. Ratcliffe, *Biochem. J.*, 1972, **129**, 683.
[1379] G. K. W. Ko and E. Raghupathy, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1704.
[1380] C. R. Brown, P. N. Srivastava, and E. F. Hartree, *Biochem. J.*, 1970, **118**, 123.
[1381] N. Galanti and R. Baserga, *J. Biol. Chem.*, 1971, **246**, 6814.
[1382] I. A. D. Bouchier and J. R. Clamp, *Clinica Chim. Acta*, 1971, **35**, 219.

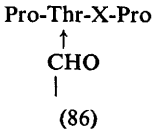


a = 2-acetamido-2-deoxy-D-galactosyltransferase
b = D-galactosyltransferase
c = L-fucosyltransferase
d = sialyltransferase

Scheme 11

Digestion with pronase of the normal material yielded two glycopeptides containing galactose (6, 6), mannose (0, 4), fucose (5, 3), 2-acetamido-2-deoxygalactose (2, 1), 2-acetamido-2-deoxyglucose (5, 6), and sialic acid (1 and 4 residues per mole, respectively).

Human bronchial mucins have been purified. All glycopeptides derived from them by proteolysis possessed the basic structure (86) and it was



(86)

suggested that the peptide codes for the attachment of carbohydrate in the biosynthesis of the glycoprotein.[1383] The biosynthesis of respiratory tract mucins has been studied.[1384] The glycoproteins of sputum from bronchitic and asthmatic patients have been studied.[1385] Gel filtration of proteolytic digests of acid-soluble and -insoluble mucins yielded three and two glycopeptide fractions, respectively. The latter two fractions were characterized by high sulphate (5—6%) and low carbohydrate contents, whereas the former three fractions contained greater amounts of carbohydrate and smaller amounts of sulphate. Typical monosaccharides were found in the glycopeptides.

The presence of sulphated glycoproteins in normal gastric juice has been attributed to contaminants from the upper digestive tract, since the saliva, but not the pure gastric juice, of oesophagectomized patients contained sulphated glycoproteins.[1386]

A gas-chromatographic method for the determination of fucose has been advocated as a means of assaying porcine gastric mucin glycoprotein.[644] Fractionation of water-soluble porcine gastric mucus by equilibrium density-gradient centrifugation in caesium chloride removed a non-covalently bound protein from the glycoprotein, which was judged to be homogeneous by other criteria.[1387] The 33S (and largest) component of the mucus was shown to contain hexose (26), fucose (11), 2-amino-2-deoxygalactose (8), and 2-amino-2-deoxyglucose (20%). Viscometric studies of the 33S and 5.7S components demonstrated that irreversible changes in the tertiary structures of these macromolecules can occur during density-gradient centrifugation.[1388] Lowering of the molecular weights of porcine gastric mucin glycoproteins by the action of 2-mercaptoethanol was found to occur in four-fold drops.[1389] Optimum conditions for the incorporation

[1383] P. Degand, R. Havez, and G. Biserte, *Compt. rend.*, 1972, **275**, *D*, 491.
[1384] D. B. Ellis, J. R. Munro, and G. H. Stahl, *Biochim. Biophys. Acta*, 1972, **289**, 108.
[1385] H. C. Ryley, *Biochem. J.*, 1972, **128**, 129P.
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[1387] B. J. Starkey, D. Snary, and A. Allen, *Biochem. J.*, 1972, **128**, 123P.
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[1389] D. Snary, A. Allen, and R. H. Pain, *Biochem. Biophys. Res. Comm.*, 1970, **40**, 844.

of radioactive monosaccharides into porcine gastric mucosal glycoproteins have been determined and 20% of the incorporated material was present in a water-soluble form.[1032] Physicochemical studies have been reported for two glycoprotein components of a water-soluble mucin from the cardiac region of porcine stomach.[1390] The relationship between the secretion of sialoglycoproteins and hydrochloric acid by rat stomach has been investigated.[1391]

Human seminal plasma has been shown to contain at least six glycoproteins, while the use of a range of lectins and protectins demonstrated the existence of terminal, non-reducing β-D-galactosyl, 2-acetamido-2-deoxy-D-galactosyl, and *N*-acetylneuraminosyl residues.[1392] 2-Acetamido-2-deoxy-D-glucose was found as an internal unit, whilst, in one case, removal of *N*-acetylneuraminic acid units exposed 2-acetamido-2-deoxy-D-galactosyl residues.

Urinary Glycoproteins

The isolation and crystallization of a λ-type Bence-Jones glycoprotein from human urine has been described[1316] and structural details of a glycoprotein from a myeloma patient have been compared with that of human immunoglobulin IgG light chains.[1329] The carbohydrate chain of human Bence-Jones glycoprotein has been shown to be attached to asparagine-93 of the protein chain and to contain neutral carbohydrate and 2-amino-2-deoxyhexose.[1393, 1394] Human κ and λ Bence-Jones glycoproteins have been examined by i.r. spectroscopy and *X*-ray diffraction[1320] and also by c.d.[1395] Amyloid fibrils have been formed from some of them.[1396] The preparation of an insolubilized form of human Bence-Jones glycoprotein has been reported.[1293]

A glycopeptide with the characteristics of 2-acetamido-1-(β-L-asparta-mido)-1,2-dideoxy-β-D-glucose has been isolated from normal human urine.[1397] In addition to this glycopeptide, urine from cases of aspartylglycosaminuria was found to contain a glycopeptide that yielded uronic acid, 2-amino-2-deoxyhexose, sialic acid, and 2-acetamido-1-(β-L-asparta-mido)-1,2-dideoxy-β-D-glucose on hydrolysis.[976, 1008] Further studies of urine from cases of aspartylglycosaminuria showed that at least nine acidic glycopeptides, most of which contain sialic acid, could be separated.[1398] Four major neutral glycopeptides also isolated were partially identified as

[1390] D. Snary, A. Allen, and R. H. Pain, *Biochem. J.*, 1972, **127**, 68P.
[1391] L. Carlborg, S. Dahlgren, H. Johansson, B. Lindquist, and B. Nordgren, *Acta Soc. Med. Upsaliensis*, 1971, **76**, 10.
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[1393] F. A. Garver and N. Hilschmann, *F.E.B.S. Letters*, 1971, **16**, 128.
[1394] F. A. Garver and N. Hilschmann, *European J. Biochem.*, 1972, **26**, 10.
[1395] H. L. Kincaid and B. Jirgensons, *Biochim. Biophys. Acta*, 1972, **271**, 23.
[1396] G. G. Glenner, D. Ein, E. D. Eanes, H. A. Bladen, W. Terry, and D. L. Page, *Science*, 1971, **174**, 712.
[1397] B. O'N. Rowley and P. B. Hamilton, *Clinical Chem.*, 1972, **18**, 951.
[1398] R. J. Pollitt and K. M. Pretty, *Biochem. J.*, 1972, **128**, 129P.

galactosyl, galactosylfucosyl, dimannosylgalactosyl, and dimannosyl-2-acetamido-2-deoxyglucosyl derivatives of 2-acetamido-1-(β-L-aspartamido)-1,2-dideoxy-β-D-glucose.

A crystalline glycopeptide containing D-galactose, xylose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and glucuronic acid has been isolated from normal human urine; the similarity of the glycopeptide to that from the glycopeptide regions of proteoglycans was noted.[911]

Digalactosylcysteine has been identified as the glycopeptide linkage region of a human urinary glycopeptide containing galactose as the only carbohydrate.[1399] The role of sialic acid in crude extracts of human urinary gonadotrophin-inhibiting substance has been investigated.[1115]

Rabbit Tamm–Horsfall glycoprotein contains sialic acid residues that were released at a slow rate under standard conditions of acid hydrolysis (0.05M-H_2SO_4, 1 h, 80 °C), but which were resistant to neuraminidase.[1378] This behaviour was attributed to the presence of *O*-acetylated sialic acid residues, since mild alkaline treatment normalized the release of sialic acid. It was found that, on average, two hydroxy-groups of the sialic acid residues are *O*-acetylated and that these groups are located either on the polyhydroxy side-chain or at O-4 and O-8. The diffusion coefficient and molecular weight (14.2×10^6) of Tamm–Horsfall glycoprotein have been determined by intensity-fluctuation spectroscopy.[1400]

A basement membrane antigen has been purified to homogeneity from the urine of nephritic rabbits.[1064] It was shown that the macromolecule contains galactose (2.1), mannose (0.8), fucose (0.5), glucose (8.8), and 2-amino-2-deoxygalactose (0.5%), and it was concluded that the antigen resembles a nephrotoxic glycoprotein isolated from rat glomerular basement membranes. The galactose and glucose contents of non-collagenous rat urinary glycoprotein were increased by the action of cyclophosphamide *in vivo*.[1063] A glycoprotein having a similar amino-acid, but a richer hexose, composition was isolated from rat urine; this macromolecule also appeared to be a fragment of glomerular basement membrane material.[1401]

A gas-chromatographic method for the assay of neuraminosyl-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose (neuraminosyl-lactose) and neuraminosyl-*O*-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose-(neuraminosyl-*N*-acetyl-lactosamine) has been reported and was used to investigate the effects of various factors upon the excretion of these compounds.[1402] Ten neuraminosyl oligosaccharides have been isolated from rat urine and, from extensive studies, the structures of five of them [(87)—(91)] were deduced.[1403, 1404] The increased excretion of these types of compounds by pregnant and lactating rats was found to involve (87) only.[1405]

[1399] C. J. Lote and J. B. Weiss, *F.E.B.S. Letters*, 1971, **16**, 81.
[1400] C. J. Oliver, E. R. Pike, A. J. Cleave, and A. R. Peacocke, *Biopolymers*, 1971, **10**, 1731.
[1401] R. M. McIntosh, H. Kihara, C. Kulvinskas, D. B. Kaufman, and S. R. Wong, *Ann. Rheumatic Diseases*, 1971, **30**, 631.
[1402] P. Maury and J. K. Huttunen, *Clinica Chim. Acta*, 1972, **37**, 433.
[1403] P. Maury, *Biochim. Biophys. Acta*, 1971, **252**, 48.
[1404] P. Maury, *Biochim. Biophys. Acta*, 1971, **252**, 472.
[1405] P. Maury, *J. Biol. Chem.*, 1972, **247**, 3153.

α-Neu*p*NAc-(2 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc
(87)

α-Neu*p*NAc-(2 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*N*Ac
(88)

α-Neu*p*NAc-(2 → 3)-β-D-Gal*p*-(1 → 4)-D-Gal*N*Ac
(89)

α-Neu*p*NAc-7 or 8-*O*-Ac-(2 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc
(90)

α-Neu*p*NG-(2 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc
(91)

Avian Egg Glycoproteins

Disulphide-linked anti-ovalbumin can be used as an immunoadsorbent for the purification of ovalbumin,[639] and the electrophoretic mobilities of hen egg albumin in various polyacrylamide gels have been examined.[1186]

β-L-Aspartamido-carbohydrate has been prepared from the egg-white ovalbumins of duck, turkey, Western gull, and chicken, demonstrating the general nature of the glycopeptide linkages.[1406] In each case, the carbohydrate was paucidisperse and the compositions of the fractions for duck and turkey indicated that more than one oligosaccharide residue is present for each polypeptide chain. The data also support the hypothesis that the series of carbohydrate fractions represents the result of biosynthetic, but not genetic, heterogeneity or subsequent degradation. A glycosylasparagine unit isolated from hen egg ovalbumin was shown to have the structure (92) on the basis of component analysis, methylation, i.r. and o.r.d.

β-D-Man*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 4)-β-D-Glc*p*NAc-Asn
(92)

spectroscopy, and experiments with glycosidases.[652, 654, 656, 1407] The β-anomeric linkage of the D-mannopyranosyl residue was confirmed by use of β-mannosidases from a number of sources and is in contrast to the α-linkage previously reported.[654–656, 1408, 1409] The linkage between 2-acetamido-2-deoxy-D-glucose and asparagine was confirmed by the formation of terminal 2-amino-2-deoxy-D-glucitol on treatment of the glycopeptide with alkaline borohydride and by the conversion of this terminal unit into 2-amino-2-deoxy-D-xylitol on periodate oxidation, reduction, and hydrolysis.[652] A (D-mannosyl)$_6$-(2-acetamido-2-deoxy-D-glucosyl)$_2$-asparagine glycopeptide [656] and a related glycopeptide [1408] also isolated from ovalbumin

[1406] C. Huang and R. Montgomery, *Carbohydrate Res.*, 1972, **22**, 83.
[1407] A. L. Tarentino, T. H. Plummer, and F. Maley, *J. Biol. Chem.*, 1972, **247**, 2629.
[1408] K. Sugahara, T. Okumura, and I. Yamashina, *F.E.B.S. Letters*, 1972, **20**, 44.
[1409] Y. T. Li and Y. C. Lee, *J. Biol. Chem.*, 1972, **247**, 3677.

were found to contain α-D-mannosyl-D-mannose bonds only, while a (D-mannosyl)$_6$-(2-acetamido-2-deoxy-D-glucosyl)$_4$-asparagine from the same source was converted into (D-mannosyl)$_6$-(2-acetamido-2-deoxy-D-glucosyl)$_2$-2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucosylasparagine by an endoglycosidase.[1407]

The native and denatured states of ovalbumin have been compared by viscosimetric measurements.[1410] Denaturation by urea was followed by aggregation [1411] and the denatured state possessed a random-coil structure.[1412] The interaction of hen ovalbumin with sperm whale metmyoglobin has been studied.[1413]

The failure to detect anti-carbohydrate antibodies in anti-ovalbumin has been discussed in terms of the orientation and the availability of oligosaccharide moieties.[1414] It has been shown that RNA from hen oviduct can direct the biosynthesis of ovalbumin in a cell-free system; the ovalbumin was identified by a number of criteria.[1415] Investigation of the biosynthesis of egg ovalbumin, conalbumin, ovomucoid, and lysozyme led to the conclusion that a single regulatory site for their production is unlikely.[1416] Ovalbumin has been insolubilized by attachment to nylon.[734]

The preparation of egg-white albumin from Embden goose eggs has been described and the glycoprotein was found to contain greater amounts of carbohydrate — mannose (12) and 2-amino-2-deoxyglucose (8 moles per mole) — than hen egg-white ovalbumin.[1417] Proteolytic digestion yielded three glycopeptides, each containing the two monosaccharides, and it was concluded that the glycoprotein contains more than one prosthetic carbohydrate moiety per molecule.

A method has been described whereby small amounts of trypsin-inhibitor ovomucoid can be isolated from either egg white or oviduct by a single precipitation and affinity chromatography on trypsin insolubilized by attachment to Sephadex.[1418] Material isolated from both sources was homogeneous on immunoelectrophoresis, but similar charge heterogeneities were observed in each. Cyanogen bromide cleavage of ovomucoid variant O1 gave two fractions (molecular weights 1.55 and 1.00×10^4).[1419] Only the former could be subfractionated after reduction and aminoethylation, two fractions (12 and 4×10^3) being obtained. Of the total fractions, the smaller two were shown to possess carbohydrate moieties of hexose (5) and 2-amino-2-deoxyglucose (8 units), whereas the carbohydrate content of the largest fragment was heterogeneous, fractions

[1410] A. A. Ansari, R. Ahmad, and A. Salahuddin, *Biochem. J.*, 1972, **126**, 447.
[1411] A. I. Shulmina and V. V. Mosolov, *Doklady Akad. Nauk S.S.S.R.*, 1972, **202**, 236.
[1412] F. Ahmad and A. Salahuddin, *Biochem. J.*, 1972, **128**, 49P.
[1413] G. A. Gilbert and G. L. Kellett, *J. Biol. Chem.*, 1971, **246**, 6079.
[1414] M. Kaplan and M. Schlamowitz, *Immunochemistry*, 1972, **9**, 737.
[1415] R. E. Rhoads, G. S. McKnight, and R. T. Schimke, *J. Biol. Chem.*, 1971, **246**, 7407.
[1416] R. D. Palmiter, *J. Biol. Chem.*, 1972, **247**, 6450.
[1417] B. M. Austen, E. J. Romanowska, and R. D. Marshall, *Biochem. J.*, 1971, **124**, 54P.
[1418] J. G. Beeley and E. McCairns, *Biochim. Biophys. Acta*, 1972, **271**, 204.
[1419] J. G. Beeley, *Biochem. J.*, 1972, **128**, 119P.

containing hexose (2 or 3) and 2-amino-2-deoxyglucose (2 or 4 units, respectively) being separated by gel filtration.

The hydrogen-ion equilibrium of ovomucoid has been examined in the acid pH range.[1420] The biosynthesis of ovomucoid has been studied *in vitro* using hen-oviduct magnum and 2-amino-2-deoxy-D-[^{14}C]glucose.[1421] From the data obtained, it was concluded that the oviduct tissue contains a pool of partially glycosylated peptide precursors and that addition of 2-amino-2-deoxy-D-glucose can occur in the absence of protein synthesis. Insolubilized forms of ovomucoid have been used as affinity matrices.[691, 1422–1424]

Ovomucin gel in hen egg-white has been separated into two species (F and S) by density-gradient column electrophoresis.[1425] The two species were shown to contain galactose (16.3, 2.3), mannose (1.3, 3.4), 2-amino-2-deoxygalactose (7.4, 0.0), 2-amino-2-deoxyglucose (10.2, 6.0), sialic acid (11.2, 1.0), and sulphate (1.2, 0.1%, respectively). Fractionation of ovomucin gel after storage for 20 days at 30 °C yielded the S species only, whereas fractionation of ovomucin sol after similar storage yielded both F and S species; it was concluded that the carbohydrate-rich component is released from the gel during storage. The pH-, ion-strength-, temperature-, and time-dependence of the weight-average molecular weight of hen ovomucin has been determined by light-scattering experiments.[1426] Ovomucin was aggregated at near-neutral pH (molecular weights 24 and 4×10^7 at pH's 6.2 and 7.9, respectively) and it was found that disaggregation of ovomucin is an important step in the process by which egg-white thins. Studies of the rate of change in the viscosity of solutions of egg-white and ovomucin as a function of pH, temperature, concentration of hydroxyl ion, and substrate concentration indicated that thinning is dependent upon exposure of ovomucin of high molecular weight to alkali, and that it is first-order in hydroxyl ion and ovomucin concentrations.[1427]

Miscellaneous Glycoproteins

A study of the simultaneous incorporation of 2-acetamido-2-deoxy-D-[^{14}C]glucose into glycoproteins and lipids of chick liver microsomes suggested that its incorporation into glycoprotein may be mediated *via* a lipid–carbohydrate intermediate.[1071] The surface glycoproteins of normal and Rous sarcoma virus-transformed chick embryo cells have been compared by incorporation studies *in vitro* with L-[^{3}H and ^{14}C]fucose.[1428] The incorporation of 2-amino-2-deoxy-D-[1-^{14}C]glucose into the glycoproteins

[1420] A. Waheed and A. Salahuddin, *Biochem. J.*, 1972, **128**, 49P.
[1421] E. McCairns and J. G. Beeley, *Biochem. J.*, 1972, **128**, 120P.
[1422] N. C. Robinson, R. W. Tye, H. Neurath, and K. A. Walsh, *Biochemistry*, 1971, **10**, 2743.
[1423] G. Feinstein, *F.E.B.S. Letters*, 1970, **7**, 353.
[1424] G. Feinstein, *Biochim. Biophys. Acta*, 1970, **214**, 224.
[1425] A. Kato and Y. Sato, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 831.
[1426] Y. Tomimatsu and J. W. Donovan, *J. Agric. Food Chem.*, 1972, **20**, 1067.
[1427] J. W. Donovan, J. G. Davies, and M. B. Wiele, *J. Agric. Food Chem.*, 1972, **20**, 223.
[1428] L. Warren, D. Critchley, and I. Macpherson, *Nature*, 1972, **235**, 275.

of chick embryo cells was depressed by cytochalasin B.[998] Stimulation by neuraminidase of the uptake of carbohydrate by chick fibroblasts has been studied.[1429]

The biosynthesis of chitin has been reviewed briefly[660] and in detail.[659] A modified method involving proteolysis has been described for the isolation of chitin from crab shells.[1430] The occurrence of chitin in Mollusca has been studied; the polysaccharide was found in species from the Cephalopoda, Gastropoda, Placophora, and Scaphopoda, and in species of *Solengastres* and *Caudofoveata*.[1431] Treatment of glycoproteins from fly (*Sarcophaga bullata*) puparia with *N*-bromosuccinimide increased the chitin : protein ratio, and the molecular weight of the chitin–protein complex was decreased by the action of pronase.[1432] It was concluded that peptide bridges exist between the polysaccharide chains of the chitin micelle. Limit chitodextrins generated by chitinase were found to be mixtures of chitin oligosaccharides and chitin glycopeptides.

The structure of α-chitin has been refined from a stereochemical viewpoint and from hydrogen-bond energy considerations by using rigid-body refinement and fibre *X*-ray intensity data.[1433] The structure was found to be free from all short contacts and to be well established by one intra-chain hydrogen bond of the O—H···O type and one inter-chain hydrogen bond of the N—H···O type.

A lipoglycoprotein fraction extracted and purified from the ovaries of the cockroach (*Leucophaea maderae*) contained mannose (6.4—7.4) and 2-amino-2-deoxyhexose, mainly as 2-amino-2-deoxyglucose (1.5—1.8%).[1434] The fraction was separable into 28.1S and 14.5S components (molecular weights 15.9×10^5 and 5.6×10^5), and disaggregation of the larger species gave the smaller species among the many products. The data supported an earlier suggestion that the larger species is formed in the follicle from the smaller species.

The origin and composition of the oligosaccharides and the polysaccharides of honey have been reviewed.[1435]

The compositions of ovorubin from the eggs of *Pomacea canaliculata*, and of carotenoid-containing lipoglycoproteins from the eggs of *Plesionika edwardsi* and from the ovaries of *Cancer pagurus*, *Homarus gammarus*, and *Pecten maximus* (aquatic invertebrates) have been studied.[1436]

Application of an isolation technique, which yielded tropocollagen from a sea anemone (*Actinia equina*), to another sea anemone (*Metridium dianthus*) yielded a 'structural' glycoprotein containing galactose (0.5), glucose

[1429] A. Vaheri, E. Ruoslahti, and S. Nordling, *Nature New Biol.*, 1972, **238**, 211.
[1430] I. F. Heneine and J. R. Kimmel, *J. Biol. Chem.*, 1972, **247**, 6589.
[1431] W. Peters, *Comp. Biochem. Physiol.*, 1972, **41B**, 541.
[1432] H. Lipke and T. Geoghegan, *Biochem. J.*, 1971, **125**, 703.
[1433] C. Ramakrishnan and N. Prasad, *Biochim. Biophys. Acta*, 1972, **261**, 123.
[1434] R. K. Dejmal and V. J. Brookes, *J. Biol. Chem.*, 1972, **247**, 869.
[1435] I. R. Siddiqui, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 285.
[1436] P. F. Zagalsky, *Comp. Biochem. Physiol.*, 1972, **41B**, 385.

(0.4), mannose (0.1), and 2-amino-2-deoxyglucose (0.1%).[1437] Disc electrophoresis fractionated the material into sixteen components (molecular weights $> 4 \times 10^4$), one of which (7.2×10^4) predominated. Sulphated polysaccharides from sea urchin (*Anthocidaris crassispina* and *Hemicentrotus pulcherrimus*) embryos inhibited RNA-polymerase activity.[974]

The anticoagulant principle of a snake (*Agkistrodon acutus*) venom has been purified and it was shown to contain 2% carbohydrate.[1438]

Six specific agglutinins have been used to identify the terminal carbohydrate residues in the surface oligosaccharides of rabbit and hamster spermatozoa.[1439] Species differences were observed in terminal D-galactosyl, α-D-mannosyl, 2-acetamido-2-deoxy-D-galactosyl, and 2-acetamido-2-deoxy-D-glucosyl residues, whereas species similarities were found in terminal L-fucosyl and *N*-acetylneuraminyl residues.

Differences have been observed in the glycopeptides derived by proteolysis of normal cells and Rous sarcoma, murine sarcoma, and polyoma virus-transformed cells.[1079]

[1437] R. L. Katzman, *Life Sciences, Part II*, 1972, **11**, 131.
[1438] C. Ouyand and C. Teng, *Biochim. Biophys. Acta*, 1972, **278**, 155.
[1439] G. L. Nicolson and R. Yanagimachi, *Science*, 1972, **177**, 276

6
Enzymes

Introduction

Books dealing with the general principles,[1440] physical principles,[1441] and the chemical reactivity and biological role of functional groups in enzymes [1442] have been published. The proceedings of the F.E.B.S. (5th) meeting on the structures, properties, and functions of enzymes have been reported.[1443] Further books, *etc.*, which have recently become available, discuss in detail the mechanistic principles of enzyme-catalysed reactions [1444] and methods for the identification of isoenzymes.[1445] The use of affinity chromatography in the purification of enzymes has been reviewed.[1446, 1447] Various methods of insolubilization of enzymes with retention of activity have been reviewed, and the stabilities and specificities of the insolubilized enzymes have been compared.[638] Kinetic aspects of the behaviour of enzymes immobilized in artificial membranes [1448] and the effect of the microenvironment on the mode of action of immobilized enzymes [1449] have been discussed. The use of enzymes in analytical chemistry has been reviewed.[1450] The use of potassium dichromate solution as a liquid photometric standard has been suggested as a means of guaranteeing standardization of enzyme assays and absolute photometric absorbance values, and of overcoming differences between spectrophotometers.[1451]

[1440] E. E. Conn and P. K. Stumf, 'Outlines of Biochemistry', 3rd edn., Wiley, New York, 1972.
[1441] H. Gutfreund, 'Enzymes: Physical Principles', Wiley, London, 1972.
[1442] Biochemical Society Symposia No. 31, ed. R. M. S. Smellie, Academic Press, London, 1970.
[1443] Proceedings of 5th meeting, Fed. European Biochem. Soc., Prague, 1968; Enzymes and Isoenzymes – Structure, Properties and Function, ed. D. Shugar, Academic Press, London, 1970, Vol. 18.
[1444] C. J. Gray, 'Enzyme-Catalyzed Reactions', Van Nostrand Reinhold, London, 1971.
[1445] G. J. Brewer, 'Introduction to Isozyme Techniques', Academic Press, New York, 1970.
[1446] R. H. Reiner and A. Walch, *Chromatographia*, 1971, **4**, 578.
[1447] D. M. Jerina and P. Cuatrecasas, Proc. Fourth International Congress on Pharmacology, Basel, 1969, ed. R. Eigenmann, Schwabe, Basel, 1970, Vol. 1, p. 236.
[1448] W. J. Blaedel, T. R. Kissel, and R. C. Boguslaski, *Analyt. Chem.*, 1972, **44**, 2030.
[1449] E. Katchalski, I. Silman, and R. Goldman, *Adv. Enzymol.*, 1971, **34**, 445.
[1450] M. M. Fishman and H. F. Schiff, *Analyt. Chem.*, 1972, **44**, 543R.
[1451] R. G. Martinek, S. L. Jacobs, and F. E. Hammer, *Clinica Chim. Acta*, 1972, **36**, 75.

Table 1 *Specificity of polysaccharide debranching enzymes*[a]

Substrate	*Amylopectin-1,6-glucosidase* (*E.C.* 3.2.1.8)	*Isoamylase*	*Pullulanase*	*Limit dextrinase*	*Oligo-1,6-glucosidase* (*E.C.* 3.2.1.10)	*Dextrin-1,6-glucosidase* (*E.C.* 3.2.1.33)
Amylopectin	+	+	+	−	−	±
Glycogen	−	+	+	−	−	±
Glycogen phosphorylase limit dextrin	−	+	+	−	−	+
Pullulan	−	−	+	+	?	−
α-Dextrins	−	−	+	+	+	+
Panose	−	−	−	−	+	−
Isomaltose	−	−	−	−	+	−

[a] (+) Readily hydrolysed; (±) slowly hydrolysed; (−) not hydrolysed.

The mechanisms, compositions, and action patterns of some glycoside hydrolases have been reviewed,[1452] and glycoside hydrolases and carbohydrate lyases and isomerases have been listed.[1453] A general method reported for distinguishing between *endo* and *exo* actions of carbohydrases is based on the use of periodate oxidation to block the *exo* activity.[1454] It was calculated that random oxidation of a polysaccharide to an extent of only 5% should lead to more than 99% of the chain being protected from *exo* activity, whereas *endo* activity should proceed normally. This theory was borne out in all the cases tested. A number of carbohydrate substrates, including comb- and star-branched polysaccharide structures in which the degree of branching can be controlled, have been described for a study of the modes of action of *exo*-, *endo*-, and debranching enzymes.[1455] Glycogen- and starch-debranching enzymes have been reviewed.[1456] The specificity of the activities of polysaccharide debranching enzymes has been discussed and a classification has been reported (Table 1).[1457]

Acetamidodeoxyhexosidases

The properties of β-acetamidodeoxyhexosidases from tissues of normals and cases of Tay–Sachs disease variants have been investigated and the relation between the A and B enzymes was discussed.[1458] In one case, form A was absent and in another B was absent, whereas in a third case the levels of both forms were elevated. Age-dependent changes of the activity of human β-acetamidodeoxyhexosidases A and B have been reported.[1459] The involvement of β-acetamidodeoxyglucosidase in the catabolism of glycosphingolipids in cases of metachromatic leukodystrophy and infantile amaurotic idiocy has been investigated.[1460]

The total β-acetamidodeoxyhexosidase activity of human brain has been shown to increase throughout life.[1461]

The distribution of β-acetamidodeoxyhexosidases A and B in the various layers of human cerebellum has been measured.[1462] β-Acetamidodeoxyhexosidases from the brain of normals and cases of Tay–Sachs disease have been studied by isoelectric focusing.[1463] Gel electrophoresis of

[1452] K. Nisizawa and Y. Hashimoto, in 'The Carbohydrates', Vol. IIA, ed. W. Pigman and D. Horton, Academic Press, New York, 1970, p. 241.
[1453] A. Herp, D. Horton, and W. Pigman, in 'The Carbohydrates', Vol. IIB, ed. W. Pigman and D. Horton, Academic Press, New York, 1970, p. 835.
[1454] G. S. Drummond, E. E. Smith, and W. J. Whelan, *F.E.B.S. Letters*, 1971, **15**, 302.
[1455] B. Pfannemüller, *Angew. Chem.*, 1972, **84**, 644; *Angew. Chem. Internat. Edn.*, 1972, **11**, 645.
[1456] E. Y. C. Lee and W. J. Whelan, in 'The Enzymes', 3rd edn., ed. P. D. Boyer, Academic Press, New York, 1972, p. 192.
[1457] D. J. Manners, *Nature New Biol.*, 1971, **234**, 150.
[1458] K. Sandhoff, K. Harzer, W. Wässle, and H. Jatzkewitz, *J. Neurochem.*, 1971, **18**, 2469.
[1459] K. Harzer and K. Sandhoff, *Z. physiol. Chem.*, 1970, **351**, 1306.
[1460] H. Jatzkewitz, *Biochem. J.*, 1972, **128**, 6P.
[1461] K. Harzer and K. Sandhoff, *J. Neurochem.*, 1971, **18**, 2041.
[1462] H. E. Hirsch, *J. Neurochem.*, 1972, **19**, 1513.
[1463] D. Robinson, T. W. Jordan, and T. Horsburgh, *J. Neurochem.*, 1972, **19**, 1975.

the same enzyme from calf brain revealed multiple forms and some components possessed other glycosidase activities. Isoelectric focusing of the calf enzyme gave four forms (p*I* values 4.9, 6.0, 6.3, and 6.8) and interconversion between the forms having p*I* 4.9 and 6.0 was noted. Using an artificial fluorogenic substrate, drastically diminished activities of β-acetamidodeoxygalactosidase were observed in Tay–Sachs brain tissue and differences were also reported for the B- and O-variants of the disease.[1464] Electrophoretic separation of β-acetamidodeoxyhexosidases of human and bovine brain, human and bovine liver, and Tay–Sachs brain revealed the presence of 2, 2, 2, 2, and 1 form(s), respectively, of β-acetamidodeoxygalactosidase and 3, 2, 2, 1, and 1 form(s), respectively, of β-acetamidodeoxyglucosidase.[1465] In some cases, both activities were found to be associated with a particular band. The involvement of β-acetamidodeoxyhexosidase in the brain lesions of metachromatic leukodystrophy and infantile amaurotic idiocy has been investigated.[1466]

The levels of β-acetamidodeoxyglucosidase in the skin of normals and cases of cystic fibrosis of the pancreas have been compared,[1467] and the levels of the enzyme in cultured fibroblasts from normals and patients with cystic fibrosis and Hurler and Maroteaux–Lamy syndromes were found to differ from those previously reported.[1468] The activities of β-acetamidodeoxyglucosidase A and of β-acetamidodeoxyglucosidases A and B in cultured fibroblasts from cases of G_{M_2}-type I (Tay–Sachs disease) and G_{M_2}-type II gangliosidoses, respectively, have been determined.[1469]

Normal patterns of β-acetamidodeoxy-galactosidases and -glucosidases of liver and other tissues from cases of Hurler and Hunter syndromes, Gaucher's disease, and mannosidosis have been reported.[1470] Complete immunological cross-reactivity has been observed between the A and B forms of the β-acetamidodeoxyhexosidase of human liver.[1471] The preparation of β-acetamidodeoxyglucosidase from human epididymus has been described [1331] and the enzyme was found to possess a pH optimum of 5.0. Human β-acetamidodeoxyhexosidase B from the placenta has been purified to homogeneity by column chromatography and isoelectric focusing.[1472] Rabbit antisera to the B form reacted equally well with the A and B forms, as shown by immunodiffusion and precipitation of the enzyme from solution.

The β-acetamidodeoxyhexosidase activity of normal human serum can be resolved into four forms by ion-exchange chromatography; the majority

[1464] J. F. Tallman, W. G. Johnson, and R. O. Brady, *J. Clinical Invest.*, 1972, **51**, 2339.
[1465] G. J. M. Hooghwinkel, W. A. Veltkamp, B. Overdijk, and J. J. Lisman, *Z. physiol. Chem.*, 1972, **353**, 839.
[1466] H. Jatzkewitz, *Z. physiol. Chem.*, 1970, **351**, 1309.
[1467] R. G. Wilson, *Clinica Chim. Acta*, 1972, **36**, 113.
[1468] J. Butterworth, F. Scott, W. M. McCrae, and A. D. Bain, *Clinica Chim. Acta*, 1972, **40**, 139.
[1469] G. Dawson, R. Matalon, and A. Dorfman, *J. Biol. Chem.*, 1972, **247**, 5951.
[1470] B. Hultberg and P. A. Öckerman, *Clinica Chim. Acta*, 1972, **39**, 49.
[1471] M. Carroll and D. Robinson, *Biochem. J.*, 1972, **126**, 17P.
[1472] S. K. Srivastava and E. Beutler, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 753.

of the activity was in a form having properties similar to the β-acetamidodeoxyglucosidase A of human tissues.[1473] Two of the forms (I_1 and I_2) were found to possess electrophoretic mobilities between those of human β-acetamidodeoxyhexosidases A and B, while the I_2 form was indistinguishable from the P form present in sera from pregnant women. A small proportion of β-acetamidohexosidase B was present in all sera examined. Application of the heat denaturation treatment commonly used in the detection of Tay–Sachs heterozygotes showed that the B and I forms have similar heat stabilities and that the heat-labile A form could be converted into the I_1, I_2, and B forms. A single, heat-stable component found in the serum of a child with Tay–Sachs disease appeared to be an intermediate form distinguishable from the B form. The β-acetamidodeoxyglucosidase activity of human maternal serum has been separated into two bands by starch gel electrophoresis; the two forms were inseparable by gel filtration, possessed identical pH optima (4.5) and inhibition characteristics, but their heat stabilities differed markedly.[1474] One form corresponded to the A form previously identified in human tissues, whereas the other (P) form could not be detected in placenta, foetal membranes, cord serum, and amniotic fluid.

An abbreviated test for serum β-acetamidodeoxyhexosidase A activity has been described and results from its application to normals, carriers of Tay–Sachs disease, and Tay–Sachs homozygotes have been compared.[1475] High values have been reported for the serum levels of β-acetamidodeoxyglucosidase in cases of Hurler and Maroteaux–Lamy syndromes.[1476] The increased serum levels of β-acetamidodeoxyglucosidase found in a number of patients with active polymyositis or dermatomyositis, systemic lupus erythematosus, and vasculitis have been correlated with the levels of lysosomal enzymes in the affected tissues.[1477] The activities of β-acetamidodeoxy-glucosidase and -galactosidase in leukocytes from normal subjects and cases of G_{M1}-gangliosidosis homozygote and heterozygous parents have been investigated.[1478]

β-Acetamidodeoxyhexosidases A and B have been detected in human blood platelets and, from measurements of the levels in platelets from normals and cases of Fabry's and Sandhoff's diseases, the value of platelet enzyme assay for diagnosis of glycosphingolipidoses was established.[1479] Two forms of β-acetamidodeoxygalactosidase and a β-acetamidodeoxyglucosidase have been purified from human blood platelets (by gel filtration) and characterized.[1480] The release of β-acetamidodeoxy-galactosidase and

[1473] R. G. Price and N. Dance, *Biochim. Biophys. Acta*, 1972, **271**, 145.
[1474] J. L. Stirling, *Biochim. Biophys. Acta*, 1972, **271**, 154.
[1475] E. Cotlier, *Clinica Chim. Acta*, 1972, **38**, 233.
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[1479] P. D. Snyder, R. J. Desnick, and W. Krivit, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1857.
[1480] H. B. Bosmann, *Biochim. Biophys. Acta*, 1972, **258**, 265.

-glucosidase by thrombin from fresh and stored human platelets [1481] and the release of β-acetamidodeoxyglucosidase from human platelets by thrombin and polystyrene latex particles [903] have been studied.

The β-acetamidodeoxyhexosidases of human amniotic fluid, serum, and white blood cells were shown to possess identical physicochemical-activity relationships and pH optima (4.4).[1482] It was suggested that the enzyme in the fluid is the same as that in the serum and, as such, its determination may be valuable in the prenatal diagnosis of Tay–Sachs disease. A component of Sanfilippo type B fibroblasts was found to have chromatographic properties identical with those of normal human urinary α-acetamidodeoxyglucosidase.[1483] Since the fibroblasts from cases of the syndrome possess no α-acetamidodeoxyglucosidase activity, it was considered that the inactive material represents a pathological defect. Skin fibroblasts from cases of Sanfilippo B disease exhibited normal activities of β-acetamidodeoxyhexosidase and α-acetamidodeoxygalactosidase.

Polyacrylamide gel electrophoresis of β-acetamidodeoxyglucosidase from bovine liver, spleen, and kidney demonstrated multiplicity of the enzyme in each case and it was concluded that at least two forms of the enzyme exist.[1484] Two enzymes, each with β-acetamidodeoxy-galactosidase and -glucosidase activities, have been purified from extracts of bovine spleen.[1485] The two enzymes were found to be physicochemically similar in a number of respects (molecular weights 1.39—1.46 × 10^5) and subunit formation was investigated. Although the amino-acid compositions are similar, the carbohydrate contents of the enzymes differ, *viz.* neutral carbohydrate (3.2, 1.4), 2-amino-2-deoxyglucose (0.3, 0.8), and sialic acid (0.4 and 0.2%, respectively). Kinetic studies showed that two 4-nitrophenyl substrates compete for the active sites of both enzymes. Both enzymes were strongly inhibited by various metal ions, but not by sulphhydryl reagents. β-Acetamidodeoxyglucosidase activities of lysosomes in various tissues of Angus cattle have been determined.[1022]

Several components with both β-acetamidodeoxy-galactosidase and -glucosidase activities have been demonstrated in extracts of ram testes and epididymi by isoelectric focusing.[1486] The major components from the two sources (p*I* values 6.2 and 7.0, respectively) exhibited pH optima in the region of pH 5 and similar K_m values.

^{1}H N.m.r. spectroscopy has been used to show that the hydrolysis of benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside by boar epididymal β-acetamidodeoxyglucosidase occurs with retention of the anomeric configuration.[1487] β-Acetamidodeoxyhexosidase has been purified from

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[1482] J. V. Murphy, *Life Sci., Pt. II*, 1972, **11**, 309.
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[1484] J. Yoshikawa, H. Koide, and M. Yamanaka, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 11.
[1485] J. A. Verpoorte, *J. Biol. Chem.*, 1972, **247**, 4787.
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[1487] A. Y. Khorlin, S. E. Zurabyan, N. I. Dubrovina, V. F. Bystrov, G. V. Vikha, and E. D. Kaverzneva, *Carbohydrate Res.*, 1972, **21**, 316.

porcine epididymus and was shown to be free from α-acetamidodeoxyhexosidase activity.[1488] From an examination of several glycosphingolipids containing 2-acetamido-2-deoxyhexose, including Tay–Sachs globoside, it was apparent that the enzyme will only release terminal, non-reducing 2-acetamido-2-deoxyhexose residues. Four enzyme forms possessing both β-acetamidodeoxy-galactosidase and -glucosidase activities have been isolated from porcine epididymus and were shown to possess the same pH optima, similar K_m values for various substrates, and similar K_i values for 2-acetamido-2-deoxy-D-glucono-1,5-lactone.[1489] Structural differences were noted between the enzymes and all four hydrolysed different types of linkage in the order (1 → 6)- > (1 → 4)- > (1 → 3)-linkages. Two distinct fractions showing both β-acetamidodeoxy-galactosidase and -glucosidase activities have been isolated from porcine kidney.[1490] Electrophoretic and ultracentrifugation studies indicated that both fractions were heterogeneous and that subunits were formed in the presence of guanidine and dithiothreitol. The two enzyme fractions possessed similar amino-acid compositions, identical K_m values, and dual maxima pH–rate profiles. Although the presence of bovine serum albumin enhanced their activities and decreased their labilities to heat, it did not alter the K_m values.

The β-acetamidodeoxy-galactosidase and -glucosidase activities of RNA tumour virus-transformed embryonic mouse fibroblasts were found to be greater than the corresponding activities of the non-transformed cells.[1491]

The β-acetamidodeoxyglucosidase of synaptic plasma membranes from rat-brain synaptosomes was released by Triton X-100, but not by salt solutions.[1492] β-Acetamidodeoxyglucosidase activities of the jejunum and ileum of adult and suckling rats have been compared with those of other glycosidases present in the same tissues[1493] and the effect of cortisone on these enzyme activities has been investigated.[1494] The distribution of β-acetamidodeoxyglucosidase among lysosomal particles of rat liver[1495] and the inhibition of rat liver and kidney β-acetamidodeoxyglucosidase by polynucleotides[1496] have been studied. β-Acetamidodeoxyglucosidase has been purified from an extract of rat epididymi.[643]

β-Acetamidodeoxy-galactosidase and -glucosidase in the brain and eyes of developing chicks have been investigated.[1497] β-Acetamidodeoxyhexosidase has been purified from hen oviduct and was shown by polyacrylamide gel electrophoresis and isoelectric focusing to comprise

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[1497] C.-L. Schengrund and A. Rosenberg, *Biochemistry*, 1971, **10**, 2424.

a major and a minor isoenzyme (molecular weights 1.18 and 1.58 × 10^5; p*I* values 6.45 and 6.86, respectively).[1498] The action patterns and rates of hydrolysis of the isoenzymes were found to be identical. Of a large number of metal ions investigated, only Hg^{2+} and Ag^+ showed selective inhibition of the enzymes.

More than sixty marine invertebrates of different systematic and ecological positions have been tested for the occurrence of β-acetamidodeoxyglucosidase activity in their organs.[1499] Enzyme activity was found in the majority of the animals and the highest specific activities were observed in the digestive systems of some Bivalvia, Crustacea, and *Halocyntia roretzi*. Extracts of the hepatopancreas of the tunicate *Pyura stolonifera* were found to contain α- and β-acetamidodeoxyglucosidase activities.[1500]

A crude preparation of β-amylase from barley was found unexpectedly to solubilize the cell-wall peptidoglycan from *Vibrio parahaemolyticus* and the cell wall of *Micrococcus lysodiekticus*.[1501] An *endo*-acetamidodeoxyglucosidase was identified among the lytic principles separated from the β-amylase preparation by isoelectric focusing. Aqueous extracts of mushrooms from the groups Agaricaceae, Boletaceae, and Lycoperdaceae have been tested for β-acetamidodeoxyglucosidase activity.[1502] The enzyme was found in most extracts and the highest activities were found in *Boletus* (*Krombholzia*) *scaber* and *Armillaria mellea*.

β-Acetamidodeoxyglucosidase isolated from *Aspergillus oryzae* Taka-diastase was found to hydrolyse partially *O*-methylated oligosaccharides from chitin to produce 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-3-*O*-methyl-D-glucose, 2-acetamido-2-deoxy-6-*O*-methyl-D-glucose, and 2-acetamido-2-deoxy-3,6-di-*O*-methyl-D-glucose.[1503] Phenyl 2-acetamido-2-deoxy-3- and -6-*O*-methyl-β-D-glucopyranosides were also hydrolysed by the enzyme, whereas phenyl 2-acetamido-2-deoxy-3,4-di-*O*-methyl-β-D-glucopyranoside, 2-acetamido-1-{(*N*-β-L-aspartyl)amino}-2-deoxy-β-D-glucopyranosylamine, 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-*N*-acetylmuramic acid, and 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucitol were not. The β-acetamidodeoxyglucosidase was inhibited by such compounds as acetamide, acetonitrile, and 2-acetamido-2-deoxy-D-glucose, and to lesser extents by 2-deoxy-2-formamido- and 2-deoxy-2-propionamido-D-glucose. It was concluded that the C-4 hydroxy- and C-2 acetamido-groups of the substrate are important in formation of the enzyme-substrate complex.

Growing cultures of *Bacillus subtilis* were found to secrete into the medium an *exo*-β-acetamidodeoxyglucosidase in a sedimentable form; extraction of the medium with aqueous sodium chloride was used to obtain

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the enzyme in a homogeneous form.[1504] The substrate specificity of an *endo*-β-acetamidodeoxyglucosidase from *Clostridium welchii* has been examined.[1505]

Myxamoebae of the cellular slime mould *Dictyostelium discoideum*, when grown in submerged culture on *Escherichia coli*, have been shown to contain a β-acetamidodeoxyglucosidase,[1506] and the specific activity of the enzyme in the myxamoebae has been determined.[1507] Of the many glycohydrolases produced by the organism, β-acetamidodeoxyglucosidase was produced preferentially in the stationary phase. The influence of changes in the myxamoebae on the synthesis of the enzyme was investigated.[1508]

An *endo*-β-acetamidodeoxyglucosidase has been obtained from the culture fluid of *Diplococcus pneumoniae*.[1345] A crude extract of a soil amoeba (*Hartmanella glebae*) grown in the presence of both *Aerobacter aerogenes* and *Alcaligenes faecalis* possibly contained an *endo*-β-acetamidodeoxyglucosidase.[1509]

Two bacteriolytic enzymes isolated from *Myxococcus virescens* have been characterized.[1510] Analysis of the products of their action on cell walls of *Micrococcus lysodiekticus* showed that the enzymes were *endo*-β-acetamidodeoxyglucosidases devoid of any *exo*-activity. Bacteriolytic enzymes produced by *Myxococcus xanthus* appeared to contain two forms of β-acetamidodeoxyglucosidase activity.[1511] Chromatography of the crude filtrate from cultures of *Streptomyces albidoflavus* on columns of chitin yielded two β-acetamidodeoxyglucosidases that were shown to possess different pH optima and thermal stabilities, but which were both active against chitin.[1512]

α-Arabinosidases

Lysosomal α-arabinosidase activity has been measured in fibroblasts cultured from normals and patients with cystic fibrosis, Hurler syndrome, and Maroteaux–Lamy syndrome.[1468]

α-Arabinofuranosidase and α-arabinopyranosidase activities have been reported to exist in wheat and flour.[1513]

The general kinetic properties of α-arabinofuranosidase from *Aspergillus niger* have been investigated.[1514] The enzyme catalysed the hydrolysis of both the α-(1 → 3)- and α-(1 → 5)-linkages of L-arabinan, cleaving L-arabinosyl units from non-reducing terminals of the substrate by a predominantly multichain mechanism. The enzyme was found to hydrolyse

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(1 → 3)-linkages more rapidly than (1 → 5)-linkages, resulting in an accumulation of a straight-chain α-(1 → 5)-L-arabinan during the course of hydrolysis. The stability of the α-arabinofuranosidase has been investigated.[1515]

The fungal plant pathogen *Colletotrichum lindemuthianum* was shown to secrete α-arabinofuranosidase selectively when cultured in the presence of L-arabinose;[1516] the enzyme was purified by ion-exchange chromatography and gel filtration. Parameters affecting the secretion of the enzyme by the organism have been studied.[1517]

An α-arabinofuranosidase, purified from the culture fluid of *Corticum rolfsii* by gel filtration and ion-exchange chromatography, was shown to be homogeneous by ultracentrifugation and disc electrophoresis.[1518] The enzyme exhibited optimum activity towards phenyl α-L-arabinofuranoside and beet arabinan at pH 2.5, and was found to be stable over a wide pH range (1.5—10). Other characteristics of the enzyme were also defined.

The α-arabinosidase of wheat-flour doughs has been investigated.[1519]

β-Fructofuranosidases

Nojirimycin and D-glucono-1,5-lactone have been shown to be powerful inhibitors of glycosidases and their inhibition of β-fructofuranosidase has been studied.[1520]

A simple method involving the use of t.l.c. and disaccharide substrates has been reported for the assay of human intestinal β-fructofuranosidase.[1521] The assay has been applied to determination of the enzyme in normals and cases of coeliac disease. A method described for the automated determination of β-fructofuranosidase of human small intestinal mucosa involved dialysis into alkaline ferricyanide solution of the reducing carbohydrates formed on incubation with the substrate.[1522] The levels of β-fructofuranosidase in jejunal biopsy specimens from American and South Indian control subjects and patients with tropical sprue have been measured.[1523]

A simplified procedure has been reported for isolation of the β-fructofuranosidase–oligo-1,6-glucosidase complex from rabbit small intestine in homogeneous form.[1524] The glycoprotein complex (molecular weight 2.2×10^5) was shown to contain galactose (26), glucose (11), fucose (17), mannose (22), 2-amino-2-deoxygalactose (12), and 2-amino-2-deoxyglucose (68 residues per mole), and values were obtained for its partial specific

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volume and hydrophobicity coefficient. The β-fructofuranosidase activity of the small intestine of genetically adipose mice was found to be greater than those of their siblings of normal weight.[1525]

A β-fructofuranosidase–oligo-1,6-glucosidase complex has been solubilized from rat intestine with papain and was purified by gel filtration and ion-exchange chromatography.[1526] Kinetic studies of the enzyme showed that it is inhibited by 4-nitrophenyl α-D-glucopyranoside and 6-bromo-2-naphthyl α-D-glucopyranoside. Diabetes [1527] and pancreatectomy [1528] have been shown to increase the activity of rat jejunal β-fructofuranosidase. The effects of actinomycin D *in vivo* on the specific activity and intracellular distribution of rat jejunal β-fructofuranosidase [1529] and of carbohydrate and corticosteroid on rat intestinal β-fructofuranosidase [1530] have been investigated.

The activity of β-fructofuranosidase in various parts of banana plants and fruit has been determined.[1531] Supplementation of the medium used for homogenization with either polyvinylpyrrolidone or Triton X-100 increased the yield of enzyme.

Three glycohydrolases have been separated by ion-exchange chromatography from extracts of the dandelion (*Taraxacum officinale*); one of these was active against sucrose (pH optimum 7.5), but not against inulin, whereas the others were found to be active against inulin only.[1532] Further studies of these enzymes have been reported.[1533]

Cultures of orchid (*Phalaenopsis* sp.) seedlings have been found to contain an extracellular β-fructofuranosidase.[1534]

The properties of bound and soluble β-fructofuranosidases of tomato cell walls have been compared.[1535] β-Fructofuranosidase has been purified from tomato fruit and ion-exchange chromatography yielded two active species, which were shown to contain neutral carbohydrate (5.3 and 9.1%).[1536] The heat-labile enzymes exhibited identical pH optima for the hydrolysis of sucrose and raffinose (4.5 and 5.1, respectively) and were inhibited by 4-chloromercuribenzoate and silver nitrate.

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The induction by sucrose of β-fructofuranosidase in dormant conidia of *Aspergillus oryzae* was stimulated by kanamycin, but not by streptomycin.[1537] Sucrose was found to induce an endocellular β-fructofuranosidase and an extracellular levansucrase in *Bacillus subtilis*.[1538] The two enzymes (molecular weights 4×10^4) were separated by ion-exchange chromatography and possessed distinctly different chemical properties.

β-Fructofuranosidase has been obtained in a purified form from the cells and culture filtrate of *Candida utilis*.[1539] Formation of the endocellular enzyme was unaffected by the nature of the carbohydrates present in the culture medium. The synthesis of D-fructose oligosaccharides by a β-fructofuranosidase from *Claviceps purpurea* has been studied.[1540] A pyridoxine glucoside-synthesizing enzyme from a *Micrococcus* sp. was found to possess β-fructofuranosidase activity.[1541]

β-Fructofuranosidases of *Neurospora* sp. and yeasts have been reviewed.[1542] β-Fructofuranosidase produced by the slime mutant of *Neurospora crassa* appeared to be serologically identical with that of the wild type.[1543] Differences were observed in the proportions of the total amount of the enzyme secreted by the two organisms. β-Fructofuranosidase was released from the cell walls of *N. crassa* by snail-gut juice, laminarinase, and chitinase, but not by cellulase, trypsin, or detergents. It was concluded that the wall-bound enzyme is only released when covalent bonds of the wall constituents are broken.[1544] The distribution of the wall-bound enzyme during the asexual life-cycle of the organism was determined.[1545]

β-Fructofuranosidase has been isolated and partially purified from rumen protozoa.[1546] The enzyme exhibited activity over a broad pH range (optimum pH 5.0), but did not possess any of the transferase activity usually associated with β-fructofuranosidases from other sources. The enzyme was inhibited by heavy metal ions and iodoacetamide, indicating the involvement of thiol groups in the active site.

The endocellular β-fructofuranosidase of *Saccharomyces cerevisiae* has been isolated and purified by ion-exchange chromatography.[1547] The properties of the enzyme and means of inhibiting its action were studied. The autolytic release of β-fructofuranosidase from the cell wall of yeast has been studied under a variety of conditions and solvents.[1548] The kinetics of

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hydrolysis of sucrose by yeast β-fructofuranosidase has been examined with particular emphasis placed on high substrate concentrations.[1549] β-Fructofuranosidase from a commercial autolysate of *S. cerevisiae* has been separated into three fractions by ion-exchange chromatography.[1550] A linear relationship between the carbohydrate content and specific activity of the enzyme was established and the purest form was shown to contain 50% carbohydrate consisting of neutral sugar and 2-amino-2-deoxyglucose (2.5%). The biosynthesis of extracellular β-fructofuranosidase by protoplasts of *Saccharomyces* sp. was inhibited by '2-deoxy-D-glucose', whereas a non-metabolized isomer (6-deoxy-D-glucose) had no effect.[1551] It was deduced from experiments on carbohydrate depletion that inhibition of the synthesis of carbohydrate moieties of the enzyme reduces formation of the active form of the enzyme. Further studies on the biosynthesis and secretion of β-fructofuranosidase by a *Saccharomyces* sp. indicated that a balance is maintained between two forms of the enzyme, one of which contains mannose and the other being devoid of carbohydrate.[1552] The effect of cycloheximide on the biosynthesis of the enzyme was studied.

Active immobilized forms of β-fructofuranosidase have been prepared and their properties examined.[1553–1555]

Fucosidases

The α-fucosidase activities of fibroblasts cultured from patients with cystic fibrosis, generalized gangliosidosis, Hurler syndrome, Hunter syndrome, Niemann–Pick disease, Maroteaux–Lamy syndrome, Sanfilippo syndrome, and Sandhoff's disease have been measured and compared with values obtained for normals.[1468, 1556] Cultured fibroblasts from cases of fucosidosis were found to contain $<7\%$ of the normal α-fucosidase activity.[1556] Cultured normal amniotic cells were found to possess greater α-fucosidase activities than normal cultured fibroblasts. A fucosidosis in which there is an apparent deficiency of the skin fibroblast enzyme has been identified. The α-fucosidase activity of cultured skin fibroblasts of a case of fucosidosis has been the subject of a further report.[1469]

α-Fucosidase activity in human brain has been shown to decrease throughout life.[1461] The preparation of α-fucosidase from human epididymus has been described;[1331] the enzyme was found to exhibit maximum activity at pH 5.0. The α-fucosidase of human placenta has been studied [1557]

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and the enzyme is active in this tissue and in human embryonic liver early in gestation.[1558] The enzyme has also been detected in amniotic fluid.

Human blood platelets have been shown to contain a β-fucosidase, which has been purified by gel filtration.[1480] The β-fucosidase activity of normal human leukocytes has been determined and abnormal amounts of the enzyme were found in leukocytes from a G_{M_1}-gangliosidosis homozygote and heterozygous parents.[1478] A new form of α-fucosidase deficiency has been found in the leukocytes of a severely retarded male.[1559]

Lysosomal α-fucosidase activities in various tissues of Angus cattle have been determined.[1022] Porcine kidney contains α-fucosidase in two forms that differ in their thermostability, storage stability, pH–activity profiles, and action patterns for natural oligosaccharide substrates.[1560]

The β-fucosidase activity of RNA tumour virus-transformed embryonic mouse fibroblasts is greater than that of the non-transformed cells.[1491] The two α-fucosidases of rat brain have different pH optima (4.0 and 6.0), but have essentially the same K_m value using 4-nitrophenyl α-L-fucopyranoside as substrate.[1054] Both enzymes were competitively inhibited by L-fucose. An α-fucosidase has been purified from extracts of rat epididymi by ion-exchange chromatography and was identified as a glycoprotein (molecular weight 2.1—2.2 × 10^5) containing galactose (10), mannose (30), fucose (3.4), and 2-amino-2-deoxyglucose (12 residues per mole).[643] The molecule dissociated into two dissimilar subunits (molecular weights 4.7 and 6.0 × 10^4) in the presence of mercaptoethanol and guanidine hydrochloride or sodium dodecyl sulphate.

Purification of α-fucosidase from the liver of *Turbo cornulus*, a marine gastropod, by gel filtration and chromatography on SE-Sephadex has been reported.[766] The purified enzyme was free from other glycosidase activities and exhibited maximum activity at pH 4.0. The action of the enzyme on blood-group substances was investigated. The α-fucosidase activity of the protozoan *Trichomonas foetus* has been investigated in order to characterize the enzymes that destroy blood-group activities.[1561] Starch-column electrophoresis and ion-exchange chromatography were used to separate the enzymes that destroy blood-group H and Le^a activities. The anti-H α-fucosidase was found to hydrolyse α-(1 → 2)-L-fucosyl linkages, but not α-(1 → 3)- or α-(1 → 4)-linkages, whereas the anti-Le^a enzyme was free from α-(1 → 2)-activity and hydrolysed α-(1 → 3)- and α-(1 → 4)-L-fucosyl linkages.

The β-D-fucosidase of wheat-flour doughs has been studied.[1519]

Galactosidases

The inhibition of β-galactosidase by nojirimycin and D-glucono-1,5-lactone has been studied.[1520]

[1558] G. Y. Wiederschain, E. L. Rosenfeld, A. I. Brusilovsky, and L. G. Kolibaba, *Clinica Chim. Acta*, 1971, **35**, 99.
[1559] V. Patel, I. Watanabe, and W. Zeeman, *Science*, 1972, **176**, 426.
[1560] G. Y. Wiederschain, *Doklady Akad. Nauk. S.S.S.R.*, 1972, **204**, 727.
[1561] J. R. Stealey and W. M. Watkins, *Biochem. J.*, 1972, **126**, 16P.

The involvement of brain β-galactosidase in the catabolism of glycosphingolipids in cases of metachromatic leukodystrophy and infantile amaurotic idiocy has been considered.[1460, 1466] The distribution of 'acid' and 'neutral' β-galactosidases in the layers of the human cerebellum has been determined.[1462]

The β-galactosidase activities of a number of tissues from the three variants of Tay–Sachs disease were found to be greater than normal,[1458] and those of the skins of normals and of patients with cystic fibrosis of the pancreas have also been compared.[1467] The activities of α- and β-galactosidases in fibroblasts cultured from patients with cystic fibrosis, Hurler syndrome, and Maroteaux–Lamy syndrome,[1468] and of α-galactosidase in fibroblasts cultured from patients with Fabry's disease,[1469] and of β-galactosidase in fibroblasts cultured from patients with lactosylceramidosis, Gaucher's disease, and G_{M_1}-gangliosidosis type I, have been compared with those for normal fibroblasts. Although one of the two forms of α-galactosidase is usually deficient in cases of Fabry's disease, both forms were found to be present in one case of the disease.[1562] This finding was ascribed to heterogeneity of the mutation affecting the structural locus coding for α-galactosidase. *myo*-Inositol appeared to be a competitive inhibitor of α-galactosidase activity in normal human skin fibroblasts, but not of the residual α-galactosidase activity of fibroblasts from patients with Fabry's disease.[1563] It was suggested that only one of the two normal enzymes is inhibited by inositol and that this form is absent in the disease. The two enzymes can also be distinguished by their K_m values and rates of inactivation by heat.

A rapid t.l.c. method using disaccharide substrates has been reported for the assay of intestinal β-galactosidase; the technique has been used for determination of the levels of the enzyme in normals and cases of coeliac disease.[1521] An improved indigogenic method employing 4-chloro-5-bromo-3-indolyl β-D-fucoside as substrate has been used to study the β-galactosidase activities of intestinal and kidney tissues of humans and a large number of animals.[1564] Three different types of human small intestinal β-galactosidases can be estimated in the presence of one another owing to their different specificities towards various substrates.[1565] The cytoplasmic enzyme is active against phenyl β-D-galactopyranoside and 6-bromo-2-naphthyl β-D-galactopyranoside, the lysosomal enzyme against lactose, phenyl β-D-galactopyranoside, and 6-bromo-2-naphthyl β-D-galactopyranoside, and the brush border enzyme against lactose. All three enzymes are active against 2-nitrophenyl β-D-galactopyranoside and 4-nitrophenyl β-D-galactopyranoside. The β-galactosidase activities of jejunal biopsy specimens from American and South Indian control subjects

[1562] G. Romeo, B. Childs, and B. R. Migeon, *F.E.B.S. Letters*, 1972, **27**, 161.
[1563] J. C. Crawhall and M. Banfalvi, *Science*, 1972, **177**, 527.
[1564] Z. Lojda and J. Kraml, *Histochemie*, 1971, **25**, 195.
[1565] N. G. Asp and A. Dahlqvist, *Analyt. Biochem.*, 1972, **47**, 527.

and patients with tropical sprue have been compared.[1523] The levels were found to be low in Indian normals.

The β-galactosidase of human liver and other tissues from cases of mannosidosis, Gaucher's disease, Hurler syndrome, and Tay–Sachs disease have been examined by isoelectric focusing.[1470] The pH–activity profiles and the isoenzyme gel-filtration patterns of β-galactosidase from liver of cases of cystic fibrosis, inborn metabolic disease, and controls have been studied.[1566] Although similarities were found to exist between parameters for the normal and cystic fibrosis enzymes, a marked variation in the level of the three β-galactosidase isoenzymes was found between individual specimens.

The preparation of β-galactosidase from human epididymus has been described.[1331] The enzyme possesses a pH optimum of 5.0.

The α- and β-galactosidases of human placenta have been studied,[1557] and the enzymes have been shown to be active in the placenta and the liver of human embryos early in gestation.[1558] Two isoenzymes (molecular weights 1.5×10^5) of α-galactosidase have been purified to homogeneity from human placenta.[1567] Although altering the electrophoretic mobility of the A enzyme, treatment with neuraminidase neither converted it into the B form nor affected its thermal stability. Immunodiffusion studies revealed that antisera against each isoenzyme did not cross-react with the other enzyme and that enzymes neutralized with antiserum were enzymically active. Neuraminidase treatment of the A form did not decrease its reactivity to anti-A nor did it allow cross-reaction with anti-B. It was concluded from these studies that the basic disorder in Fabry's disease is not due to a failure to convert isoenzyme B into A, but to the fact that the residual enzyme found in Fabry's fibroblasts is normal B, and not an altered A, isoenzyme.

The α-galactosidase activity of plasma from a case of Fabry's disease has been investigated.[1568] It was found that the enzyme activities measured using such artificial substrates as 4-nitrophenyl and 4-methylumbelliferyl α-D-galactosides and *O*-α-D-galactopyranosyl-(1 → 4)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucopyranosylceramide are different, thus demonstrating the high degree of substrate specificity of the α-galactosidases.

The α- and β-galactosidase activities of normal human leukocytes have been determined.[1478] The well-known deficiency of β-galactosidase was demonstrated in the leukocytes from a G_{M_1}-gangliosidosis homozygote and a less severe deficiency was also apparent in the heterozygous parents. Both α- and β-galactosidases have been identified in human blood platelets and, from measurements of their levels in platelets from normals and cases of Fabry's and Sandhoff's diseases, the value of the platelet enzyme assay for diagnosis of glycosphingolipidoses was established.[1479] The α- and β-galactosidases of human platelets have been purified by gel filtration and the

[1566] J. Butterworth, A. D. Bain, and W. N. McCrae, *Clinica Chim. Acta*, 1972, **41**, 367.
[1567] E. Beutler and W. Kuhl, *Nature New Biol.*, 1972, **239**, 207.
[1568] C. A. Mapes and C. C. Sweeley, *F.E.B.S. Letters*, 1972, **25**, 279.

latter enzyme gave two active peaks.[1480] The release of β-galactosidase from human platelets by the action of thrombin has been studied under various conditions.[1481] The enzyme was inhibited by ADP and ATP.

The *in utero* diagnosis of globoid cell leukodystrophy (Krabbe's disease) has been achieved by the assay of β-galactosidase in cultured amniotic fluid cells from a foetus at risk – enzymic activity was found to be 5% of the control level.[1569] Similar low activities were demonstrated in brain and liver tissues of the aborted foetus.

Normal human urine has been shown to contain an α-galactosidase that is active against ceramide trihexoside and synthetic D-galactosides.[1570] The enzyme, which exhibits maximum activity at pH 4.6, was weakly inhibited by D-galactose and *myo*-inositol. Its measurement is useful in elucidation of the nature of the genetic defect in cases of Fabry's disease.

In an investigation of the genetics of α-galactosidase deficiency in Fabry's disease, the production of α-galactosidase in man–Chinese hamster clones and subclones has been examined by gel electrophoresis to determine whether the structural locus for the enzyme is located on the X-chromosome.[1571]

Partially purified preparations of α-galactosidase have been obtained from monkey small intestine.[1572] The enzyme exhibits maximum activity at pH 4.3 and is more active against synthetic substrates than against either melibiose or raffinose. The lysosomal activities of α- and β-galactosidases in various tissues of Angus cattle have been determined.[1022]

β-Galactosidases extracted from porcine small intestine have been fractionated into three types, according to their substrate specificity, by ion-exchange chromatography and gel filtration.[1573] The first type was more active against lactose than synthetic β-D-galactosides such as 2-nitrophenyl β-D-galactopyranoside. The second type hydrolysed synthetic β-D-galactosides, but was inactive against lactose, and could be fractionated into four forms with similar enzymic activities. The third type was more active against synthetic β-D-galactosides than lactose and was the only type to exhibit activity against galactosylglycoproteins and galactosylglycopeptides. Porcine kidney has been shown to contain two types of α-galactosidase and four types of β-galactosidase.[1560] The latter four enzymes were active against 4-nitrophenyl β-D-galactopyranoside, whereas only three of them were active against lactose.

The α- and β-galactosidase activities of jimpy mouse brain have been shown to be the same as those in normal mice,[1574] whereas those of RNA

[1569] K. Suzuki, E. L. Schneider, and C. J. Epstein, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 1363.
[1570] P. J. G. M. Rietra, J. M. Tager, and P. Borst, *Biochim. Biophys. Acta*, 1972, **279**, 436.
[1571] K. H. Grzeschik, A. M. Grzeschik, S. Banhof, G. Romeo, H. van Someren, P. M. Khan, A. Westerveld, and D. Bootsma, *Nature New Biol.*, 1972, **240**, 48.
[1572] B. Seetharam and A. N. Radhakrishnan, *Indian J. Biochem. Biophys.*, 1972, **9**, 59.
[1573] M. Sato and I. Yamashina, *J. Biochem. (Japan)*, 1971, **70**, 683.
[1574] D. S. Deshmukh, T. Inoue, and R. A. Pieringer, *J. Biol. Chem.*, 1971, **246**, 5695.

tumour virus-transformed embryonic mouse fibroblasts are greater than those of non-transformed fibroblasts.[1491]

The presence of a galactosidase in rat brain has been demonstrated using as substrate galactosylsphingosine specifically labelled with tritium at C-6 of the D-galactosyl residue.[1575] The enzyme was partially purified and exhibited maximum activity at pH 4.2—4.5. Changes in the specific activity of rat intestinal β-galactosidase during development have been related to the nutritional environment of the animal.[1576] β-Galactosidase has been obtained in a purified form from rat small intestine by gel filtration and ion-exchange chromatography of an extract solubilized by papain;[1577] the enzyme possesses a pH optimum of 5.2. The effects of carbohydrates and corticosteroid on the activity of rat intestinal β-galactosidase have been studied.[1530]

Kinetic data have been obtained for the β-galactosidase activities of the jejunum of infant rats.[1578] The α- and β-galactosidase activities of the jejunum and ileum of adult and suckling rats have been compared with those of other glycosidases present.[1493] The effects of pancreatectomy[1528] and actinomycin D[1529] on rat jejunal β-galactosidase have been investigated. Dilution of a solution of partially purified 'acid' α-galactosidase from ileal mucosa of suckling rats resulted in a decrease of the specific activity of the enzyme.[1496] This effect appeared to be the result of dissociation of the molecule and could be prevented by the addition of several proteins to the solution. DNA, tRNA, and oligonucleotides inhibited the enzyme, but several proteins were also able to prevent this inhibi tion. Inhibitions by nucleic acid species occurred only at pH value less than 4.0, whereas nucleotides had no effect. The effect of DNA on the activity of α-galactosidase from the same source was also investigated. The cortisone-evoked decrease of 'acid' β-galactosidase in the ileum of suckling rats has been studied.[1494]

The distribution of β-galactosidase among lysosomal particles of rat liver has been investigated,[1495] while that in rat renal cortex has been found to be typical of a lysosomal enzyme.[1579] Purification of β-galactosidase from a rat epididymal extract has been described and the activity of the enzyme against a series of substrates was investigated.[643]

The activity of β-galactosidase in chick brain and eyes has been followed during development.[1497] Extracts of the hepatopancreas of the tunicate *Pyura stolonifera* have been shown to contain α- and β-galactosidase activities.[1500]

[1575] T. Miyatake and K. Suzuki, *J. Biol. Chem.*, 1972, **247**, 5398.
[1576] C. Raychaudhuri and I. D. Desai, *Comp. Biochem. Physiol.*, 1972, **41B**, 343.
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[1578] J. Kraml, J. Kolínská, D. Ellederová, and D. Hiršová, *Biochim. Biophys. Acta*, 1972. **258**, 520.
[1579] D. G. Taylor, R. G. Price, and D. Robinson, *Biochim. Biophys. Acta*, 1972, **273**, 237.

The extent of transfer of β-D-galactofuranose from various aryl β-D-galactofuranosides to ethanol and methanol by almond emulsin β-galactosidase has been studied.[1580] The α-galactosidase present in green coffee beans has been isolated.[1581] Evidence has been presented to show that *myo*-inositol is a highly stereospecific and competitive inhibitor of α-galactosidase from glandless cotton (*Gossypium hirsutum*) seeds, germinating chicken peas (*Cicer arietinum*), peanuts (*Arachis hypogaea*), and pinto beans (*Phaseolus vulgaris*), but not of other similar glycohydrolases.[1582] Similarity between *myo*-inositol and α-D-galactopyranosides is considered to be responsible for inhibition.

Ficin, a crude proteolytic enzyme preparation from the fig, has been shown to contain α-galactosidase as the only glycohydrolase and the latter enzyme has been purified from ficin by gel filtration and ion-exchange chromatography.[1583] The enzyme was shown to be completely free from β-galactosidase activity. Evidence has been reported for the existence of α- and β-galactosidases in the culture fluids of orchid (*Phalaenopsis* sp.) seedlings.[1534] β-Galactosidase activity (pH optimum 5.3) has been detected in the 'Marianna' plum (*Prunus cerasifera* × *P. munsoniana*).[1584] Both α- and β-galactosidase activities have also been detected in wheat and flour [1513] and in wheat-flour doughs.[1519]

Growth of a fungal plant pathogen (*Collectotrichum lindemuthianum*) in the presence of D-galactose as the sole carbon source resulted in the selective secretion of α-galactosidase.[1516] Parameters affecting the secretion of the enzyme have been investigated.[1517]

The effect of anaerobiosis and decouplers on the stimulation of β-galactosidase synthesis by adenosine 3′,5′-cyclic phosphate in *Escherichia coli* has been studied.[1585] Inducible and constitutive β-galactosidase formation has been measured in *E. coli* cells recovering from various treatments that inhibited cell-protein synthesis.[1586] Delayed and immediate enzyme formation was observed in different strains, but all recovering cells exhibited a decreased rate of enzyme formation. Kinetic data have been obtained for the hydrolysis of β-D-galactosides by the β-galactosidase from *E. coli*.[1587] Using a series of substrates varying only in the identity of the leaving group, a constant ratio of products was obtained with both ethanol and methanol as acceptors. It was concluded that a common intermediate, which reacts in turn with the two acceptors (water and alcohol) available, is involved in the hydrolytic reaction of the enzyme. β-D-Galactopyranosyl azide is hydrolysed by *E. coli* β-galactosidase, whereas methyl 1-thio-β-D-galactopyranoside is a competitive inhibitor of the hydrolyses of the

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[1582] C. B. Sharma, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 572.
[1583] S. Hakomori, B. Siddiqui, Y. Li, S. Li, and C. G. Hellerqvist, *J. Biol. Chem.*, 1971, **246**, 2271.
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[1587] T. M. Stokes and I. B. Wilson, *Biochemistry*, 1972, **11**, 1061.

azide and 2-nitrophenyl β-D-galactopyranoside.[1588] It was concluded from the data obtained that the enzyme can hydrolyse a range of substrates of general structure β-D-galactopyranosyl-X(Y), where the atom X has a lone pair of electrons on which the enzyme may act as a Lewis or Brønsted acid, but in which the length of the bond cleaved varies significantly – a theory inconsistent with the orbital-steering hypothesis.

The β-galactosidase of *E. coli* has been shown to convert D-galactal into the corresponding 2-deoxy-D-hexose.[1589] In the presence of glycerol as acceptor, glyceryl 2-deoxy-β-D-*lyxo*-hexopyranoside was formed. The hydration is irreversible and hydrolysis of glyceryl 2-deoxy-β-D-*lyxo*-hexopyranoside by the enzyme gave rise to 2-deoxy-D-*lyxo*-hexose. The mechanisms of enzymic hydration and glycosylation of glycals was discussed.

A kinetic study of the effect of Mg^{2+} ions on the activity of β-galactosidase from *E. coli* indicated that activation operates through a binding with the free enzyme involving two steps.[1590] The first, more rapid step resulted in formation of the metal–protein bond and the second step in a conformational change induced by the bound metal ion. The inter-relationships between the effects of Na^+ and Mg^{2+} ions on the enzyme have been studied, as well as inhibition of the enzyme by high concentrations of the two ions and its reactivation on dilution.[1591] Labelling of the active site of *E. coli* β-galactosidase by *N*-bromoacetyl-β-D-galactopyranosylamine has been investigated.[1592] Sequence data have been obtained for *E. coli* β-galactosidase.[1593, 1594]

Active β-galactosidase has been obtained from thirteen mutant strains of *E. coli*, whereas the enzyme from a fourteenth strain, in which the site of mutation is close to the operator locus, was inactive.[1595] α-Complementation *in vivo* of the enzymes was studied and permitted some structural comparisons. Gel filtration of an extract from a mutant *E. coli* gave a number of peaks showing *in vitro* complementation of β-galactosidase; further observations suggested that the 'inactive' β-galactosidase consists of a number of units possessing molecular weights of less than 1.35×10^5.[1596] The rates of synthesis and degradation of incomplete chains of β-galactosidase by mutant strains of *E. coli* have been determined under a number of conditions.[1597] Incomplete chains were degraded at rates that are not directly proportional to their lengths, but which are apparently determined by their conformation. Synthesis of the different chains

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[1594] I. Zabin and A. V. Fowler, *J. Biol. Chem.*, 1972, **247**, 5432.
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occurred at similar rates, indicating the absence of a polar effect within the gene.

Subunits in hybrid β-galactosidases, reconstituted from β-galactosidases from wild type and mutant forms of *E. coli*, behaved as independent units of activity.[1598] Under certain conditions for renaturation or denaturation of the hybrid enzyme, subunits were found to exert an influence on neighbouring subunits, thereby rendering the whole hybrid molecule active or inactive. The purification and properties of an antigen cross-reacting with *E. coli* β-galactosidase have been described.[1599]

The involvement of lactose in the biosynthesis of β-galactosidase by cultures of *Klebsiella aerogenes* has been studied.[1600] The highest specific activities induced by lactose could not be increased further by addition of methyl β-D-1-thiogalactoside. The action of α-naphthyl acetic acid on the activity of β-galactosidase of *Lactobacillus acidophilus* during culture has been studied.[1601]

The α-galactosidase activities of subcellular fractions of particles from *Saccharomyces carlsbergensis* have been investigated by high-speed zonal fractionation.[1602] β-Galactosidase has been induced in the cells of *S. cerevisiae* using D-galactose;[1603] the failure of lactose to act as an inducer was attributed to a block in the permeability of the cells to β-D-galactosides. D-Galactose and lactose were found to be the best inducers of β-galactosidase in *S. fragilis*.[1604] The intra- and extra-cellular enzymes, which accumulated in equal amounts, were found to have common properties. β-Galactosidase isolated from the cells of *S. fragilis* has been purified by gel filtration and ion-exchange chromatography.[1605] The enzyme (molecular weight $> 5 \times 10^5$) possesses a pH optimum of 6.7—7.1 and is separated into four bands by polyacrylamide gel electrophoresis and by ultracentrifugation. The enzyme purified from the same source by isoelectric focusing was found to be crystallizable.[1606] This 10.0S β-galactosidase (molecular weight 2.03×10^5, p*I* 4.4) is activated by K^+, Mn^{2+}, Mg^{2+}, and Co^{2+} ions; it is thiol-dependent and is completely inactivated by Hg^{2+} ions and 4-chloromercuribenzoate.

An enzyme induced in a *Streptomyces* sp. and which degraded blood-group substance B has been identified as an α-galactosidase.[1607] The enzyme has been freed from other glycosidases and was shown to cleave α-(1 → 3)- and α-(1 → 6)-D-galactosyl linkages; it was inhibited by D-galactose, L-arabinose, D-xylose, melibiose, and raffinose.

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[1605] A. K. Kulikova, A. S. Tikhomirova, and R. V. Feniskova, *Biokhimiya*, 1972, **37**, 405.
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[1607] K. Oishi and K. Aida, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 578.

A thermostable β-galactosidase was inducible in an extreme thermophile resembling *Thermus aquaticus* by addition of lactose, D-galactose, or melibiose to growing cultures.[1608] The purified enzyme was found to possess an optimum pH of 5.0, a temperature optimum of 80 °C, and a molecular weight of 5.7×10^5. The enzyme is activated by Mn^{2+} and Fe^{2+} ions and is thiol-dependent.

β-Galactosidase has been used as an antigen directly or in conjugated form for the enumeration of lymphoid cells with specific surface-receptors for antigen.[1609] β-Galactosidase has been insolubilized by trapping in polyacrylamide gels [1610, 1611] and kinetic studies of the immobilized enzyme have been reported.[1610]

Glucosidases

A method described for the determination of β-glucosidase is based on measurement of the rate of enzymic hydrolysis of amygdalin as monitored with an electrode sensitive for cyanide ion under controlled conditions.[1612] As little as 10 ng of the enzyme can be determined precisely within one minute. A rapid, fluorometric method has also been developed for the measurement of β-glucosidase activity;[1613] 4-methylumbelliferyl β-D-glucopyranoside is used as a non-fluorescent substrate, with the enzyme activity determined from the amount of fluorescent aglycone released. Sweet clover, corn leaf, and almond emulsin were used as enzyme sources for developing the method, which gave linear Lineweaver–Burk plots.

In an attempt to identify the role of component C_1 in cellulolytic systems, it has been shown that C_1 hydrolysed native cellulose to cellobiose, which is then further hydrolysed by β-glucosidase to D-glucose.[1614] β-Glucosidase was found to have no action on cellulose.

The response patterns of β-glucosidase and glucoamylase activities of a purified glucoamylase in the presence of urea differed from one another according to the urea concentration.[1615] The effect was fully reversible.

Nojirimycin and D-glucono-1,5-lactone have been shown to be powerful inhibitors of glucosidases, but poor inhibitors of *exo-* and *endo*-glucanases.[1520] Thus, nojirimycin can be used to distinguish between α-glucosidase and glucoamylase, and between β-glucosidase and oligo-1,3-glucosidase. α-Glucosidase activity can be inhibited by tris(hydroxymethyl)-methylamine.[1522]

The activity of human brain β-glucosidase throughout life has been determined.[1461] The involvement of β-glucosidase in the catabolism of

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[1614] G. Halliwell, M. Griffin, and R. Vincent, *Biochem. J.*, 1972, **127**, 43P.
[1615] D. M. Belenkii and E. L. Rozenfeld, *Doklady Akad. Nauk S.S.S.R.*, 1971, **199**, 708.

brain glycosphingolipids in cases of metachromatic leukodystrophy and infantile amaurotic idiocy has been investigated.[1460, 1466] The β-glucosidase activities of tissues from three variants of Tay–Sachs disease were all found to be greater than normal.[1458] 'Acid' and 'neutral' α-glucosidases of human tissues and leukocytes can be separated by cellulose acetate electrophoresis.[1616] An additional isoenzyme was detected in kidney extracts and two variants of the 'acid' enzyme were found in two cases of α-glucosidase deficiency (glycogen-storage disease).

The β-glucosidase activities of the skins of normals and patients with cystic fibrosis of the pancreas have been compared.[1467] The α-glucosidase of human fibroblasts was inhibited non-competitively by αα-trehalose.[818] The β-glucosidase activity of human fibroblasts cultured from a case of Gaucher's disease has been determined.[1469] The lysosomal β-glucosidases of fibroblasts cultured from cases of cystic fibrosis, Hurler syndrome, and Maroteaux–Lamy syndrome and normals have been studied.[1468] A study of the electrophoretic patterns and activities of 'acid' and 'neutral' α-glucosidases of cultivated fibroblasts from controls and patients with the Type II variant (Pompé's disease) of glycogen-storage disease and their heterozygous parents has shown that the fibroblasts of the patients have dramatically reduced amounts of the enzyme, so that the disease can be diagnosed from the cultured cells.[1617] Similar results were obtained for amniotic fluid cells, indicating the feasibility of *in utero* diagnosis.

A rapid method for the assay of intestinal glycosidases has been applied to the measurement of intestinal α-glucosidase in normals and cases of coeliac disease.[1521] α-Glucosidase levels have been determined for jejunal biopsy specimens from American and South Indian control subjects and for patients with tropical sprue.[1523] Gel filtration and isoelectric focusing were used for the analysis of the isoenzyme patterns of β-glucosidase in liver and other tissues from cases of mannosidosis, Gaucher's disease, Tay–Sachs disease, and Hurler syndrome.[1470] The enzyme from Hurler tissue was found to transform more easily into high molecular weight forms. The pH–activity profiles and isoenzyme patterns of β-glucosidase from liver of controls have been compared with those of cases of cystic fibrosis and inborn metabolic disease.[1566]

Lysosomal 'acid' α-glucosidases of human placenta and of mice and rat livers have been purified.[823] The kinetic properties of the enzyme from humans were found to be similar to those of the rodent enzymes, although it was not inhibited by an excess of maltose. The ability of the enzyme to hydrolyse glycogen or to catalyse transglucosylation from maltose to glycogen was inhibited by an antibody to the enzyme; the antibody also inhibited the ability of the enzyme to hydrolyse substrates of low molecular weight, but to a lesser extent. Partial immunological cross-reactivity

[1616] J. C. Dreyfus and Y. Alexandre, *Biochem. Biophys. Res. Comm.*, 1971, **48**, 914.
[1617] J. F. Koster, R. G. Slee, W. C. Hülsmann, and M. F. Niermeijer, *Clinica Chim. Acta*, 1972, **40**, 294.

between the enzymes from the three species was recognized, but no immunologically reactive protein could be detected in tissues from patients with Type II glycogenosis. The properties of a purified α-glucosidase from human placenta have been investigated in the treatment of human Type II glycogenosis.[820]

The specific activities of α-glucosidase in leukocytes from normals and cases of Type II glycogen-storage disease have been reported.[1618] The β-glucosidase activities of blood platelets from controls and cases of Fabry's and Sandhoff's diseases have been measured, and the value of the platelet-enzyme assay for diagnosis of glycosphingolipidoses was discussed in the light of the results.[1479] β-Glucosidase from human blood platelets has been purified by gel filtration and its characteristics have been determined.[1480]

The lysosomal activities of α- and β-glucosidases in various tissues of Angus cattle have been determined.[1022] α-Glucosidase has been extracted from bovine spleen and was separated into two fractions by gel filtration.[1619] One of the fractions was shown to contain only 'acid' α-glucosidase that hydrolysed maltose and glycogen at comparable rates. The other fraction contained both 'acid' and 'neutral' α-glucosidases; the latter was separated by polyacrylamide gel electrophoresis into four forms, all of which hydrolysed maltose much faster than glycogen. The second 'acid' enzyme was inactivated by electrophoresis.

α-Glucosidase from porcine spleen has been purified to homogeneity by adsorption on Sephadex.[1620] The enzyme hydrolysed maltose, nigerose, kojibiose, isomaltose, glycogen, amylose, amylopectin, nigeran, and dextran. Dextrans containing α-(1 → 6)- and α-(1 → 3)-linkages were completely hydrolysed by the enzyme, whereas dextrans containing α-(1 → 2)-linkages were only hydrolysed partially. An enzyme (molecular weight 1.8×10^5) purified from canine serum by gel filtration appeared to possess both α-glucosidase and glucoamylase activities.[1621] The maximum α-glucosidase activity was manifested at pH 6.1 and was inhibited by turanose, erythritol, and tris(hydroxymethyl)methylamine.

The action pattern and kinetics of an 'acid' α-glucosidase purified from rabbit muscle have been defined using maltodextrins and glycogen as substrates.[831] The enzyme was shown to possess a pH optimum of 4.5—5.1 and to contain certain glucanase activities. Brief treatment of a membranous fraction from rabbit renal cortex with papain released most of the α-glucosidase activity.[1622] The gel-filtration properties of the enzyme were investigated. An enzyme purified from rabbit liver extracts by ion-exchange chromatography and gel filtration appeared to possess β-glucosidase and oligo-1,6-glucosidase activities.[1623]

[1618] S. R. Wyss, J. F. Koster, and W. C. Hülsmann, *Clinica Chim. Acta*, 1971, **35**, 277.
[1619] K. Fujimori, T. Fukui, and Z. Nikuni, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 483.
[1620] M. E. Preobrazhenskaya and E. L. Rosenfeld, *Biokhimiya*, 1972, **37**, 974.
[1621] K. F. McGeeney and D. O'Donnell, *Biochem. J.*, 1971, **125**, 115P.
[1622] F. K. Stevenson, *Biochim. Biophys. Acta*, 1972, **266**, 144.
[1623] N. A. Ushakova and I. S. Lukomskaia, *Doklady Akad. Nauk S.S.S.R.*, 1971, **199**, 719.

High activities of α-glucosidase have been detected in mouse pancreatic islets and the activities were compared with those of liver, skeletal muscle, and alloxan diabetic animals.[1624] The enzyme was found to exhibit maximum activity at pH 5.0—5.5. α-Glucosidase activities of the small intestine of genetically adipose mice were found to be greater than those of their siblings of normal weight.[1525] The α- and β-glucosidase activities of RNA tumour virus-transformed embryonic mouse fibroblasts were shown to be greater than those of the non-transformed fibroblasts.[1491]

Glucagon had no effect on the levels of 'acid' α-glucosidase of rat tissues.[835] The 'acid' α-glucosidase activity of rat liver and other tissues was inhibited by cortisone *in vivo* and *in vitro*.[1625] The effects of carbohydrate and corticosteroid upon the α-glucosidase activity of the intestines of infant rats have been examined.[1530] Gel filtration revealed the presence of two β-glucosidases in the supernatant obtained from treatment of a mucosal homogenate from the jejunum of infant rats with papain.[1578] These enzymes possessed pH optima of 5—6 and 3.5, and kinetic data obtained for them were compared with those for the β-galactosidases obtained from the same source. The effects of DNA *in vitro*,[1496] and of pancreatectomy [1528] and actinomycin D *in vivo*,[1529] upon rat jejunal α-glucosidase have been investigated.

α-Glucosidase has been demonstrated in the ovarian, fallopian, uterine, cervical, and vaginal tissues of female rats.[1626] In most of these tissues, the enzyme activity exhibited four different pH optima (pH 4.3, 5.6, 6.3, and 7.1) and these enzymic forms differed in their heat stabilities. The involvement of the enzymes in the normal physiological processes of the female reproductive tract was discussed.

Extracts of the hepatopancreas of the tunicate *Pyura stolonifera* have been shown to contain α- and β-glucosidases.[1500]

α-Glucosidase activities of lysosome-rich fractions from livers of normal and fasting rats have been measured.[840] Accelerated liver glycogenolysis was paralleled by a decrease in enzyme activity. The enzyme exhibited maximum activity at pH 4.0. Lysosomal α-glucosidase activities have been determined in foetal and neonatal rat liver.[839] α-Glucosidase has been found associated with the plasma membranes of rat renal cortex.[1579]

The extents of transfer of β-D-glucofuranose from various aryl β-D-glucofuranosides to ethanol and methanol catalysed by β-glucosidase from almond emulsin have been measured.[1580] The β-glucosidase of sweet almond oil converted D-glucal into the corresponding 2-deoxy-D-hexose.[1589] In the presence of glycerol as acceptor, glyceryl 2-deoxy-β-D-*arabino*-hexopyranoside was formed; unidentified glyceryl side-products were also produced. Enzymic hydration was found to be irreversible. Studies of the

[1624] I. Lundquist, *Hormone Metab. Res.*, 1972, **4**, 245.
[1625] E. J. Bourne, K. Clarke, J. B. Pridham, and J. J. M. Rowe, *Biochem. J.*, 1971, **121**, 663.
[1626] M. C. Rao, K. P. Gunaga, A. R. Sheth, and S. S. Rao, *Indian J. Biochem. Biophys.*, 1971, **8**, 232.

effect of gibberellic acid on the β-glucosidases ('cellobiase' and 'laminaribiase') of the aleurone cells of barley suggested that this compound is not involved in development of the enzyme.[1627]

A study of the distribution of β-glucosidase activity in fractionated homogenates of bean (*Phaseolus vulgaris*) hypocotyls and sweet clover (*Melilotus alba*) leaves suggested that binding of the enzyme to cell walls is not the only factor that influences its distribution.[1628]

Cell-wall preparations of *Convolvulus arvensis* have been shown to contain an α-glucosidase possessing a pH optimum of 4.7.[1629] The bulk of the activity could be solubilized by solutions of high ionic strength and it was concluded that the enzyme is located at the cell surface. β-Glucosidase prepared from the stems of 'Marianna' plum (*Prunus cerasifera* × *P. munsoniana*) has been characterized and shown to be free from α-galactosidase activity.[1584] The enzyme was shown to possess a pH optimum of 5.3 and highest activity was exhibited when D(−)-mandelonitrile β-D-glucopyranoside (prunasin) was used as substrate.

The α-glucosidase activity found in three mutants of sweet corn (*Zea mays*) and in a commercial preparation of malt amylase has been separated into three fractions by ion-exchange chromatography and gel filtration.[1630] The three forms of the enzyme exhibited their maximum activities at pH 3.0 and brought about hydrolysis of maltodextrin substrates with retention of anomeric configuration of the liberated D-glucose residues. The rate of hydrolysis of maltodextrins was found to be dependent on their molecular weight. The α- and β-glucosidases of wheat and flour [1513] and wheat flour doughs [1519] have been investigated.

Cell-free extracts of buffalo calf rumen fluid micro-organisms have been prepared and were able to hydrolyse carboxymethylcellulose and cellobiose.[1631] Inhibition studies with D-glucono-1,4-lactone indicated that two enzymes were present, the β-glucosidase being completely inhibited by the lactone.

β-Glucosidase (molecular weight 1.7×10^5, p*I* 3.8) obtained from *Aspergillus wentii* was shown to contain 21% carbohydrate consisting of neutral sugars (156 moles per mole, mannose with smaller amounts of glucose and fucose) and 2-amino-2-deoxyglucose (52 moles per mole).[1632] The formation by the enzyme of a complex with concanavalin A suggested that a number of glucose and mannose residues are present as terminal, non-reducing α-glycosides. The enzyme is apparently unable to form subunits. O.r.d. and c.d. measurements indicated that only 15% of the molecule is present in a helical conformation and that conformational changes occur below pH 2.2 and above pH 7.5.

[1627] D. J. Manners, G. H. Palmer, G. Wilson, and D. Yellowlees, *Biochem. J.*, 1971, **125**, 30P.
[1628] T. A. Jaynes, F. A. Haskins, H. J. Gorz, and A. Kleinhofs, *Plant Physiol.*, 1972, **49**, 277.
[1629] F. M. Klis, *Physiol. Plant.*, 1971, **25**, 253.
[1630] J. J. Marshall and P. M. Taylor, *Biochem. Biophys. Res. Comm.*, 1971, **42**, 173.
[1631] S. R. Anrad and K. K. Gandhi, *Indian J. Biochem. Biophys.*, 1971, **8**, 39.
[1632] G. Legler, M. Von Radloff, and M. Kempfle, *Biochim. Biophys. Acta*, 1972, **257**, 40.

Using 4-nitrophenyl β-D-glucopyranoside as substrate, it was found that the high molecular weight β-glucosidase obtained from the culture fluid of *Botryodiplodia theobromae* is inhibited by high concentrations, and is stimulated by low concentrations, of cellobiose.[1633] The enzyme was also inhibited by maltose and D-glucose, but these inhibitions were abolished by low concentrations of cellobiose. Further characteristics of the enzyme, including its ability to catalyse transglucosylation, were also investigated.

Press supernatants from blastospore and mycelial forms of *Candida albicans* have been found to contain β-glucosidase.[1634] A β-glucosidase has been purified from cultures of *Collectotrichum lindemuthianum*[1516] and factors affecting the secretion of the enzyme by the organism have been studied.[1517] Maltose has been shown to induce an enzyme, which appeared to have amylase and α-glucosidase activities, in cultures of *Corynebacterium diphtheriae*.[1635] The enzyme (molecular weight 6.12×10^4) was inhibited by Hg^{2+} ions, 4-chloromercuribenzoate, and iodoacetic acid.

β-Glucosidase activity has been detected in myxamoebae of the cellular slime mould *Dictyostelium discoideum* during growth in axenic culture[1507] and the influence of changes in the myxamoebae on the biosynthesis of the enzyme has been studied.[1508] α-Glucosidase has been purified from *Mucor rouxii* by a series of treatments followed by ion-exchange chromatography and gel filtration.[1636] The enzyme (pH optimum 3.5), which gave one band only on polyacrylamide gel electrophoresis, was shown to contain 40% carbohydrate consisting of glucose and, to a lesser extent, galactose and fucose. It behaved as a cation at low pH, but did not behave as an anion at high pH. The enzyme was highly specific, hydrolysing maltose and starch, but not synthetic α-D-glucosides and a number of other disaccharides.

The biosynthesis of α-glucosidase by protoplasts of a *Saccharomyces* sp. was inhibited by '2-deoxy-D-glucose', but not by 6-deoxy-D-glucose.[1551] The α-glucosidase activities of subcellular fractions of *S. carlsbergensis* have been investigated by high-speed zonal fractionations.[1602] A method for the decryptification of α-glucosidase in *S. cerevisiae* employed DMSO and permitted the direct assay of the enzyme on small samples of yeast suspensions.[1637] The synthesis of 1-, 3-, and 4-*O*-α-D-glucopyranosyl-L-sorbose by the transglucosidation reaction of brewer's yeast α-glucosidase has been reported.[1638] The influence of the α-glucosidase of baker's yeast on leavening has been studied.[1639] β-Glucosidase and laminarinase activities of *Saccharomyces lactis* have been compared.[1640]

[1633] G. M. Umezurike, *Biochim. Biophys. Acta*, 1971, **250**, 182.
[1634] F. W. Chattaway and F. C. Odds, *J. Gen. Microbiol.*, 1971, **67**, 255.
[1635] B. L. Howington and A. D. Larson, *J. Microbiol. Serol.*, 1972, **36**, 265.
[1636] A. Flories-Carreón and J. Ruiz-Herrera, *Biochim. Biophys. Acta*, 1972, **258**, 496.
[1637] B. G. Adams, *Analyt. Biochem.*, 1972, **45**, 137.
[1638] S. Chiba and T. Shimomura, *Agric. and Biol. Chem. (Japan)*, 1971, **9**, 1363.
[1639] H. Suomalainen, J. Dettwiler, and E. Sina, *Process Biochem.*, 1972, **7**, 16.
[1640] M. A. Tingle and H. O. Halvorson, *Biochim. Biophys. Acta*, 1971, **250**, 165.

β-Glucosidase activities have been identified in exudates and sclerotial extracts from *Sclerotinia sclerotiorum*.[1641]

A water-insoluble derivative of β-glucosidase has been formed by covalent attachment to poly(allyl cyclic carbonate).[1642] Instability of the enzyme on storage can be prevented by lyophilization in the presence of a polyol.

Glucuronidases

The formation of artefacts during incubation of neutral steroids with commercial β-glucuronidase preparations has been described.[1643]

Lower than normal levels of β-glucuronidase in skin from patients with cyctic fibrosis of the pancreas have been discussed in relation to the raised levels of uronic acid-containing macromolecules.[1467] The lysosomal activities of cultured fibroblasts from patients with cystic fibrosis, Hurler syndrome, Hunter syndrome, and controls have been measured.[1468]

Gel filtration and isoelectric focusing have been used to examine the β-glucuronidase isoenzymes of liver and other tissues from normals and cases of Hurler syndrome, Gaucher's disease, and Hunter syndrome; patterns from the patients were found to be normal.[1470] Characterization of β-glucuronidase purified from human placental lysosomes showed that only one isoenzyme exists.[1644] This isoenzyme was found to be the same as that in the endoplasmic reticulum, but to differ from that found in normal human plasma. A number of activators and inhibitors of the placental enzyme have been recognized. The high levels of β-glucuronidase found in human pregnancy serum were attributed solely to increased amounts of the normal enzyme.

The levels of β-glucuronidase in sera of cases of Hurler and Maroteaux–Lamy syndromes have been found to be elevated above normal.[1476] Serum and urinary levels of β-glucuronidase are above normal in cases of aspartylglucosaminuria.[976] Human blood platelets have been shown to contain β-glucuronidase and the enzyme has been purified by gel filtration.[1480] The release of the enzyme by the action of thrombin on the platelets has been determined for a variety of conditions.[1481] The activity of the released enzyme was inhibited by adenosine triphosphate, cytidine triphosphate, and guanosine triphosphate.

The normal levels of β-glucuronidase in tissues of horse and sheep have been reported.[1645] The elevated β-glucuronidase activity in mutant sheep was considered to reflect lysosomal retention related to hepatic pigmentation. Studies were also carried out on the variation of the levels of plasma β-glucuronidase with age.

[1641] N. Colotelo, J. L. Sumner, and W. S. Voegelin, *Canad. J. Microbiol.*, 1971, **17**, 1189.
[1642] J. F. Kennedy, S. A. Barker, and A. Rosevear, *J.C.S. Perkin I*, 1972, 2568.
[1643] M. A. Manson, L. Nocke-Fink, J. Å. Gustafsson, and C. H. L. Shackleton, *Clinica Chim. Acta*, 1972, **38**, 45.
[1644] S. F. Contractor and B. Shane, *Biochem. J.*, 1972, **128**, 11.
[1645] M. S. Anwer, A. S. Mia, and R. Gronwall, *Comp. Biochem. Physiol.*, 1972, **43B**, 929.

The β-glucuronidase activities of tissues of Angus cattle have been determined.[1022] The inhibition of bovine liver β-glucuronidase by 5-amino-5-deoxy-D-glucaro-1,5-lactam and D-glucaro-1,4-lactone has been compared.[1646] A similar comparison has been made *in vivo* for mouse liver and rat serum β-glucuronidases. The effect of DNA at various pH values on the activity of bovine liver β-glucuronidase has been investigated.[1496]

The levels of β-glucuronidase in skeletal muscle of normal and B10 14.6 dystrophic hamsters have been determined.[1647] The properties of mouse kidney microsomal and lysosomal β-glucuronidases have been compared and the induction of intracellular transport of the enzyme by gonadotrophins has been studied.[1648] The β-glucuronidase activity of B16 melanoma in mice has been investigated.[1649]

The relation between β-glucuronidase activity and bilirubin in the skin, liver, and blood has been studied in normal rats and those with experimental obstructive jaundice.[1650] Changes observed in the specific activity of β-glucuronidase in the intestine of rats during development have been related to a response to the nutritional environment of the animal.[1576] The activities of β-glucuronidase in the jejunum and ileum of adult and suckling rats have been compared with those of other glycosidases present.[1493] The effects of cortisone *in vivo* on the β-glucuronidase activity in the ileum of suckling rats have been investigated.[1494]

The distribution of β-glucuronidase among lysosomal particles of rat liver has been investigated [1495] and β-glucuronidase activities in subcellular fractions of rat kidney have been determined.[1065] The behaviour of the kinetic parameters of rat-liver β-glucuronidase as a function of pH has been examined;[1651] the dependence of maximal velocity on pH suggested that two dissociable groups are involved in the active site of the enzyme. p*K* Values of 4.14 and 6.21 were calculated for these groups and, together with values for the heats of dissociation, suggested the involvement of carboxy- and carboxylate-groups in the catalysis. Inactivation of the enzyme by a carbodi-imide and isoxazolium salts was considered to be compatible with this theory. Interaction of the β-glucuronidase with phenolphthalein β-D-glucopyranuronoside was found to involve ionic forces and the ionic group involved in the binding appeared to be an imidazolium group, since the enzyme was inactivated by both photo-oxidation and diazonium salts.

The relative rates of hydrolysis of monosubstituted phenyl β-D-glucopyranosiduronic acids catalysed by β-glucuronidase from rat-liver lysosomes

[1646] T. Niwa, T. Tsuruoka, S. I. Nouye, Y. Naito, T. Koeda, and T. Niida, *J. Biochem. (Japan)*, 1972, **72**, 207.
[1647] K. Wrogemann, B. E. Jacobson, and M. C. Blanchaer, *Enzyme*, 1971, **12**, 322.
[1648] K. Kato, I. Hirohata, W. H. Fishman, and H. Tsukamoto, *Biochem. J.*, 1972, **127**, 425.
[1649] C. Vaquero, M. Guigon, C. Masson, J. Lecordier, and J. Hewitt, *Compt. rend. Soc. Biol.*, 1971, **165**, 1217.
[1650] E. A. R. Garay, M. D. R. Spetale, and L. S. Morisoli, *Clinica Chim. Acta*, 1972, **37**, 171.
[1651] C. Wang and O. Touster, *J. Biol. Chem.*, 1972, **247**, 2644.

have been correlated with the corresponding Hammett values.[1652] A similar relationship has been observed for other enzymes. The relative rates of hydrolysis of 4-methoxyphenyl, 4-chlorophenyl, 4-nitrophenyl, and phenolphthalein β-D-glucopyranosiduronic acids by the enzyme were compared in water and deuterium oxide. It was observed that the pH optimum is shifted to a higher value in the case of deuterium oxide, whereas the rates of hydrolysis in this solvent are decreased. The results suggested that a rate-determining proton transfer is involved in the catalytic steps of the enzymic reaction.

Analysis of the pH and temperature optima, K_m values, heat denaturation data, and effects of Triton X-100 on the activities of various rat-liver fractions suggested that the β-glucuronidases from the endoplasmic reticulum and lysosomes have similar properties.[1653] Compounds inducing hepatic drug-metabolizing enzymes were found to reduce β-glucuronidase activity *in vivo*. The effect of sex hormones and intrauterine contraceptive suture on β-glucuronidase activity of rat uterus has been investigated.[1654]

β-Glucuronidase activity in the excreta of tadpoles of *Rana tigrina* increased considerably with advance of metamorphosis and the enzyme was found to be localized in the degenerated intestinal mucosa.[1655] The properties of the enzyme (pH optimum 4.2) were compared with those of enzymes present in various tissues of tadpoles.

Both α- and β-glucuronidase activities have been detected in the hepatopancreas of the tunicate *Pyura stolonifera* and the two activities were separable by gel filtration.[1500] The α-glucuronidase was shown to be active against aryl glycosides and the α-phosphate ester of D-glucuronic acid. Extracts of the embryogenesis stage, larva, and freshly emerged adults of the blowfly (*Crysomyia rufiferacis*) have been shown to contain both heat-labile and heat-stable β-glucuronidases (pH optima 5.2—5.4 and 4.2—4.4, respectively).[1656] Changes in the relative activities of the enzymes with development of the insect were determined.

The transfer of D-glucofuranuronic acid from 2-naphthyl β-D-glucofuranosiduronic acid to ethanol and methanol by the catalytic action of a β-glucuronidase from *Escherichia coli* has been studied.[1580]

An α-glycuronidase, which acted on degradation products of heparin and heparan sulphate containing terminal, non-reducing α-L-*threo*-4-deoxyhex-4-enopyranosyluronic acid residues, has been isolated and purified from *Flavobacterium heparinum*.[1657] The α-L-*threo*-hex-4-enopyranosyluronidase was separated from a heparinase and a sulphatase produced by the same organism by gel filtration and was found to be inactive against terminal, non-reducing, saturated uronosyl residues. The activity of the

[1652] C. Wang and O. Touster, *J. Biol. Chem.*, 1972, **247**, 2650.
[1653] G. W. Lucier and O. S. McDaniel, *Biochim. Biophys. Acta*, 1972, **261**, 168.
[1654] M. Ghose, J. K. Pande, P. R. Dasgupta, and A. B. Kar, *Current Sci.*, 1972, **41**, 217.
[1655] A. T. Varute and V. A. Sawant, *Compt. Biochem. Physiol.*, 1972, **41B**, 675.
[1656] A. T. Varute and V. A. Sawant, *Insect Biochem.*, 1971, **1**, 327.
[1657] C. T. Warnick and A. Linker, *Biochemistry*, 1972, **11**, 568.

enzyme was greatest against disaccharides and fell off markedly with increase in the chain length of oligosaccharide substrates. The enzyme, in conjunction with eliminases produced by *F. heparinum*, degraded heparin and heparan sulphate to monosaccharides.

The activities of β-glucuronidase of subcellular fractions of *Saccharomyces carlsbergensis* have been investigated by high-speed zonal fractionation.[1602]

Iduronidases

Extracts of skin fibroblasts and liver from normal individuals and patients with Hurler syndrome on incubation with desulphated dermatan sulphate released L-iduronic acid.[1658] Extracts from normal fibroblasts liberated larger amounts of this acid than those from Hurler fibroblasts. Similar studies with desulphated heparan sulphate as substrate showed that L-idurono-6,3-lactone was released. Measurement of the fibroblast α-iduronidase in cases of Hurler syndrome has been reported.[1469]

A deficiency of α-iduronidase in extracts from cultured fibroblasts, liver, and urine of patients with Hurler syndrome has been claimed, whereas no such deficiency exists in fibroblasts and liver from cases of Hunter or Sanfilippo syndromes.[1659] Thus, the absence of α-iduronidase is believed to be the underlying enzymic defect of the Hurler syndrome. Similar results and conclusions have been reported by others; culture of the deficient cells in the presence of an α-iduronidase (prepared from normal human urine) resulted in correction of the cells, accompanied by efficient uptake of the enzyme from the medium.[1660] A purified macromolecular fraction from normal human urine accelerated the degradation of radioactively labelled endogenous dermatan sulphate and added dermatan sulphate proteoglycan by the fibroblasts in a case of Hunter syndrome.[1661] The factor was shown to be free from common lysosomal glycosidases and sulphatases, and was separated by polyacrylamide gel electrophoresis into two isofactors.

Phenyl α-L-idopyranosiduronic acid has been used to detect an α-iduronidase in rat-liver lysosomal extracts.[1662] The enzyme was shown to be distinct from a β-glucuronidase present, which did not hydrolyse the L-*ido*-substrate.

Mannosidases

The inhibition of β-mannosidase by nojirimycin and D-glucono-1,5-lactone has been investigated.[1520]

[1658] R. Matalon, J. A. Cifonelli, and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1971, **42**, 340.
[1659] R. Matalon and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 959.
[1660] G. Bach, R. Friedman, B. Weissmann, and E. F. Neufeld, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2048.
[1661] M. Cantz, A. Chrambach, G. Bach, and E. F. Neufeld, *J. Biol. Chem.*, 1972, **247**, 5456.
[1662] B. Weissmann and R. Santiago, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1430.

α-Mannosidase activity of human brain has been shown to decrease throughout life.[1461] The levels of α-mannosidase in normal human skin have been compared with those from patients with cystic fibrosis of the pancreas.[1467] α-Mannosidases of fibroblasts cultured from patients with cystic fibrosis, Hurler syndrome, and Maroteaux–Lamy syndrome, and from controls have been studied.[1468]

The α-mannosidase of human liver and other tissues has been examined by gel filtration and isoelectric focusing, and the patterns were found to be normal for cases of Hurler syndrome, Hunter syndrome, Gaucher's disease, and Tay–Sachs disease.[1470] Normal human liver α-mannosidase has been shown to exist in three forms that are separable by ion-exchange chromatography.[1663] Two of the forms (A and B) possessed pH optima of 4.4, whilst the third form (C) exhibited maximum activity at pH 6.0. Examination of the α-mannosidase in cases of mannosidosis by ion-exchange chromatography and isoelectric focusing showed that the A and B forms are absent and that residual α-mannosidase activity is due to the presence of the C form in normal amounts.

α-Mannosidase activity has been shown to be present in human placenta and embryonic liver early in gestation;[1558] the enzyme was also found in amniotic fluid. A study of placental α-mannosidase has been reported.[1557] The preparation of α-mannosidase from human epididymus has been described and the enzyme was found to possess a pH optimum of 4.5.[1331]

The activities of α-mannosidase in leucocytes from normals and G_{M_1}-gangliosidosis homozygotes and heterozygous parents have been compared.[1478] The release of α-mannosidase from human blood platelets by thrombin has been studied for a variety of conditions.[1481] The purification of α- and β-mannosidases from human blood platelets by gel filtration has been described.[1480]

A disease of Angus cattle, previously known as pseudolipidosis, has been diagnosed as an inherited lysosomal-storage disease in which a glycopeptide material containing 2-amino-2-deoxyglucose and mannose is stored.[1022] Diseased animals have a near absolute deficiency of lysosomal α-mannosidase, whereas heterozygotes have a partial deficiency of the enzyme. Partially purified preparations of α-mannosidase have been obtained from monkey small intestine.[1572] The enzyme was inhibited by H_4edta, and Zn^{2+} ions were the only metal ions capable of reversing the inhibition; Zn^{2+} ions were also able to reverse inhibition of the enzyme caused by pre-incubation with Cu^{2+} and Cd^{2+} ions.

The α- and β-mannosidase activities of RNA tumor virus-transformed embryonic mouse fibroblasts have been shown to be greater than those of the non-transformed cells.[1491] Both α- and β-mannosidases from rat epididymus, β-mannosidase from hen-oviduct, and α-mannosidase from Jack-bean meal have been used in structural studies of glycoproteins.[655] α-Mannosidase has been purified from a rat epididymal extract.[643]

[1663] M. Carroll, N. Dance, P. K. Masson, D. Robinson, and B. G. Winchester, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 579.

The α- and β-mannosidases (molecular weights 2.5×10^5 and 1.0×10^5, respectively) have been purified from hen oviduct extracts by gel filtration.[656] The β-mannosidase was stable to high pH and the α-mannosidase was stable to heat; these properties were utilized to remove contaminating glycosidases from the preparations. Ag^+ ions inhibited completely the α-mannosidase, but were without effect on the β-enzyme; on the other hand, the α-enzyme was more labile to guanidine hydrochloride. The activities of both enzymes were impaired to the same degree by Hg^{2+} ions and the β-mannosidase was more susceptible to inhibition by D-mannono-1,5-lactone.

A β-mannosidase has been purified from the viscera of snails (*Achatina fulica*) by ion-exchange chromatography.[1408] The final product was free from other glycosidases (including α-mannosidase) and its specificity was demonstrated using a mannosyl glycopeptide from Taka-amylase as substrate.[1664] Extracts from the hepatopancreas of the tunicate *Pyura stolonifera* have been shown to contain α- and β-mannosidases.[1500]

A method described for the purification of α-mannosidase from the seeds of *Phaseolus vulgaris* involved ion-exchange chromatography, gel filtration, and isoelectric focusing.[1665] Two forms of the enzyme (I and II, p*I* values 5.1 and 6.1, respectively) were separated and form I (11.2S, molecular weight 1.94×10^5) was predominant. This form was Zn^{2+}-dependent and exhibited maximum activity at pH 4.6. Its properties were compared with those of a partially purified form II.

Both α- and β-mannosidases from crude pineapple bromelain have been isolated and purified.[1409] A method has been described for the isolation of α-mannosidase from soy-bean seeds and the properties of the enzyme were investigated.[1666] Germinated seeds appeared to contain two forms of α-mannosidase that exhibited different electrophoretic mobilities. The α- and β-mannosidases of wheat-flour doughs [1519] and the α-mannopyranosidase of wheat and flour [1513] have been investigated.

An α-mannosidase, purified from *Aspergillus niger* by means of gel filtration and ion-exchange chromatography, was shown to hydrolyse *O*-α-D-mannopyranosyl-(1 → 2)-D-mannose and *O*-α-D-mannopyranosyl-(1 → 2)-*O*-α-D-mannopyranosyl-(1 → 2)-D-mannose, but not *O*-α-D-mannopyranosyl-(1 → 3, 4, or 6)-D-mannose.[1667] The enzyme was also inactive against yeast mannan and a variety of D-mannose-containing glycopeptides. It exhibited maximum activity at pH 4.8 and was inhibited only slightly by D-mannono-1,5-lactone. Another α-mannosidase, purified from *A. niger* by gel filtration and ion-exchange chromatography, was obtained free from other glycosidases and the preceding α-(1 → 2)-mannosidase.[1668]

[1664] K. Sugahara, T. Okumura, and I. Yamashina, *Biochim. Biophys. Acta*, 1972, **268**, 488.
[1665] E. Paus and T. B. Christensen, *European J. Biochem.*, 1972, **25**, 308.
[1666] M. Saita, T. Kenaka, and Y. Matsushima, *J. Biochem.* (*Japan*), 1971, **70**, 827.
[1667] N. Swaminathan, K. L. Matta, L. A. Donoso, and O. P. Bahl, *J. Biol. Chem.*, 1972, **247**, 1775.
[1668] K. L. Matta and O. P. Bahl, *J. Biol. Chem.*, 1972, **247**, 1780.

Whereas the enzyme hydrolysed 4-nitrophenyl α-D-mannopyranoside very slowly, it readily hydrolysed α-(1 → 4)- and α-(1 → 6)-D-mannosyl linkages to adjoining D-mannose and 2-acetamido-2-deoxy-D-glucose residues. The enzyme (pH optimum 4.2) was strongly inhibited by D-mannono-1,5-lactone.

The specific activity of α-mannosidase in myxamoebae of the cellular slime mould *Dictyostelium discoideum* during growth in axenic culture has been determined.[1507] The influence of changes in the myxamoebae on the biosynthesis of the enzyme was studied.[1508]

Sialidases

The neuraminidases have been reviewed.[1669]

Similar levels of neuraminidase have been found in brain tissues from normals and cases of Tay–Sachs disease.[1464] Human blood leukocytes have been shown to contain a neuraminidase exhibiting maximum activity at pH 4.0 and which is inhibited by Hg^{2+} ions.[1670] Lower molecular weight substrates such as sialoglycopeptides, *N*-acetylneuraminyllactose, and its sulphated derivative were hydrolysed at higher rates than higher molecular weight substrates such as gangliosides and glycoproteins. A procedure for determining leukocyte neuraminidase for clinical purposes has been developed. Neuraminidase activities in patients infected with *Diplococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* have been investigated.[1671]

Endogenous and added gangliosides have been confirmed as substrates for calf-brain sialidase.[1021] The enzyme was also found to be active against endogenous sialoglycoproteins and sialoglycopeptides derived from them, whereas extraneous glycoproteins were not hydrolysed. The sialidase activity appeared to be enhanced by Triton X-100. The synthesis of neuraminidase by monkey-heart cells infected with Newcastle disease virus has been investigated.[1672]

A major sialidase fraction of rat liver which is active against gangliosides has been located in the plasma membrane of the cell.[1673]

The levels of sialidase have been followed in chick embryonic eyes during development.[1497] The liver of developing chicks has been found to contain soluble and bound forms of neuraminidase (pH optima 4.4 and 3.8, respectively).[1674] The soluble, but not the bound, enzyme was inhibited by Cu^{2+} and Hg^{2+} ions. Pre-incubation resulted in a decrease in activity of the soluble enzyme and an increase in activity of the bound enzyme.

[1669] A. Gottschalk and A. S. Bhargava, in 'The Enzymes', 3rd edn., ed. P. B. Boyer, Academic Press, New York, 1972, p. 321.
[1670] A. K. Yeh, D. R. P. Tulsiani, and R. Carubelli, *J. Lab. Clinical Med.*, 1971, **78**, 771.
[1671] R. D. O'Toole, L. Goode, and C. Howe, *J. Clinical Invest.*, 1971, **50**, 979.
[1672] L. Wassilewa, *Zentrallbatt Veterinärmedizin*, 1971, **18**, 824.
[1673] C. Schengrund, D. S. Jensen, and A. Rosenberg, *J. Biol. Chem.*, 1972, **247**, 2742.
[1674] D. R. P. Tulsiani and R. Carubelli, *Biochim. Biophys. Acta*, 1972, **284**, 257.

Terminal, *O*-acetylated sialic acid residues occurring naturally in rabbit Tamm–Horsfall glycoprotein were not hydrolysed by neuraminidases from *Clostridium welchii* and *Vibrio cholerae*.[1378] The abilities of neuraminidases from *C. welchii*, *V. cholerae*, and PR-8 influenza virus to cleave terminal *N*-acetylneuraminic acid units of human α_1-acid glycoprotein, which had been modified to contain eight- and seven-carbon analogues of the acid by sequential periodate oxidation and borohydride reduction, were investigated.[1166] *N*-Acetylneuraminosyl-2-acetamido-2-deoxy-D-galactitol and analogues having the *N*-acetylneuraminosyl units replaced with 5-acetamido-3,5-dideoxy-D-*galacto*-2-octulosonic acid and 5-acetamido-3,5-dideoxy-L-*arabino*-2-heptulosonic acid units were also used as substrates. It was found that 5-acetamido-3,5-dideoxy-D-*galacto*-2-octulosonic acid residues of the modified glycoprotein and disaccharide were hydrolysed by the enzymes at an initial rate of 30% of that found for complete *N*-acetylneuraminic acid units. 5-Acetamido-3,5-dideoxy-L-*arabino*-2-heptulosonic acid units were hydrolysed from the macromolecule at an initial rate of 10% of that for the complete units, but the corresponding disaccharide was not hydrolysed by the enzymes. The results of modification of neuraminidases obtained (by affinity chromatography) from *C. welchii* and from influenza A_2/Asia/57 virus (by ion-exchange chromatography) using *N*-bromosuccinimide and 2-hydroxy-5-nitrobenzylbromide in the presence and absence of substrate suggested an essential role of tryptophan in either the active-site region or in maintaining the intact structure of the protein chain necessary for enzymic activity.[1675]

Lactobacillus bifidus var. *pennsylvanicus*, which requires human milk for growth, has been shown to contain a neuraminidase.[1676] The purified enzyme was found to be active principally against *O*-(*N*-acetyl-α-neuraminosyl)-(2 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose and significantly less active against *O*-(*N*-acetyl-α-neuraminosyl)-(2 → 6)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose and *O*-(*N*-acetyl-α-neuraminosyl)-(2 → 8)-*O*-(*N*-acetyl-α-neuraminosyl)-(2 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose.

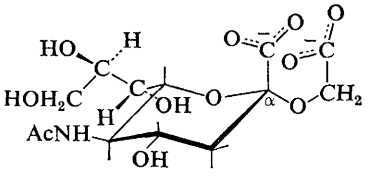
Neuraminidase has been detected in some strains of *Pasteurella haemolytica* and in most strains of *P. multocida*.[1677] In the latter organism, the enzyme was found to be bound to the cell wall and to be inducible by 2-acetamido-2-deoxy-D-mannose and by free and bound sialic acid. On solubilization, the enzyme (pH optimum 6.0) was shown to possess a molecular weight of 2.5×10^4. Pneumococcal neuraminidase has been purified by ion-exchange chromatography and gel filtration.[1678] The enzyme has been characterized by polyacrylamide gel electrophoresis and microisoelectric focusing, and it was estimated to possess a molecular weight of 6.98×10^4 and a p*I* of 4.90.

[1675] H. Bachmayer, *F.E.B.S. Letters*, 1972, **23**, 217.
[1676] H. von Nicolai and F. Zilliken, *Z. physiol. Chem.*, 1972, **353**, 1015.
[1677] R. Drzeniek, W. Scharmann, and E. Balke, *J. Gen. Microbiol.*, 1972, **72**, 357.
[1678] W. L. Stahl and R. D. O'Toole, *Biochim. Biophys. Acta*, 1972, **268**, 480.

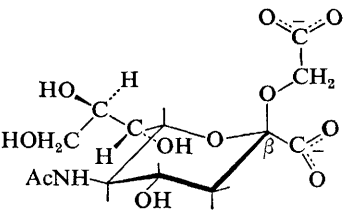
Neuraminidase activity has been detected in culture filtrates of some *Streptomyces* sp. including *S. albus* and *S. albidoflavus*.[1679] The enzyme hydrolysed *N*-acetylneuraminosyllactose and various sialoglycoproteins; in the presence of an excess of substrate, the reaction rate is proportional to the enzyme concentration. The enzyme (molecular weight 4.0—4.5 × 10^4) exhibited maximum activity at pH 6.0 with *N*-acetylneuraminosyllactose as substrate, but was neither stimulated by bivalent metal ions nor inhibited by H_4edta.

The neuraminidase activities of various *Vibrio* species have been investigated.[1680] The action of sialidase from *V. cholerae* has been investigated using gangliosides, sialoglycosaminoglycans, and fibrinogen as substrates; the enzyme was stimulated by Ca^{2+}, Mn^{2+}, and Co^{2+} ions, and possessed a pH optimum of 5.5.[1681] The activity of sialidase from *V. cholerae* has been studied as a function of the physical state of ganglioside substrates.[1682] Kinetic investigations showed that the substrate is hydrolysed both in disperse and micellar forms. Neuraminidases from *V. cholerae* and strains of *Myxovirus* and *Paramyxovirus* selectively liberated different types of neuraminic acid derivatives from a glycoprotein fraction of human serum.[1152]

The role of the anionic site in substrates of *V. cholerae* neuraminidase has been studied using α- and β-ketosides of *N*-acetylneuraminic acid.[1683] High K_m values for the 2-aminoethyl and 2-pyridyl α-ketosides, and the inability of the 2-aminoethyl α-ketoside to inhibit cleavage of the benzyl α-ketoside, indicated a low affinity of substrate for the enzyme. The formation of an internal, ionic bond in the substrate was shown to disturb the activity of the neuraminidase. The results support the assumption that a bond between a carboxy-group of the substrate and the enzyme is necessary for cleavage of α-ketosidic linkages in the substrate. However, the carboxymethyl α-ketoside of *N*-acetylneuraminic acid (93) was completely hydrolysed by *V. cholerae* neuraminidase and it was concluded



(93)



(94)

[1679] M. K. Myhill and T. M. Cook, *Canad. J. Microbiol.*, 1972, **18**, 1007.
[1680] V. D. Soloviev, I. V. Domaradsky, N. Y. Shimanyuk, K. G. Bichul, and I. I. Kurennaya, *Biull. Eksper. Biol. Medit.*, 1972, **73**, 61.
[1681] A. S. Balasubramanian, *Indian J. Biochem. Biophys.*, 1971, **8**, 77.
[1682] V. Lipovac, G. Bigalli, and A. Rosenberg, *J. Biol. Chem.*, 1971, **246**, 7642.
[1683] L. Holmquist and R. Brossmer, *Z. physiol. Chem.*, 1972, **353**, 1346.

that the additional anionic site in the aglycone has no drastic effect upon the activity of the enzyme.[1684] The β-ketoside (94) was not cleaved by the enzyme nor did it inhibit enzymic activity. *V. cholerae* neuraminidase neither cleaved the benzyl α-ketosides of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulose (5-*N*-acetyl-D-nonulosamine) and 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonamide (5-*N*-acetyl-D-neuraminide) nor was it inhibited by these compounds.[1685] The free β-amide, however, showed an inhibitory action when benzyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-nonulosidonic acid (benzyl *N*-acetyl-α-D-neuraminidic acid) was used as substrate.

The neuraminidase of fowl plague virus was specifically active toward *O*-(*N*-acetyl-α-neuraminosyl)-(2 → 3)-D-galactose linkages, whereas *O*-(*N*-acetyl-α-neuraminosyl)-(2 → 8)-*N*-acetylneuraminic acid linkages were cleaved only at very slow rates;[1686] similar properties for the enzyme from this source have been reported by others.[1687] Whereas both types of linkage were hydrolysed by Newcastle disease virus neuraminidase, neither enzyme was capable of hydrolysing *O*-(*N*-acetyl-α-neuraminosyl)-(2 → 4)-2-acetamido-2-deoxy-D-glucose linkages. Differences in the activities of the enzymes against hematosides were recognized. The synthesis of neuraminidase by influenza virus was inhibited by 2-deoxy-D-*arabino*-hexose and 2-amino-2-deoxy-D-glucose.[742]

Xylosidases

The inhibition of β-xylosidase by nojirimycin and D-glucono-1,5-lactone has been investigated.[1520]

Human blood platelets have been shown to contain a β-xylosidase and the enzyme has been purified by gel filtration.[1480] The α- and β-xylosidases of wheat and flour [1513] and the β-xylosidase of wheat-flour dough [1519] have been investigated.

4-Aminobenzyl 1-thio-β-D-xylopyranoside, coupled as an inhibitor ligand to Sepharose, has been used for the purification of β-xylosidase from *Bacillus pumilus* by affinity chromatography.[1688] No detectable binding of the enzyme occurred when 4-aminophenyl 1-thio-β-D-xylopyranoside was used as the ligand.

Factors affecting the secretion of β-xylosidase by cultures of *Colletotrichum lindemuthianum* have been examined.[1517] β-Xylosidase has been detected in Enterobacteriaceae sp. including strains of *Enterobacter aerogenes*, *E. agglomerans*, *E. cloacae*, *Klebsiella pneumoniae*, *K. oxytoca*, *K. ozenae*, *Yersinia pestis*, and *Y. pseudo-tuberculosis*.[1689]

[1684] L. Holmquist and R. Brossmer, *F.E.B.S. Letters*, 1972, **22**, 46.
[1685] R. Brossmer and L. Holmquist, *Z. physiol. Chem.*, 1971, **352**, 1715.
[1686] I. Ishizuka and H. Wiegandt, *Biochim. Biophys. Acta*, 1972, **260**, 279.
[1687] R. T. C. Huang and M. Orlich, *Z. physiol. Chem.*, 1972, **353**, 318.
[1688] M. Claeyssens, H. Kersters-Hilderson, J. Van Wauwe, and C. K. de Bruyne, *F.E.B.S. Letters*, 1970, **11**, 336.
[1689] B. Brisou, C. Richard, and A. Lenriot, *Ann. Inst. Pasteur*, 1972, **123**, 341.

α-Amylases

Animal and plant,[1690] and bacterial and mould amylases[1691] have been reviewed and the action patterns of amylases have been described.[1692] The inhibition of α-amylase by nojirimycin and D-glucono-1,5-lactone has been studied.[1520]

Amylopectin dyed with Reactone Red 2B has been used as a substrate for the determination of amylase.[1693] After incubation with the enzyme, residual polymeric substrate could be precipitated by methanol containing tannic acid, benzoic acid, and sodium benzoate; the colour density of the supernatant was then determined spectrophotometrically. Amylose dyed with Cibachron Blue has also been used as a basis for the determination of amylase.[1694]

The principle of radial diffusion in agar gels containing the substrate has been applied to the quantitative assay of α-amylase.[1695] The appearance of clear zones in the opalescent agar, or the staining of incorporated substrate or its hydrolysis products, indicated the diffusion zone of the enzyme. A linear relationship was found between the logarithm of the enzyme concentration and the corresponding diameters of the diffusion zones. The method is applicable not only to free enzymes in solution, but also to diffusible enzymes in small pieces of tissues and homogenates.

It has been shown that random oxidation with periodate of a polysaccharide substrate to an extent of 5% is sufficient to block the action of an *exo*-enzyme, but not an *endo*-enzyme, upon the substrate.[1454] Such modified substrates can be used for distinguishing between α- (*endo*) and β-amylases (*exo*).

A method involving dialysis into alkaline ferricyanide solution of the reducing sugars liberated from the substrate during incubation has been described for the automated determination of α-amylase in human small intestinal mucosa.[1522] The action of the α-glucosidase present was inhibited by tris(hydroxymethyl)methylamine, whereas the α-amylase was inactivated by incubation of the mucosal homogenates in the presence of H_4edta. The isoenzymes of human, equine, porcine, ovine, feline, canine, and rabbit pancreatic α-amylases, and human and rat serum α-amylases have been investigated by isoelectric focusing.[1696] The characteristics of the enzymes were studied. The hydrolyses of soluble starch by human and rat pancreatic α-amylases appeared to be biphasic reactions.[1697] Amylolysis by the rat

[1690] J. A. Thoma, J. E. Spradlin, and S. Dygert, in 'The Enzymes', 3rd edn., ed. P. D. Boyer, Academic Press, New York, 1972, p. 115.
[1691] T. Takagi, H. Toda, and T. Isemura, in 'The Enzymes', 3rd edn., ed. P. D. Boyer, Academic Press, New York, 1972, p. 235.
[1692] K. Hiromi, *Seikagaku*, 1972, **44**, 233.
[1693] A. L. Babson, S. A. Tenney, and R. E. Megraw, *Clinical Chem.*, 1970, **16**, 39.
[1694] B. Klein, J. A. Foreman, and R. L. Searcy, *Clinical Chem.*, 1970, **16**, 32.
[1695] W. B. Schill and G. F. B. Schumacher, *Analyt. Biochem.*, 1972, **46**, 502.
[1696] W. Berndt, H. Kolhoff, and U. Staudt, *Science Tools*, 1970, **17**, 45.
[1697] A. Vandermeers, M.-C. Vandermeers-Piret, and J. Christophe, *Biochimie*, 1971, **53**, 859.

enzyme was characterized by a primary phase, which corresponded to the rapid hydrolysis of starch to maltodextrins larger than maltodecaose, followed abruptly by a secondary phase in which maltodextrins were hydrolysed slowly to end-products in the maltopentaose–maltose range. The findings have been applied to an automated single-point determination of human and rat α-amylases using 3,5-dinitrosalicylic acid as the oxidizing agent, the assays being conducted during the primary phase. The method was also adapted to measure human urinary α-amylase.

The determination of α-amylase activity in fluids from normals and patients using 3,5-dinitrosalicylic acid has been described.[1698] A chromogenic method, which employs Cibachron Blue F_3G-A–amylose as a substrate, for the determination of serum α-amylase was found to compare favourably in its precision with the amyloclastic procedure.[1699] Cross-linking of this dyed substrate with butane-1,4-diol glycide ether gave a material suitable for inclusion into agar gels for use as a substrate in the determination (by a gel-diffusion technique) of α-amylase in normal blood, sera, and plasma.[1700] Similarly, dyeing of diepoxide cross-linked starch with Cibachron Blue F_3G-A yielded a material that could be incorporated into agar gels.[1701] Such substrates were used for the determination of α-amylase in sera and urine from cases of pancreatitis. The use of Reactone Red 2B coupled to amylopectin as a substrate for the assay of serum α-amylase has also been described and it was concluded from the analyses that the enzymic digestion is not a random process.[1702] A kinetic procedure for the determination of human serum amylase has been developed using nephlometry.[1703] Light scattering from the substrate (a stable suspension of starch) was decreased as the enzyme converted the substrate into soluble products. Good correlation was found between results obtained from this method and those in which a starch–dye complex was used.

A procedure using a u.v.-spectrometric finish and based on the measurement of maltose has been described for the determination of α-amylase activity in human sera and urine.[1704] α-Glucosidase and hexokinase acted as auxiliary enzymes and glucose 6-phosphate dehydrogenase as the indicator enzyme, the reduced nicotine adenine dinucleotide phosphate formed being measured. A plate method using an agar medium for the measurement of human serum and plasma α-amylase gave high correlations with results obtained from saccharogenic, amyloclastic, and chromogenic methods when applied to samples from normals and cases of acute pancreatitis.[1705]

[1698] S. Scharpé, *Clinica Chim. Acta*, 1972, **37**, 301.
[1699] F. R. Dalal and S. Winsten, *Clinica Chim. Acta*, 1971, **32**, 327.
[1700] M. Ceska, *Clinica Chim. Acta*, 1972, **36**, 453.
[1701] M. Ceska, *Clinica Chim. Acta*, 1972, **36**, 463.
[1702] C. D. Voorhorst and K. de Kloep, *Clinica Chim. Acta*, 1972, **39**, 391.
[1703] J. R. Shipe and J. Savory, *Clinical Chem.*, 1972, **18**, 1323.
[1704] H. W. Schinara, *Z. klin. Chem. klin. Biochem.*, 1972, **10**, 12.
[1705] M. Ceska, *Clinica Chim. Acta*, 1972, **36**, 419.

A comparison of serum amylase activities in normals and cases of liver disfunction showed that values for the patients were low and that the amount of residual activity was related to the degree of liver disfunction.[1706] It has been shown by gel-filtration studies that normal human serum amylase can form a complex with glycogen.[1025] Similar studies and the use of concanavalin A have shown that the macroamylase in sera from cases of macroamylasemia may be a complex of normal-sized amylase and a glycoprotein. Incubation of the macroamylases with human salivary [^{131}I]amylase resulted in an increase in the molecular size of the latter, thus substantiating previous results.[1325] The compound bound to amylase differed from subject to subject and was found to be an immunoglobulin in one case. However, the inability of the compound to bind fungal α-amylase suggested that the complexes are not the result of enzyme–substrate interactions.

Studies on one of the starch-degrading enzymes of bovine serum using amylose as substrate indicated that the enzyme is an *endo*-amylase rather than an *exo*-amylase.[1707] The enzyme exhibited a pronounced degree of multiple attack in its mode of action.

The mechanism of action of α-amylase from porcine pancreas and *Bacillus subtilis* has been investigated by determining the concentrations of oligomeric and polymeric substrate chains during the course of amylolysis.[1708] Relationships between the degree of multiple attack and various parameters were derived. Primary structural data have been reported for porcine pancreatic α-amylase;[1709] two forms of the enzyme have been isolated and the activities, molecular weights, compositions, and partial structures were indicated to be similar.[1710] Crystalline porcine pancreatic α-amylase has been separated by ion-exchange chromatography into four fractions, each of which hydrolysed amylose in a manner identical with that of the parent enzyme; *i.e.* at optimal pH, the reaction pattern corresponded to multiple attack, whereas in the presence of glycerol or at a high pH, it changed to multi-chain attack.[1711] The molecular weights (5×10^4) of the parent and the four forms were identical and amino-acid analyses failed to reveal any gradation in properties that would result in fractionation during chromatography. It was concluded that the multiple forms are artefacts arising during isolation of the enzyme. Porcine pancreatic α-amylase has been used for the preparation of polysomes free from glycogen.[1712]

[1706] I. H. Bhutta and M. A. Rahman, *Clinical Chem.*, 1971, **17**, 1147.
[1707] W. Banks, N. K. Mazumder, and R. L. Spooner, *Comp. Biochem. Physiol.*, 1971, **40B**, 983.
[1708] K. M. Bendetskii, V. L. Yarovenko, and L. N. Luk'yanova, *Biokhimiya*, 1972, **37**, 117.
[1709] P. Cozzone and G. Marchis-Mouren, *Biochim. Biophys. Acta*, 1972, **257**, 222.
[1710] P. Cozzone, L. Paséro, B. Beaupoil, and G. Marchis-Mouren, *Biochimie*, 1971, **53**, 957.
[1711] W. Banks, C. T. Greenwood, and K. M. Khan, *Carbohydrate Res.*, 1971, **20**, 233.
[1712] S. Gamulin, C. H. Gray, and M. R. Norman, *Biochim. Biophys. Acta*, 1972, **259**, 239.

From a study of the digestibility of hydroxypropyl-starches by a relatively large amount of porcine pancreatin, it was found that digestibility decreased exponentially with increases in the degree of substitution.[1713] This relationship was explained by means of a statistical model based on the assumption that the hydroxypropyl groups are distributed randomly over the starch molecule.[1714] In this case, the reducing power of the porcine pancreatic digests is proportional to the function (1 − degree of substitution) and, from the data obtained, the value of the proportionality constant was estimated to be 5.3. This finding indicated that the pancreatic enzymes act only on α-(1 → 4)-linked chains containing five or more D-glucopyranose units.

Although the secretion of both parotid and pancreatic mouse amylases is mediated by adenosine 3′,5′-cyclic phosphate, the secretion of the parotid enzyme only is influenced by isoproterenol.[1715] Purified α-amylases from rat pancreas and parotid glands were distinguishable by disc gel electrophoresis and isoelectric focusing, and the latter technique resolved the pancreatic enzyme into two major species (p*I* values 8.77 and 8.95).[1716] The molecular weights (5.6×10^4) of the two enzymes were identical and this value was maintained in the presence of dissociating agent. The parotid enzyme was found to contain somewhat less carbohydrate than the pancreatic enzyme and it was concluded that the two enzymes are products of different genes and not of subunit hybridization. Thyroxine has been shown to modulate the adenosine 3′,5′-cyclic phosphate-dependent secretion of rat parotid α-amylase by parotid glands *in vitro* by enhancing the sensitivity of membrane-bound adenyl cyclase to epinephrine.[1717] Thyroxine was shown to be without effect.

The levels of α-amylase in sera of a number of monotremes and marsupials have been determined.[1204] The occurrence of amylase in flies (*Drosophila melanogaster*) has been found to vary according to the genotype and nature of the food medium.[1718] Amylase activity has been detected in a xylophagic insect *Rhagium inquisitor*.[1719]

α-Amylase is considered to be produced in the aleurone cells of barley by a mechanism that is sensitive to exogenous gibberellic acid,[1627] which has been shown to induce *de novo* synthesis of the enzyme in aleurone tissue by modifying a post-transcriptional control point.[1720] Using radioactive labelling techniques, it has been shown that there is an association between the gibberellic acid-enhanced synthesis of RNA and that of

[1713] D. C. Leegwater and J. B. Luten, *Stärke*, 1971, **23**, 430.
[1714] D. C. Leegwater, *Stärke*, 1972, **24**, 11.
[1715] D. Malamud, *Biochim. Biophys. Acta*, 1972, **279**, 373.
[1716] T. G. Sanders and W. J. Rutter, *Biochemistry*, 1972, **11**, 130.
[1717] T. E. Nelson and J. E. Stouffer, *Biochem. Biophys. Res. Comm.*, 1972, **48**, 480.
[1718] G. de Jong, A. J. W. Hoorn, G. E. W. Thörig, and W. Scharloo, *Nature*, 1972, **238**, 453.
[1719] V. Levaditou, M. J. Foglietti, and J. E. Courtois, *Compt. rend. Soc. Biol.*, 1971, **165**, 796.
[1720] P. S. Carlson, *Nature New Biol.*, 1972, **237**, 39.

α-amylase in barley aleurone layers, and it was concluded that synthesis of the enzyme does not occur without the production of gibberellic acid-RNA.[1721]

A disc-electrophoretic system has been described for the separation of the α-amylase components of malted barley at low pH.[1722] α-Amylase extracted from the same source has been purified to homogeneity by gel filtration.[1723] The enzyme was found to be a glycoprotein in which the carbohydrate moiety contains xylose, galactose, mannose, fucose, and 2-amino-2-deoxyglucose; the carbohydrate appears to act as a protective agent against denaturation and proteolysis. Separation of the α- and β-amylases of barley malt by ion-exchange chromatography led to the identification of two α-amylase components.[1724] Heat treatment of the components affected neither their activity nor their relative proportions nor chromatographic behaviour.

The site of α-amylase in developing barley kernels has been studied and the enzyme has been purified by ion-exchange chromatography followed by selective precipitation with glycogen.[1725] The properties of the enzyme were determined using amylose as substrate. The α-amylase of extracts of germinated barley has been separated from the debranching enzymes.[1726]

The hydrolytic and branching activities of an enzyme fraction obtained by ion-exchange chromatography of a corn extract have been separated by either zonal ultracentrifugation or membrane ultrafiltration into two fractions.[1727] The action of one of the enzymes (molecular weight 7×10^4) simulated that of α-amylase in by-passing branch points. The other enzyme (molecular weight 2×10^4) was found to introduce barriers to β-amylolysis without causing hydrolysis. It was concluded that the hydrolytic and branching activities are manifested by two separate molecules.

Starch-degrading enzymes of the leaves of a temperate grass, orchard grass (*Dactylis glomerata*), tropical grasses, 'Pangola' digit grass (*Digitaria decumbens*), pearl millet (*Pennisetum glaucum*), and sugar cane (*Saccharum officinarum*) have been separated by polyacrylamide gel electrophoresis and their properties have been examined.[1728] Densitometer tracings of the gels negatively stained by starch–iodine showed distinct patterns for each species and it was concluded that α-amylase is the major starch-degrading enzyme of these grasses.

The induction of amylase in mustard seedlings by phytochrome has been investigated and the properties of the amylase bands obtained on gel

[1721] J. A. Zwar and J. V. Jacobsen, *Plant Physiol.*, 1972, **49**, 1000.
[1722] A. W. MacGregor and W. O. S. Meredith, *J. Inst. Brewing*, 1971, **77**, 510.
[1723] E. D. Mitchell, *Phytochemistry*, 1972, **11**, 1673.
[1724] A. W. MacGregor, D. E. LaBerge, and W. O. S. Meredith, *Cereal Chem.*, 1971, **48**, 490.
[1725] A. W. MacGregor, A. G. Gordon, W. O. S. Meredith, and L. Lacroix, *J. Inst. Brewing*, 1972, **78**, 174.
[1726] D. J. Manners and K. L. Rowe, *J. Inst. Brewing*, 1971, **77**, 358.
[1727] H. L. Griffin and Y. V. Wu, *Biochemistry*, 1971, **10**, 4330.
[1728] J. L. Carter, L. A. Garrard, and S. H. West, *Phytochemistry*, 1972, **11**, 2423.

electrophoresis were examined.[1729] In an investigation of the biochemical changes occurring in rice grains during germination, it was found that α-amylase activity increased progressively.[1730] Two isoenzymes were separated by polyacrylamide gel electrophoresis and a third isoenzyme appeared during germination.

α-Amylase has been prepared from an extract of malted rye flour by heat treatment and continuous electrophoresis.[1731] Two isoenzymes differing in physical properties were obtained, but their action patterns appeared to be identical.

The location of α-amylase in developing wheat grains has been studied.[1732] The preparation of α-amylase from wheat has been described [1184] and a large number of isoenzymes have been identified by gel electrophoresis.[1733] Changes in the amylases of hard red spring wheat during growth and maturation have been investigated by polyacrylamide gel electrophoresis.[1734] Three α-amylase isoenzymes are formed during early growth and persist until maturation, whereafter their quantities decrease rapidly. The location of the enzyme was investigated. Two sets of α-amylase isoenzymes are formed during germination.[1735] One set is composed of three isoenzymes, which are electrophoretically identical with those found in immature kernels, and the other set is composed of four isoenzymes, the lower electrophoretic mobilities and intensities of which changed during germination. Antibodies specific for the α-amylase of germinated wheat seeds have provided evidence that the α-amylase of maturing seeds is antigenically different from that synthesized during germination.[1736]

It has been shown that the concentrations of product and substrate chains do not exceed the initial concentration of substrate chains in the hydrolysis of starch, amylose, and maltodextrins by glucoamylase from *Aspergillus awamori*.[1737] Thus, the presence of α-amylase activity in the enzyme preparation was detectable by an increase in the concentration of substrate chains above the initial concentration during the course of the reaction.

The amylase activity of *A. nidulans* has been investigated.[1738] From an investigation of the function of sulphhydryl groups in the acid-stable and acid-labile α-amylases from *A. niger*, it was concluded that some differences exist in the neighbourhood of this group in each enzyme, but that it is not present at the active site of either enzyme.[1739]

[1729] H. Drumm, I. Elchinger, J. Möller, K. Peter, and H. Mohr, *Planta*, 1971, **99**, 265.
[1730] E. P. Palmiano and B. O. Juliano, *Plant Physiol.*, 1972, **49**, 751.
[1731] D. J. Manners and J. J. Marshall, *Stärke*, 1972, **24**, 3.
[1732] W. Banks, A. D. Evers, and D. D. Muir, *Chem. and Ind.*, 1972, 573.
[1733] S. V. Iliev, *Doklady Bolg. Akad. Nauk*, 1972, **25**, 833.
[1734] J. E. Kruger, *Cereal Chem.*, 1972, **49**, 379.
[1735] J. E. Kruger, *Cereal Chem.*, 1972, **49**, 391.
[1736] J. Daussant and M. Renard, *F.E.B.S. Letters*, 1972, **22**, 301.
[1737] K. M. Bendetsky, V. L. Yarovenko, L. N. Ermakova, and L. N. Luk'yanova, *Biokhimiya*, 1970, **35**, 627.
[1738] B. J. M. Zonneveld, *Biochim. Biophys. Acta*, 1972, **273**, 174.
[1739] K. Aoki, M. Arai, Y. Minoda, and K. Yamada, *Agric. and Biol. Chem.* (*Japan*), 1971, **35**, 1913.

The role of the cell envelope in regulation of the synthesis of α-amylase in *A. oryzae* has been investigated,[1740] and sulphhydryl and disulphide groups of the enzyme have been enumerated.[1741] Treatment of an asparagine-oligosaccharide from *A. oryzae* α-amylase with alkaline borohydride resulted in complete cleavage of the 2-amino-2-deoxy-D-glucose-asparagine linkage with concomitant production of terminal 2-amino-2-deoxy-D-glucitol. Sequential periodate oxidation, borohydride reduction, and hydrolysis yielded 2-amino-2-deoxy-D-xylitol, indicating the presence of an *O*-(2-acetamido-2-deoxy-D-glucosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose unit.[652] Using the same technique, the penultimate 2-acetamido-2-deoxy-D-glucose unit was found to be substituted at O-4. These assignments were confirmed by methylation studies and it was concluded that the glycopeptide structure involves (95), which has been also recognized in hen

β-D-Man-(1 → 4)-β-D-Glc*N*Ac-(1 → 4)-β-D-Glc*N*Ac-Asn

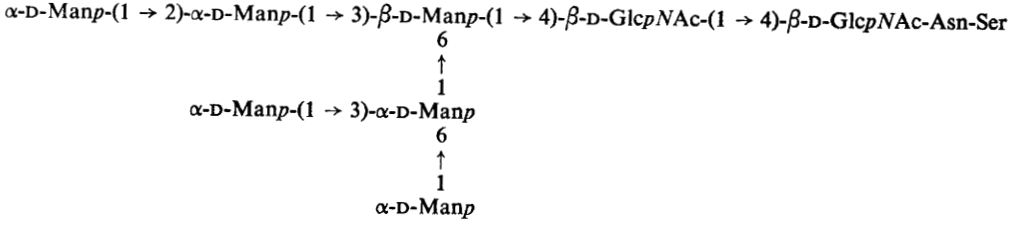
(95)

ovalbumin and pineapple bromelain. The β-configuration of the D-mannosyl residue was demonstrated by experiments with β-mannosidase and by physicochemical measurements.[1409]

The inhibitory effects of '2-deoxy-D-glucose', methyl α-D-glucopyranoside, and 2-amino-2-deoxy-D-glucose on the hydrolytic reaction catalysed by Taka-amylase A (one of the α-amylases of *A. oryzae*) have been studied using phenyl α-maltoside and linear malto-oligosaccharides as substrates.[1742] In experiments with a mixture of analogues that inhibited hydrolysis of phenyl 4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside non-competitively, the analogues were found to compete with each other for the same site *X* on the enzyme molecule. In similar studies with mixtures of a non-competitive inhibitor ('2-deoxy-D-glucose' or methyl α-D-glucopyranoside) and a competitive inhibitor (D-glucose or D-xylose), no interaction was detected between site *X* and the binding site of the competitive inhibitors. When the natural linear oligosaccharides maltotetraose and maltoheptaose were used as substrates, analogues that inhibited hydrolysis of phenyl 4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside non-competitively were now shown to exhibit competitive inhibition. The location of site *X* in the specificity region of the enzyme was proposed on the basis of the effect of the chain length of linear substrates on alteration of the type of inhibition and of previous results for subsite affinities.

The substrate specificity of Taka-amylase A has been investigated further using a number of modified phenyl α-maltosides. Phenyl 4-*O*-α-D-glucopyranosyl-α-D-mannopyranoside, phenyl 2-deoxy-4-*O*-α-D-glucopyranosyl-α-D-*arabino*-hexopyranoside, and phenyl 4-*O*-α-D-glucopyranosyl-2-*O*-methyl-α-D-glucopyranoside were not hydrolysed by the enzyme,

[1740] V. V. Iurkevich and G. T. Kozyreva, *Doklady Akad. Nauk S.S.S.R.*, 1972, **204**, 740.
[1741] J. F. Robyt, R. J. Ackerman, and C. G. Chittenden, *Arch. Biochem. Biophys.*, 1971, **147**, 262.
[1742] Y. Nitta, K. Hiromi, and S. Ono, *J. Biochem.* (*Japan*), 1971, **70**, 973.


α-D-Manp-(1 → 2)-α-D-Manp-(1 → 3)-β-D-Manp-(1 → 4)-β-D-GlcpNAc-(1 → 4)-β-D-GlcpNAc-Asn-Ser
6
↑
1
α-D-Manp-(1 → 3)-α-D-Manp
6
↑
1
α-D-Manp

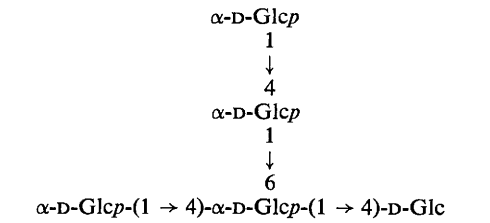
(96)

and the latter two, but not the former, glycosides inhibited the hydrolysis of phenyl 4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside.[1743] Using phenyl 4-*O*-(4-deoxy-α-D-*xylo*-hexopyranosyl)-α-D-glucopyranoside, phenyl 4-*O*-(4-*O*-methyl-α-D-glucopyranosyl)-α-D-glucopyranoside, and related compounds as substrates, it was shown that the terminal C-4 hydroxy-group is almost indifferent to enzymic action, but that inversion of the configuration at this position considerably retards the enzyme's action.[1744] That the hydroxy-group at C-6 of phenyl 4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside is involved in enzymic reaction was deduced from the fact that phenyl 6-deoxy-6-fluoro-4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside is hydrolysed comparatively slowly.[1745]

A glycopeptide obtained from Taka-amylase A by proteolysis has been shown to possess the structure (96) on the basis of methylation, partial acetolysis, and periodate oxidation.[1746] The α-D-configurations of the outer mannosyl residues can be assumed from the susceptibility of a glycopeptide from the same enzyme to α-mannosidase, whereas the β-D-configuration of the innermost mannosyl residue was demonstrated using a β-mannosidase.[1664] The β-D-configuration of the 2-acetamido-2-deoxyglucosyl residues can be assumed by analogy with its presence in other glycopeptides.

A *Bacillus* sp. isolated from water-stored Sitka spruce (*Picea sitchensis*) was found to contain an inducible amylase on growth in the presence of a number of carbohydrates.[1747] A reappraisal of the nature of the immediate precursor of an extracellular α-amylase of *B. amyloliquefaciens* indicated that there is no reason to suppose that it is formed from a high molecular weight precursor.[1748] The same situation appears to hold for related organisms.

Crystalline α-amylase from *B. subtilis* hydrolysed amylopectin to a mixture of D-glucose, maltose, and branched oligosaccharides (α-limit dextrins), the smallest of which was shown to be (97).[1749] Catalysis of the hydrolyses of phenyl and 4-nitro-, 4-chloro-, 4-methyl-, 4-ethyl-, and



(97)

[1743] H. Arita, T. Ikenaka, and Y. Matsushima, *J. Biochem.* (*Japan*), 1971, **69**, 401.
[1744] H. Arita and Y. Matsushima, *J. Biochem.* (*Japan*), 1971, **70**, 795.
[1745] H. Arita and Y. Matsushima, *J. Biochem.* (*Japan*), 1971, **69**, 409.
[1746] H. Yamaguchi, T. Ikenaka, and Y. Matsushima, *J. Biochem.* (*Japan*), 1971, **70**, 587.
[1747] O. P. Ward and W. M. Fogarty, *Biochem. J.*, 1971, **125**, 109P.
[1748] M. A. Grant and G. Coleman, *Biochem. J.*, 1972, **129**, 483.
[1749] D. French, E. E. Smith, and W. J. Whelan, *Carbohydrate Res.*, 1972, **22**, 123.

4-(t-butyl)-phenyl 4-*O*-α-D-glucopyranosyl-α-D-glucopyranosides by α-amylase from *B. subtilis* has been studied at the normal optimum pH of 5.3.[1750] The K_m values were found to decrease slightly on increasing the electron-withdrawing capacity of the phenyl substituent. The effects of isotopic solvents and temperature on the hydrolyses were also investigated and the results suggested that the reaction mechanism depends on the nature of the phenyl substituent. By simultaneous measurement of the reducing value and optical rotation in the initial phase of the enzymic reaction, it was shown that the anomeric form of maltose produced by *B. subtilis* α-amylase from the substrates is changed from the α- to the β-form by substitution of the aglycone with either electron-withdrawing or electron-repelling groups.[1751] Kinetic studies by stopped-flow methods of the hydrolysis of 2-nitrophenyl 4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside by the enzyme led to the conclusion that a Michaelis–Menten, rather than a three-step, mechanism accounts for the hydrolysis catalysed.[1752]

Calcium ions present in *B. subtilis* α-amylase have been shown to be replaceable by rare-earth metal ions.[1753] The lanthanide α-amylases were enzymically active and the ions activated apo-α-amylase in the order $Ca^{2+} \sim Lu^{3+} > Yb^{3+} > Er^{3+} \sim Y^{3+} \sim Dy^{3+} \sim Tb^{3+} > Sm^{3+} > Nd^{3+} > Pr^{3+} > La^{3+}$, with the smallest ions being most effective. The c.d. spectra of native calcium-containing α-amylase, apoamylase, and Nd^{3+}-α-amylase are identical in the region 200—250 nm, suggesting that there are no large structural changes in the enzyme on removal of Ca^{2+} ions from the native form.

Maltose was found to be the best inducer of the starch-degrading enzyme of *Corynebacterium diphtheriae*.[1635] The properties of the enzyme resembled those of an amylase and α-glucosidase. The enzyme (molecular weight 6.12×10^4) was partially purified and was inhibited by Hg^{2+}, 4-chloromercuribenzoate, and iodoacetate ions.

Extracellular amylase produced by a halophilic *Micrococcus* sp. exhibited maximum activity in the pH range 6—7; Ca^{2+} ions and a high concentration of sodium or potassium chlorides were found to be essential for the activity and the stability of the enzyme.[1754] The salt response of the amylase depended greatly upon the pH and temperature of the enzyme assay used.[1754, 1755] Only a few of a large number of *Nocardia* species and strains scanned for amylase activity were able to hydrolyse starch.[1756]

Insolubilized forms of α-amylase have been produced by attaching the enzyme to either *Saccharomyces cerevisiae* cells[1757] or poly(allyl cyclic

[1750] N. Suetsugu, K. Hiromi, and S. Ono, *J. Biochem. (Japan)*, 1971, **70**, 595.
[1751] T. Shibaoka, N. Suetsugu, K. Hiromi, and S. Ono, *F.E.B.S. Letters*, 1971, **16**, 33.
[1752] N. Suetsugu, K. Hiromi, and S. Ono, *J. Biochem. (Japan)*, 1971, **69**, 421.
[1753] G. E. Smolka, E. R. Birnbaum, and D. N. Darnall, *Biochemistry*, 1971, **10**, 4556.
[1754] H. Onishi, *J. Bacteriol.*, 1972, **109**, 570.
[1755] H. Onishi, *Canad. J. Microbiol.*, 1972, **18**, 1617.
[1756] H. Mordarska, J. Wieczorek, B. Jaworska, and M. Mordarski, *Acta Microbiol. Polon. Ser. A*, 1972, **IV**, 27.
[1757] J. S. Hough and T. P. Lyons, *Nature*, 1972, **235**, 389.

carbonate).[1642] The enzyme has also been insolubilized by reaction with titanium chelates of various materials [1555] and by attachment to reactive derivatives of a number of water-soluble polysaccharides.[1758]

β-Amylases

A new theory to explain the extraordinarily strong effectiveness of β-amylase has been developed from kinetic data on the hydrolysis of starch by the enzyme.[1759] According to the theory, β-amylase gradually fits into the spiral structure of the substrate and this adaptation induces the gradual development of hydrogen bonds with the side-chains of the enzyme molecule. In this development, the bond to be cleaved is more easily annexed and acted upon by the enzyme than in the non-deformed case. The effect of nojirimycin and D-glucono-1,5-lactone on β-amylase activity has been investigated.[1520] The activity of β-amylase was not inhibited by non-ionic detergents such as Spans and Tweens, but the latter inhibited the activity when the enzyme solution had been stored for some time.[1760]

Random oxidation with periodate of a polysaccharide substrate to an extent of 5% has been found to be sufficient to block the action of an *exo*-, but not an *endo*-, enzyme upon the polysaccharide.[1454] Substrates modified in this way may be used for distinguishing between β-amylases (*exo*) and α-amylases (*endo*).

The activities of β-amylase in homogenates prepared from various parts of banana plants and fruit have been determined.[1531] Supplementation of the medium used for homogenization with Triton X-100 increased the yield of enzyme obtained.

Barley malt α- and β-amylases have been separated by ion-exchange chromatography and the β-amylase, which consists of two components, was completely inactivated by heat treatment.[1724] Two β-amylases obtained from barley malt have been purified further by ion-exchange chromatography; whereas each enzyme migrated as a single band during disc electrophoresis at pH 4.5, two active bands were observed at pH 8.9.[1761] Crystalline β-amylase has been prepared from ungerminated *Pirkha* barley in the presence of either mercaptoethanol or dithiothreitol. The preparation, which consisted of four isoenzymes of different p*I* values, was monodisperse and possessed a molecular weight of 5.72×10^4.[1762] The involvement of thiol groups in its activity was deduced from the observations that the enzyme was reversibly inhibited by, and reacted with, 4-chloromercuribenzoate and that it polymerized in the absence of reducing agents. The enzyme was found to be similar to wheat β-amylases. A commercial preparation of crude barley β-amylase was found unexpectedly

[1758] J. R. Wykes, P. Dunnill, and M. D. Lilly, *Biochim. Biophys. Acta*, 1971, **250**, 522.
[1759] J. Holló and E. László, *Stärke*, 1971, **23**, 272.
[1760] M. Kojima and M. Narahara, *Yakugaku Zasshi*, 1971, **91**, 1073.
[1761] D. E. Laberge and W. O. S. Meredith, *J. Inst. Brewing*, 1971, **77**, 436.
[1762] K. Visuri and M. Nummi, *European J. Biochem.*, 1972, **28**, 555.

to solubilize cell-wall peptidoglycan; this was shown to be due to the presence of an *endo*-acetamidodeoxyglucosidase and a lysozyme.[1501]

Determinations of total β-amylase activity and gibberellic acid-enhanced activation of zymogen β-amylase during the germination of barley have been reported.[1763] The changes of salt- and papain-soluble and -insoluble zymogen β-amylases during the germination of barley have also been investigated.[1764] All four zymogen levels were found to decrease progressively, whereas the level of salt-soluble active β-amylase increased. It was concluded that the active form is derived from the zymogen form(s). Two zymogen β-amylase fractions (*A* and *B*, molecular weights 2.8 and 1.6×10^5, respectively) and an active β-amylase fraction (*C*, 5.6×10^4) have been purified from a urea extract of ground barley by gel filtration and ion-exchange chromatography.[1765] It appeared that *A* and *B* are heteropolymers involving *C*, and that two activation mechanisms (proteolysis and disulphide-bond cleavage) are necessary for full activation of the β-amylase.

The hydrolytic and branching activities of an enzyme fraction obtained by ion-exchange chromatography of a potato extract can be separated by either zonal ultrafiltration or membrane filtration.[1727] The action of one enzyme (molecular weight 7×10^4) resembled that of β-amylase, stopping near branch points in the substrate. The other enzyme (molecular weight 2×10^4) was found to introduce barriers to β-amylolysis without causing hydrolysis. It was concluded that the hydrolytic and branching activities are manifested by two separate molecules.

The amylase activity induced in mustard seedlings by phytochrome has been separated into three bands by gel electrophoresis; the action pattern of the three enzymes is similar to that of barley β-amylase.[1729] In an investigation of the biochemical changes occurring in rice grains during germination, it was found that β-amylase activity increased progressively.[1730] The β-amylase was separated into two isoenzymes by polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis of sweet potato β-amylase in the presence of sodium dodecyl sulphate indicated a monomer molecular weight of 5.62×10^4 for the enzyme,[1766] which was inactivated by riboflavin-sensitization.[1767]

β-Amylase, purified from extracts of the leaves of *Vicia faba* by gel filtration, was found to be homogeneous by a number of criteria.[1768] The 6.5S peak obtained by ultracentrifugation corresponded to a molecular weight of 1.07×10^5 and a Stokes' radius of 31.8 Å. The hydrolytic action pattern of the enzyme on a number of maltodextrins was shown to be

[1763] R. Shinke and N. Mugibayashi, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1391.
[1764] R. Shinke and N. Mugibayashi, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 378.
[1765] R. Shinke and N. Mugibayashi, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1381.
[1766] Y. Takeda and T. Murakami, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 778.
[1767] K. Uehara, T. Mizoguchi, K. Kishida, and S. Mannen, *J. Biochem. (Japan)*, 1971, **69**, 27.
[1768] G. W. Chapman, J. E. Pallas, and J. Mendicino, *Biochim. Biophys. Acta*, 1972, **276**, 491.

independent of the molecular size of the substrate and the only products detected during the course of hydrolysis of maltodextrins with chain lengths of 9—198 were maltose and very small amounts of D-glucose and maltotriose. The findings are consistent with a mechanism in which the maltodextrin combines with β-amylase to form a loose complex, which rearranges to a tightly bound intermediate that becomes completely hydrolysed.

Isoelectric focusing of the free forms of β-amylase from mature wheat indicated the presence of four forms (p*I* values 4.8, 4.9, 5.6, and 5.8).[1769] The bound form of the enzyme, however, was homogeneous (p*I* 5.0) and certain of the free forms were absent during germination. The location of β-amylases in hard red spring wheat has been studied.[1734] Two major β-amylase components formed in early growth increase, whereas the minor β-amylase component decreases. Four minor β-amylase components are formed during final maturation. However, all of these β-amylase forms disappear on germination and are replaced by an electrophoretically slower β-amylase.[1735]

It has been shown that the concentrations of product and substrate chains do not exceed the initial concentration of substrate chains on the hydrolysis of starch, amylose, and maltodextrins by glucoamylase from *Aspergillus awamori*.[1737] Thus, the presence of β-amylase activity in the enzyme preparation was detectable by an increase in the concentration of substrate chains during the course of the reaction.

A *Bacillus* sp. has been found to produce an amylase in alkaline media.[1770] The enzyme was partially inactivated by H_4edta, and possesses pH and temperature optima of 9.2 and 50 °C, respectively. Products from the action of the *endo*-enzyme on amylose and amylopectin possessed the β-configuration and were shown to be principally maltose, together with turanose and small quantities of D-glucose and maltotetraose. The product predominating at early stages of hydrolysis was maltotetraose. Cyclohepta-amylose was hydrolysed by the enzyme to maltotriose, maltose, and D-glucose.

Glucoamylases

The purification of glucoamylase by means of adsorption on Sephadex has been described.[1615] The enzyme was subsequently desorbed from the column using a specific competitive inhibitor of glucoamylase, *viz.* methyl α-D-glucopyranoside. Treatment of glucoamylase by various concentrations of urea revealed significant differences in the patterns of hydrolysis of maltose and glycogen, and this effect was fully reversible.

Nojirimycin and D-glucono-1,5-lactone were shown to be powerful inhibitors of glucosidases, but poor inhibitors of *exo*-glucanases, *endo*-glucanases, and related enzymes.[1520] Thus, nojirimycin can be used to

[1769] M. Niku-Paavola, M. Nummi, A. Kachkin, J. Daussant, and T. Enari, *Cereal Chem.*, 1972, **49**, 580.

[1770] E. W. Boyer and M. B. Ingle, *J. Bacteriol.*, 1972, **110**, 992.

differentiate between α-glucosidase and glucoamylase (γ-amylase). The structural similarity of the two inhibitors was discussed in relation to the mechanisms of action of the enzymes. Methodology has been described for the determination of starch with glucoamylase.[1771]

A method involving dialysis of the reducing sugars formed from the substrate into alkaline ferricyanide solution has been described for the determination of glucoamylase in homogenates of human small intestinal mucosa.[1522] The α-amylase activity was inactivated by prior heating of the mucosal homogenate in the presence of H_4edta. A technique has also been reported for the assay of glucoamylase in isolated pancreatic islets using glycogen as the substrate.[1772] Effects of the type and concentration of buffer, pH, incubation time, and concentrations of tissue and substrate on the rate of hydrolysis were described.

A 'glucoamylase' separated from canine serum by gel filtration appeared to possess both glucoamylase and α-glucosidase activities, and to attack α-(1 → 6)- as well as α-(1 → 4)-linkages.[1621] The enzyme (molecular weight 1.8×10^5) exhibited maximum activities of both types at pH 6.1 and both activities were inhibited by erythritol, turanose, and tris(hydroxymethyl)-methylamine.

The kinetics and action pattern of a purified enzyme from rabbit muscle have been defined using maltodextrins and glycogen as substrates.[831] The enzyme exhibited optimum activity at pH 4.5—5.1 and appeared to have dextranase, glucoamylase, and α-glucosidase activities. The physiological role of the enzyme in lysosomal glycogen catabolism has been discussed.

High glucoamylase activity (pH optimum 5.0—5.5) has been found in pancreatic islets of mice and the activity was compared with those of the liver, skeletal muscle, and alloxan diabetic animals.[1624]

Although affecting other enzymes involved in the breakdown of glycogen, glucagon has been shown to have no effect on the glucoamylase activities of rat tissues.[835] Glucoamylase (pH optimum 6.5) has been obtained in a highly purified form from rat small intestine by papain solubilization followed by gel filtration and ion-exchange chromatography.[1577] Glucoamylase obtained similarly from rat intestine has been studied kinetically using various substrates; 4-nitrophenyl and 6-bromo-2-naphthyl α-D-glucopyranosides were found to be competitive inhibitors of the enzyme.[1526]

The substrate specificity of glucoamylase from *Aspergillus awamori* has been investigated using a wide range of oligo- and poly-saccharide substrates.[1773] The enzyme is capable of hydrolysing α-(1 → 4)- and α-(1 → 6)-linkages, but not α-(1 → 1)-, β-(1 → 4)-, or β-(1 → 6)-linkages. Starch, glycogen, amylose, and amylopectin were hydrolysed much more rapidly than maltose or isomaltose. It has been shown that during the hydrolyses of starch, amylose, and maltodextrins by *A. awamori* glucoamylase, the concentrations of poly- and oligo-saccharide chains do not exceed the

[1771] P. Thivend, C. Mercier, and A. Guilbot, *Methods Carbohydrate Chem.*, 1972, **6**, 100.
[1772] I. Lundquist, *Enzyme*, 1971, **12**, 647.
[1773] V. G. Ryzhakova, *Doklady Akad. Nauk S.S.S.R.*, 1972, **204**, 737.

initial concentration of the chains.[1737] The presence of other amylolytic enzymes in preparations of glucoamylase may be revealed, therefore, by an increase in the number of substrate chains during enzymic hydrolysis.

A detailed study of the formation of glucoamylase by *A. niger* has shown that many mono-, oligo-, and poly-saccharides stimulate production of the enzyme.[1774] In certain circumstances, production of the two glucoamylase isoenzymes I and II was stimulated to different extents by a single stimulant. *A. niger* glucoamylase has been purified by ion-exchange chromatography and gel filtration to electrophoretic homogeneity, and the effect of the enzyme upon glycogen metabolism and insulin secretion in rats has been studied.[833] Glucoamylases I and II from *A. niger* have been purified to homogeneity by ion-exchange chromatography.[1775] The enzymes (molecular weights 7.49 and 5.43 $\times$ 10^5) have been shown to contain galactose (0.3, 0.7), glucose (2.1, 4.4), and mannose (6.8 and 10.6%, respectively). Treatment of the enzymes with alkaline borohydride yielded a trisaccharide, which on acidic hydrolysis gave a disaccharide and mannitol. The carbohydrate moieties of the glycoproteins appear to be linked glycosidically through mannose to the hydroxy-groups of serine and threonine in the polypeptide chain. Evidence was presented to indicate that the two enzymes contain the same carbohydrate moieties, but different protein chains. Photo-oxidation of glucoamylase I (obtained from *A. niger*) in the presence of Methylene Blue has permitted deductions concerning the conformation of the molecule to be made.[1776]

Glucoamylases have been insolubilized by covalent attachment to DEAE-cellulose [1777] and Sepharose,[1778] and by adsorption on to activated charcoal, clay, and diatomaceous earth.[1779] The kinetics of these insolubilized enzymes have been studied. Glucoamylase has also been attached to metal chelates of a number of materials [1555, 1780, 1781] and to cells of *Bacillus subtilis*, *Escherichia coli*, and *Saccharomyces cerevisiae*.[1757]

Lysozymes

The topic of phage lysozyme has been reviewed [1782] and crystal-structure data for the enzyme–substrate complexes of lysozyme have been discussed.[1783]

[1774] L. L. Barton, C. E. Georgi, and D. R. Lineback, *J. Bacteriol.*, 1972, **111**, 771.
[1775] D. R. Lineback, L. A. Aira, and R. L. Horner, *Cereal Chem.*, 1972, **49**, 283.
[1776] S. A. Barker, C. J. Gray, and M. E. Jolley, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 654.
[1777] S. P. O'Neill, P. Dunnill, and M. D. Lilly, *Biotechnol. and Bioeng.*, 1971, **13**, 337.
[1778] S. Gestrelius, B. Mattiasson, and K. Mosbach, *Biochim. Biophys. Acta*, 1972, **276**, 339.
[1779] S. Usami and H. Shirasaki, *J. Ferment. Technol.*, 1970, **48**, 506.
[1780] S. A. Barker, A. N. Emery, and J. M. Novais, *Process Biochem.*, 1971, **6** (10), 1971, 11.
[1781] A. N. Emery and J. S. Hough, *Birmingham University Chemical Engineer*, 1971, **22**, 37.
[1782] A. Tsugita, in 'The Enzymes', 3rd edn., ed. P. D. Boyer, Academic Press, New York, 1972, p. 344.
[1783] G. Strahs, *Adv. Carbohydrate Chem. Biochem.*, 1970, **25**, 53.

Disulphide-linked antibodies to lysozyme have been used in purification of the enzyme and nearly quantitative recoveries were obtained on elution from the antibody column.[639]

A modified method based on determining the reducing power of liberated 2-amino-2-deoxy-D-glucose from a glycol–chitin substrate has been reported for the measurement of lysozyme.[1784] The principle of radial diffusion of a substrate contained in agar gel has been applied to the quantitative assay of lysozyme.[1695] Clearing zones in the opalescent agar, or staining of the substrate incorporated into the agar, indicated the diffusion zone of the enzyme. A linear relationship was found between the logarithm of the enzyme concentration and the corresponding diameter of the diffusion zone. In addition to measuring free enzymes in solution, the method is applicable to the determination of diffusible enzymes in tissue homogenates and small pieces of tissue. An automated method for the assay of lysozyme depends on continuous monitoring at 505 nm of the lysis of cells of *Micrococcus lysodeikticus* by the enzyme.[1785] Good correlations with manual methods were observed in determinations of human serum and urinary lysozyme.

Changes occurring in the lysozyme molecule during roasting have been studied.[1142]

The actions of human lysozyme and of duck, goose, and hen egg-white, and turnip lysozymes on [*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-]$_4$-2-amino-2-deoxy-D-glucose (chitopentaose) have been studied.[1786] The patterns and optimum conditions for the human, hen, and duck enzymes were found to be similar (pH optimum 4.7) and all possible degradation products (1—4 units) were observed. Small amounts of transglycosylation products were also formed under conditions of optimal activity. The behaviour of turnip lysozyme was different; although the same optimum conditions were found, the enzyme was less sensitive, and di- and tri-saccharides were the main reaction products.

The fluorescence of human lysozyme is enhanced by addition of chito-oligosaccharides (3—6 units).[1787] On the basis of the similarity of the primary structures of human and hen egg-white lysozymes, the pH-dependence of the fluorescence of human lysozyme–saccharide complexes has been ascribed to conformational changes affecting the interactions taking place with two pairs of amino-acid residues. Spectrofluorometric measurement of the association constants of chito-oligosaccharides (2—6 units) complexed with the lysozyme suggested that the active site of the human enzyme, like that of hen-egg lysozyme, is made up of a series of subsites with different affinities for the substrate.

[1784] T. Imoto and K. Yagishita, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1154.
[1785] J. M. Terry, J. D. Blainey, and M. C. Swingler, *Clinica Chim. Acta*, 1971, **35**, 317.
[1786] D. Chalemagne and P. Jollès, *F.E.B.S. Letters*, 1972, **23**, 275.
[1787] V. I. Teichberg, T. Plasse, S. Sorell, and N. Sharon, *Biochim. Biophys. Acta*, 1972, **278**, 250.

Fragments of human lysozyme obtained by cleavage with cyanogen bromide retained their immunological, but not enzymic, activity.[1788] Methods have been reported for the extraction and measurement of lysozyme from human cartilage.[899] The ability of cations to extract the enzyme correlated with their ability to bind to chondroitin sulphate and it was suggested that the enzyme occurs in cartilage as a counterion to glycosaminoglycans. Degradation of cartilage may contribute, therefore, to the increased levels of serum and synovial fluid lysozyme often present in patients with rheumatoid arthritis.

The kinetics of lysozymes from human plasma, leucocytes, milk, tears, and leukaemic urine and also from the whites of duck, goose, guinea-fowl, and hen eggs have been studied as functions of pH and ionic strength.[1789] The relationship of the structure of lysozyme from a case of human monocytic leukaemia to that from other sources has been investigated.[1790] Whereas no cross-reaction was observed between native human leukaemic and hen egg-white lysozymes despite identical sequences, the reduced, carboxymethylated derivatives were found to cross-react.[1791]

An improved method for the purification of lysozyme in high yield, involving chromatography on Bio Gel–asbestos and ion-exchangers, has been applied to the preparation of human-milk, mare's milk, and hen egg-white lysozymes.[1792] The properties of the enzymes were compared and evidence for the occurrence of a second isoenzyme in human milk was presented.

Human-milk and urinary lysozymes have been purified; diffusion and sedimentation experiments showed that the hydrodynamic shape and molecular weight of human lysozyme and α-lactalbumin are strikingly comparable.[1793] Other physicochemical properties were also compared and it was observed by o.r.d. and c.d. spectroscopy that the human enzyme displays a slightly more helical conformation than human α-lactalbumin. Reduction of the disulphide bonds in human-milk and bovine-milk lysozymes with 2-mercaptoethanol destroyed the lytic activities.[1794] However, enzymic activity (84 and 328% !, respectively) was rapidly regenerated during re-oxidation. Whereas the re-oxidized human species was apparently identical with the native enzyme, the re-oxidized bovine enzyme differed in spectral properties, although it exhibited the same electrophoretic mobility as the original material. Possible explanations for the altered properties of the bovine lysozyme were discussed. The sequences of human-milk and hen egg-white lysozymes have been compared.[1795]

[1788] B. Bonavida, *Immunochemistry*, 1971, **8**, 829.
[1789] J. Saint-Blanchard and P. Jollès, *Biochimie*, 1972, **54**, 7.
[1790] J. Thomsen, E. H. Lund, K. Kristiansen, K. Brunfeldt, and J. Malmquist, *F.E.B.S. Letters*, 1972, **22**, 34.
[1791] N. Arnheim, J. Sobel, and R. Canfield, *J. Mol. Biol.*, 1971, **61**, 237.
[1792] J. Jáuregui-Adell, *Biochimie*, 1971, **53**, 1167.
[1793] A. O. Barel, J. P. Prieels, E. Maes, Y. Looze, and J. Léonis, *Biochim. Biophys. Acta*, 1972, **257**, 288.
[1794] B. A. Friend, R. R. Eitenmiller, and K. M. Shahani, *Arch. Biochem. Biophys.*, 1972, **149**, 435.
[1795] J. Jollès and P. Jollès, *F.E.B.S. Letters*, 1971, **22**, 31.

Simultaneous studies of tear and serum lysozyme levels of humans with a wide range of serum lysozyme activities have been reported.[1796] Failure to establish a relationship between the activities of the enzymes from the two locations accords with the concept that lysozyme is synthesized *de novo* by lacrimal cells. The occurrence of lysozyme in human seminal plasma has been confirmed and comparisons of the levels in samples from normals and patients with infertility and/or genital glandular infections suggested a role for the enzyme in the normal liquefaction of human semen.[1797]

Purified human urinary and hen egg-white lysozymes have been shown to possess chitinase activities.[1505] The chitinase and lysozyme activities of these two enzymes were compared using glycol–chitin, chitin–agar, chitopentaose, and whole cells, cell walls, and a cell-wall glycan of *Micrococcus lysodeikticus*. It was concluded that the lysozyme activities are much stronger than the chitinase activities and that the enzymes may be regarded as true lysozymes. The effects of pH and various inhibitors on the c.d. spectra of human urinary and hen egg-white lysozymes have been compared.[1798] Whereas *NN'*-diacetylchitobiose and *NN'N"*-triacetylchitotriose had similar effects on the spectra of the two enzymes, differences were observed when a mixture of 2-acetamido-2-deoxy-D-glucose and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside was employed. These differences were related to interactions with certain amino-acids in the molecular chains. The immunological cross-reactivity of purified lysozymes from human urine and the whites of chicken, bobwhite quail, turkey, Japanese quail, ring-necked pheasant, and duck eggs has been investigated.[1799] C.d. data have been obtained for human leukaemic urinary lysozyme and lysozymes from duck, goose, and hen eggs.[1800] Sequence similarities were observed between human and hen lysozymes and α-lactalbumins from a number of sources.

The isolation and characterization of mare's milk lysozyme have been described and the composition of the enzyme was compared with those of the enzymes from human milk and hen egg-white.[1801] Lysozyme is present in the extracellular matrix of bovine nasal cartilage from which it can be extracted with guanidinium hydrochloride solutions at concentrations lower than those required for the extraction of proteoglycans.[1802] Since lysozyme migrated to the top of the caesium chloride density gradients used to purify proteoglycans, it appears that lysozyme is not involved in

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[1797] C. Hirschhäuser and R. Eliasson, *Life Sci., Part II*, 1972, **11**, 149.
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[1799] E. M. Prager and A. C. Wilson, *J. Biol. Chem.*, 1971, **246**, 5978.
[1800] D. A. Cowburn, K. Brew, and W. B. Gratzer, *Biochemistry*, 1972, **11**, 1228.
[1801] J. Jáuregui-Adell, G. Cladel, C. Ferraz-Pina, and J. Rech, *Arch. Biochem. Biophys.*, 1972, **151**, 353.
[1802] N. Sorgente, V. C. Hascall, and K. E. Kuettner, *Biochim. Biophys. Acta*, 1972, **284**, 441.

the aggregation of proteoglycans *in vitro*. Furthermore, chondroitin sulphate, but not lysozyme, was effectively solubilized from cartilage by the action of chondroitin sulphate lyase ABC.

The distribution of lysozyme activity in subcellular components of rat liver, spleen, and kidney has been investigated.[1803] The enzyme from the latter two sources exhibits two pH optima (6.2 and 9.2), whereas that from the liver exhibits optimal activity at pH 5.4.

Lysozymes have been purified from pooled samples of Peking, Kaki, and wild-type duck egg-whites by gel filtration and ion-exchange chromatography, and their complete primary structures have been determined.[1804] Guinea-hen egg-white lysozyme has been purified to homogeneity by ion-exchange chromatography and its primary structure has been compared with those of other avian egg-white lysozymes.[1805]

The lysozyme activities of cardiac muscles of chick have been measured.[1806] The cuticle and shell of hen eggs contain little lysozyme and most of the enzyme is concentrated in the membranes.[1807] The outer and inner membranes contain the same amounts of enzyme and the presence of salt during extraction was found to be essential for the detection of activity.

The substrate specificity of hen egg-white lysozyme has been investigated.[1808] The 3,4-dinitrophenyl β-glycoside of *NN'N''N'''*-tetra-acetylchitotetraose has proved to be a good chromophoric substrate for hen egg-white lysozyme.[1809] Cleavage of *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-D-glucose bonds occurred at the same rate as the release of 3,4-dinitrophenol and, although this made analysis of the time course of the hydrolysis difficult, it was considered that the rate of release of 3,4-dinitrophenol at zero time should arise solely from direct hydrolysis, since no induction periods were observed. The specificities of a number of related substrates for hen egg-white lysozyme were compared.

The amount of water adsorbed by lysozyme at vapour pressures near to saturation was insufficient to form a monolayer on a model with minimum surface area.[1810] There is a good correlation between the number of surface polar groups (as evaluated from *X*-ray crystallographic data) on the tertiary structure of the enzyme and the amount of vapour adsorbed.

The effects of ionic strength, concentration, and temperature on the association–dissociation properties of hen egg-white lysozyme have been

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[1804] J. Hermann, J. Jollès, and P. Jollès, *European J. Biochem.*, 1971, **24**, 12.
[1805] J. Jollès, E. van Leemputten, A. Mouton, and P. Jollès, *Biochim. Biophys. Acta*, 1972, **257**, 497.
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[1807] D. V. Vadehra, R. C. Baker, and H. B. Naylor, *Comp. Biochem. Physiol.*, 1972, **43B**, 503.
[1808] S. Hara, *Seikagaku*, 1972, **44**, 146.
[1809] F. W. Ballardie and B. Capon, *J.C.S. Chem. Comm.*, 1972, 828.
[1810] Y. I. Khurgin, V. Y. Roslyakow, A. L. Klyachko-Gurwitch, and T. P. Brueva, *Biokhimiya*, 1972, **37**, 485.

studied by gel filtration.[1811] Increasing ionic strength led to increased interconversion between monomeric and associated forms (including higher oligomers as well as dimers), and the data could be fitted by a theory of indefinite association having the same equilibrium constant for the addition of each successive monomer. Thermodynamic quantities ($\Delta G°$, $\Delta H°$, and $\Delta S°$) for the association were calculated from the equilibrium ratio of the slow polymerization process at various temperatures and the data are consistent with a hydrophobic interaction between lysozyme monomers and the Sephadex–gel matrix. It is suggested that adsorption involves the tryptophanyl residues of the active centre. The enthalpy value for the polymerization process may be interpreted in terms of a conformational change of the same order of magnitude as that occurring on the binding of 2-acetamido-2-deoxy-D-glucose to the enzyme.

The Raman spectra of native and thermally denatured hen egg-white lysozymes have been compared in aqueous solutions at various temperatures.[1812] Other studies on thermal denaturation of the enzyme have led to the conclusion that the breakdown of lysozyme proceeds according to a typical one-by-one mechanism.[1813] The denaturation of lysozyme by mixtures of guanidine and hydrochloric acid has been studied at 25 °C using a dilatometric technique, and volume changes for partial and complete denaturation by hydrochloric acid at a constant concentration of guanidine hydrochloride were calculated.[1814] The volume changes due to unfolding *per se* in 3M- and 6M-guanidine hydrochloride were calculated to be 47 and 54 ml mol^{-1}, respectively.

The kinetics of hydrogen–deuterium exchange in hen egg-white lysozyme have been followed in aqueous solutions at various pHs and in solutions of lithium chloride by means of an i.r. technique.[1815] The results obtained were related to hydrogen-bonding and fluctuations in the lysozyme structure. The tertiary structure of lysozyme has been refined using electron-density maps and energy parameters.[1816]

The number of thiol and disulphide groups in hen egg-white lysozyme has been studied with the aid of Ellman's reagent.[1741] Using a new technique involving ^{35}Cl n.m.r., it has been found that the disulphide groups of lysozyme are less accessible for reduction than those of α-lactalbumin.[1817] The effects of ionic strength on the ionization behaviour of the catalytic carboxy-groups of lysozyme have been investigated by pH-difference titration of the native enzyme and the β-ethyl ester of aspartic acid-52 of lysozyme.[1818] The results indicated that lysozyme has a net positive charge

[1811] O. G. Hampe, *European J. Biochem.*, 1972, **31**, 32.
[1812] H. Brunner and H. Sussner, *Biochim. Biophys. Acta*, 1972, **271**, 16.
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[1815] M. Nakanishi, M. Tsuboi, and A. Ikegami, *J. Mol. Biol.*, 1972, **70**, 351.
[1816] R. Diamond and M. Levitt, *Biochem. J.*, 1971, **125**, 92P.
[1817] J. A. Magnuson and N. S. Magnuson, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 1513.
[1818] S. M. Parsons and M. A. Raftery, *Biochemistry*, 1972, **11**, 1623.

on its surface in potassium chloride solutions of pH 4—7. The parameters obtained from similar titrations at different temperatures have been interpreted with the aid of *X*-ray crystallographic data to be indicative of the interaction of each carboxy-group with the surrounding enzyme structure.[1819] The bulk of the fluorescence of lysozyme has been located in tryptophans-62 and -108, and a pH-dependent interaction between these residues was detected by examination of the fluorescence of derivatives in which these residues were specifically oxidized.[1820] The interaction is considered to involve an energy transfer.

The u.v., c.d., and o.r.d. spectra of hen egg-white lysozyme have been compared with those of the di-isopropylphosphoryl derivative of the enzyme.[1821] Comparison of the extrinsic fluorescence of hen egg-white lysozyme and α-lactalbumin dyed with 2-(4-toluidinyl)naphthalene 6-sulphonate has shown that the binding site of lysozyme is less hydrophobic than that of α-lactalbumin.[1822] The effects of 2,4-dinitrophenylation on the enzymic and physicochemical properties of lysozyme have been investigated.[1823–1825] The effects of modifying the L-arginyl residues in lysozyme on the conformation, enzymic activity, and immunochemistry of the molecule have been studied; it appeared that L-arginine-61 is not located in the antigenic site and that not all conformational changes influence antigenic reactivity.[1826] Retention of enzymic activity by lysozyme in the presence of non-ionic detergent micelles (Tween 20, polyoxyethylene-sorbitan nonolaurate) has been examined.[1827]

Gel filtration of a heavily damaged, salt-insoluble fraction from dry hen egg-white lysozyme after γ-irradiation *in vacuo* revealed the presence of species with larger hydrodynamic volumes than native lysozyme.[1828] Molecular weights determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis indicated the presence of altered monomers and polymers of integral multiples of the monomeric lysozyme. From these results and those obtained for γ-irradiated, reduced, alkylated lysozyme, it was proposed that heavily damaged molecules with exposed disulphide bonds can undergo a free-radical polymerization initiated by $-CH_2\dot{S}$ radicals. A salt-soluble fraction resembled native lysozyme in a number of ways.

Inhibition of hen egg-white lysozyme by various imidazole and indole derivatives has been examined by kinetic and crystallographic methods.[1829]

[1819] S. M. Parsons and M. A. Raftery, *Biochemistry*, 1972, **11**, 1630.
[1820] T. Imoto, L. S. Forster, J. A. Rupley, and F. Tanaka, *Proc. Nat. Acad. Sci., U.S.A.*, 1972, **69**, 1151.
[1821] T. Murachi, T. Miyake, and K. Kato, *J. Biochem.* (*Japan*), 1971, **69**, 209.
[1822] A. O. Barel, M. Turneer, and M. Dolmans, *European J. Biochem.*, 1972, **30**, 26.
[1823] D. N. Rao and S. Gurnani, *Indian J. Biochem. Biophys.*, 1972, **9**, 36.
[1824] D. N. Rao and S. Gurnani, *Indian J. Biochem. Biophys.*, 1972, **9**, 43.
[1825] D. N. Rao and S. Gurnani, *Indian J. Biochem. Biophys.*, 1972, **9**, 49.
[1826] M. Z. Atassi, A. M. Suliman, and A. F. S. A. Habeeb, *Immunochemistry*, 1972, **9**, 907.
[1827] F. R. Bernath and W. R. Vieth, *Biotechnol. and Bioeng.*, 1972, **14**, 737.
[1828] D. J. Marciani and B. M. Tolbert, *Biochim. Biophys. Acta*, 1972, **271**, 262.
[1829] I. D. A. Swan, *J. Mol. Biol.*, 1972, **65**, 59.

The results were consistent with the formation of charge-transfer complexes between the imidazole derivatives and the L-tryptophan residues in lysozyme. Comparison of the kinetic and crystallographic data suggested that direct interpretation of inhibition studies is open to question when the inhibitor is not a close analogue of the natural substrate.

Computer simulation of sedimentation equilibrium distributions for systems involving heterogeneous associations has been applied to interacting mixtures of hen egg-white lysozyme (monomer and dimer) and 2-acetamido-2-deoxy-D-glucose.[1830] It was shown that the apparent weight–average molecular weight approaches that of the monomer–inhibitor complex at saturating concentrations of the inhibitor, which is behaviour consistent with the effective loss of binding sites on dimerization. Direct comparison of simulated distributions with those obtained experimentally for various concentrations of inhibitor indicated that monomeric and dimeric forms of lysozyme each bear one binding site with essentially identical affinities for the inhibitor.

The effects of 2-acetamido-2-deoxy-D-glucose and its derivatives and of chito-oligosaccharides on the c.d. spectrum of hen egg-white lysozyme have been studied and conditions useful for differentiating the interactions of various reagents with lysozyme were recommended.[1831] The binding of 2-acetamido-2-deoxy-D-glucose, *NN'N''*-triacetylchitotriose, and *NN'N''N'''*-tetra-acetylchitotetraose as inhibitors of lysozyme has been studied by microcalorimetry and values for $\Delta G°$, $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ were derived for the binding process.[1832]

Application of thin-film dialysis to a study of the conformational mobility of lysozyme in solution showed that an increase in the ionic strength of the solvent system slightly increased the diffusional size of the enzyme.[1833] This change was reversible and did not inactivate the enzyme. Using the same method, it was found that the binding constant for 2-acetamido-2-deoxy-D-glucose increases with a decrease in the concentration of lysozyme. The dissociation of lysozyme dimer was found to be a function of the concentrations of both enzyme and 2-acetamido-2-deoxy-D-glucose.

From an n.m.r. study of effects of fluorine substituents on the association between hen egg-white lysozyme and derivatives of 2-amino-2-deoxy-D-glucose, it emerged that ^{19}F chemical shifts are more sensitive to intermolecular changes in environment than are ^{1}H chemical shifts, but the enhancement is not as marked for intramolecular changes.[1834] Although a change in the chemical shift of a small, reversible inhibitor at a probe site can be readily interpreted in terms of a molecular association, the absence of any observed shift does not necessarily indicate the absence of molecular

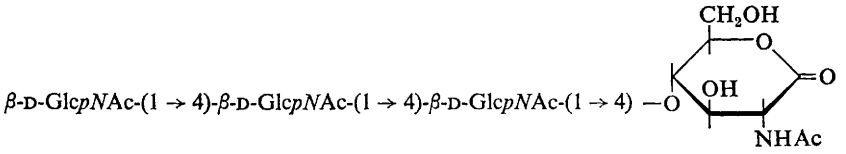
[1830] G. J. Howlett and L. W. Nichol, *J. Biol. Chem.*, 1972, **247**, 5681.
[1831] K. Ikeda and K. Hamaguchi, *J. Biochem. (Japan)*, 1972, **71**, 265.
[1832] C. Bjurulf and I. Wadsö, *European J. Biochem.*, 1972, **31**, 95.
[1833] H. C. Chen and L. C. Craig, *Bioorganic Chem.*, 1972, **1**, 51.
[1834] L. D. Hall and C. W. M. Grant, *Carbohydrate Res.*, 1972, **24**, 218.

association. It was also concluded that introduction of a fluorine substituent can cause changes in the nature of a molecular association, implying that specifically fluorinated substrates cannot necessarily be used as models for normal substrates. Other workers have advocated the use of 2-deoxy-2-trifluoroacetamido-D-glucose as a molecular probe for lysozyme with n.m.r. techniques.[1835–1837] This fluorinated monosaccharide is inhibitory towards lysozyme and its addition to solutions of hen egg-white lysozyme at pH 5.4 resulted in a concentration-dependent broadening and down-field shift of the ^{19}F resonance of both anomers, the shift for the α-anomer being the greater.[1835] Determination of the ^{19}F chemical shifts of several fluorinated monosaccharides in the inhibitor–lysozyme complex confirmed these results and also showed different shifts for methyl 2-deoxy-2-trifluoroacetamido-α- and -β-D-glucopyranosides.[1836] The ^{19}F resonances of the inhibitors were broadened in the presence of lysozyme by addition of Gd^{3+} ions and the Gd^{3+}–F distances have been determined. Reference to a molecular model of lysozyme indicated that ^{19}F chemical shifts of the 2-deoxy-2-trifluoroacetamido-D-glucose–enzyme complexes arise from electrostatic effects involving two L-tryptophan residues. A combination of the Gd^{3+}–F distance and chemical-shift data for the complexes suggested that the co-ordinates of the $-CF_3$ group are almost identical in the crystal phase and in solution. Similar shifts have been reported for the binding of 2-deoxy-2-trifluoroacetamido-D-glucose and analogous derivatives of chito-biose, -triose, and -tetraose.[1837] A study of the pH sensitivity of the ^{19}F n.m.r. spectrum of the fluorinated chitotriose–lysozyme complex revealed that the chemical shift of the trifluoroacetyl group of the non-reducing, terminal unit changed owing to ionization of L-aspartic acid-101, whereas that of the reducing, terminal unit changed owing to ionization of L-glutamic acid-35. The former change was attributed to a translation of the bound chitotriose relative to the binding cleft of the enzyme upon formation of a hydrogen bond with the ionized aspartic acid-101 residue.

The use of chitotriose as a 'tritium-exchange probe' of the cleft of lysozyme is based upon the principle that a molecule which binds with the enzyme's active site may be labelled by means of exchange with tritiated water; the rates of exchange back into water in the presence and in the absence of enzyme can be determined by a freeze-drying technique.[1838] Using this technique, information was obtained on the interactions between the active site of the enzyme and the bound molecule.

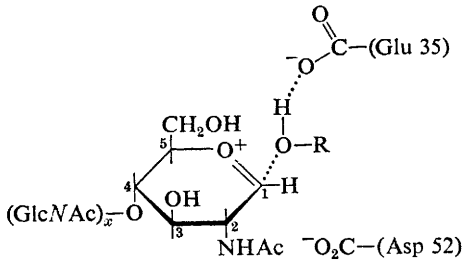
The binding of the 1,5-lactone (98) (derived from *NN'N''N'''*-tetra-acetylchitotetraose) to hen egg-white lysozyme has been investigated by means of its ability both to inhibit the lysozyme-catalysed lysis of cells of

[1835] R. A. Dwek, P. W. Kent, and A. V. Xavier, *European J. Biochem.*, 1971, **23**, 343.
[1836] C. G. Butchard, R. A. Dwek, P. W. Kent, R. J. P. Williams, and A. V. Xavier, *European J. Biochem.*, 1972, **27**, 548.
[1837] F. Millett and M. A. Raftery, *Biochemistry*, 1972, **11**, 1639.
[1838] P. W. Linder and W. P. Bryan, *J. Amer. Chem. Soc.*, 1971, **93**, 3061.



(98)

Micrococcus lysodiekticus and to perturb the tryptophanyl fluorescence spectrum of lysozyme.[1839] On the basis of these results and the similarity between the known conformation of the lactone ring and the proposed conformation of the transition state (99) for lysozyme-catalysed reactions



(99)

(both half-chairs), it was concluded that (98) is a transition-state analogue for lysozyme. It was also estimated that the affinity of one of the subsites of lysozyme for the half-chair conformation of the pyranose ring of 2-acetamido-2-deoxy-D-glucose is greater than its affinity for the chair conformation by a factor of 6×10^3.

The action of hen egg-white lysozyme on three isomeric trisaccharides, 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside, 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranoside, and 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranoside, has been investigated.[1840] The three isomers exhibited the same inhibitory effect on the lysozyme-catalysed hydrolysis of *Micrococcus lysodeikticus* cell walls and the latter two isomers were slowly hydrolysed by the enzyme with a marked lag period. It was concluded that the specificity of lysozyme, closely associated with the conformation of the active site of the enzyme, can vary extensively.

[1839] I. I. Secemski, S. S. Lehrer, and G. E. Lienhard, *J. Biol. Chem.*, 1972, **247**, 4740.
[1840] A. Y. Khorlin, E. A. Shashkova, and S. E. Zurabyan, *Carbohydrate Res.*, 1972, **21**, 269.

N.m.r. studies of the conformation of the D-glucose ring of 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-β-D-glucopyranoside and the reducing muramic acid ring of (100) on binding to the active site of

β-D-Glc*N*Ac-(1 → 4)-β-Mur*N*Ac-(1 → 4)-β-D-Glc*N*Ac-(1 → 4)-Mur*N*Ac
(100)

lysozyme indicated that, when the former is bound, only a small ring-distortion occurs, but that, when the latter is bound, the conformation of the reducing muramic acid residue changes towards a half-chair conformation.[1841] No substantial difference was observed between the abilities of *p*-tolyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranoside and its isologues (in which the aryl glycosidic oxygen atom is replaced by either sulphur or selenium atoms) to form non-productive complexes with hen egg-white lysozyme.[1842] However, the isologues differed in their rates and patterns of hydrolysis and transglycosylation. Relative reactivities of the isologues for enzymic hydrolysis followed the order (O ≥ S > Se) of the acid-catalysed hydrolysis of model compounds. These findings are in agreement with the proposed carbonium-ion mechanism for lysozyme catalysis.

pH-Difference titrations of lysozyme and its β-ethyl ester in the presence of several inhibitors and the substrate glycol–chitin permitted some assessment of the ionization behaviour of the cleft carboxy-groups in lysozyme–substrate complexes.[1843] Microcalorimetric studies of the interactions of hen egg-white lysozyme with such neutral carbohydrates as D-glucose, cellobiose, and D-mannose indicated that the reactions involve the formation of hydrogen bonds at the active site of the enzyme and solvation effects.[1844] Values were estimated for the thermodynamic parameters of these stages. The interaction of hen egg-white lysozyme with F2 bacteriophage has been investigated.[1845]

The proximity of the antigenic site of hen egg-white lysozyme to the catalytic site has been studied and an immunologically active peptide has been isolated from the enzyme by means of limited digestion with thermolysin.[1846] Lysozyme irreversibly inhibited by an affinity label (2,3-epoxypropyl β-*NN'N''*-triacetylchitotrioside) reacted with anti-(hen egg-white lysozyme) in the same way as the native enzyme.[1847] However, antibodies directed against the active-site region of the native enzyme reacted to a much lesser extent with the affinity-labelled enzyme. The reversible complex formed between hen lysozyme and *NN'*-diacetylchitobiose reacted

[1841] B. D. Sykes, S. L. Platt, and D. M. Dolphin, *Cold Spring Harbor Symp. Quant. Biol.*, 1971, **36**, 29.
[1842] C. S. Tsai, C. Reyes-Zamora, and R. Otson, *Biochim. Biophys. Acta*, 1971, **250**, 172.
[1843] S. M. Parsons and M. A. Raftery, *Biochemistry*, 1972, **11**, 1633.
[1844] B. Y. Zaslavsky, A. A. Vichutinsky, and A. Y. Khorlin, *Biofizika*, 1972, **17**, 385.
[1845] K. S. Mathews and R. D. Cole, *J. Mol. Biol.*, 1972, **68**, 173.
[1846] N. Sakato, H. Fujio, and T. Amano, *Biken J.*, 1972, **15**, 135.
[1847] E. Maron, V. Eshdat, and N. Sharon, *Biochim. Biophys. Acta*, 1972, **278**, 243.

with antibodies directed against the active site in an identical manner with the affinity-labelled enzyme, suggesting that, in both cases, the disaccharide occupies the same site on the enzyme. Correlations have been found between the immunological reactivity of α-lactalbumin, human leukaemic urinary, and ring-necked pheasant, turkey, and duck egg lysozymes with antibodies directed against the active site and the primary sequences of the enzymes, *etc.* The immunological reactivities of hen [1848] and several other egg-white lysozymes [1849] have been investigated after acetylation. The immunological reactivities of lysozyme and derivatives obtained by acetylation, guanidation, succinylation, and maleylation have also been compared.[1850] The immunological [1851] and charge [1852] properties of antibodies specifically directed to regions of hen egg-white lysozyme have been studied.

The preservative effect of egg-white lysozyme has been investigated.[1853] Biosynthetic incorporation studies with cultured hen-oviduct magnum explants suggested that a single regulatory site for the control of egg-white synthesis and that of ovalbumin, conalbumin, ovomucoid, and lysozyme is unlikely, since several of these molecules appear to be under independent control.[1416]

The validity of a prediction, based on immunological data, that there are sequence differences between bobwhite quail and hen egg-white lysozymes has been tested by obtaining primary structural data for the quail lysozyme.[1854]

A commercial preparation of crude β-amylase from barley was found unexpectedly to solubilize cell-wall peptidoglycan from *Vibrio parahaemolyticus* and *Micrococcus lysodiekticus*, and this property was shown to be partially due to the presence of a lysozyme.[1501] The lysozyme was isolated by isoelectric focusing and its properties were investigated. A modified method for the isolation of papaya lysozyme has been reported.[1430] The enzyme, by virtue of its ability to react with a variety of sulphhydryl reagents, has been shown to possess four sulphhydryl groups per molecule. These groups are available for reaction in the native enzyme, but reacted more rapidly when denaturants were present. Evidence from mercaptide-exchange analysis and studies of Hg^{2+}–lysozyme derivatives indicated that one sulphhydryl group is essential for activity. Titration of the sulphhydryl groups of the enzyme was associated with increased lability to heat and with decreased solubility.

The distribution of yeast cell-wall lytic activity among micro-organisms has been investigated.[1855] Identification of the products arising from

[1848] M. Kurata, S. Otodani, and J. Kawada, *J. Pharm. Soc. Japan*, 1972, **92**, 64.
[1849] M. Kurata, S. Otodani, and T. Shioda, *Yakugaku Zasshi*, 1972, **92**, 64.
[1850] A. F. S. A. Habeeb and M. Z. Atassi, *Immunochemistry*, 1971, **8**, 1047.
[1851] H. Fujio, N. Sakato, and T. Amano, *Biken. J.*, 1971, **14**, 395.
[1852] N. Sakato, H. Fujio, and T. Amano, *Biken. J.*, 1971, **14**, 405.
[1853] A. Akashi and A. Oono, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 177.
[1854] E. M. Prager, N. Arnheim, G. A. Mross, and A. C. Wilson, *J. Biol. Chem.*, 1972, **247**, 2905.
[1855] T. Sugimori, Y. Uchida, and Y. Tsukada, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 669.

digestion of purified bacterial sacculi with the extracellular, bacteriolytic activity of *Chondrococcus coralloides* demonstrated that the main extracellular enzyme possesses the substrate specificity of a lysozyme.[1856]

Amoebae of the cellular slime mould *Dictyostelium discoideum*, when grown in submerged culture on *Escherichia coli*, have been shown to contain a lysozyme.[1506] Lysozyme has been purified to homogeneity from lysates of T4-infected *E. coli* cells.[1857] In the purification procedure, advantage was taken of the affinity of the enzyme for chitin and, under certain conditions, the binding was complete. The enzyme could be desorbed from chitin columns by increasing the ionic strength of the eluent at a low pH; conditions for adsorption and desorption differed from those for hen egg-white lysozyme.

Crude extracts from a soil amoeba (*Hartmanella glebae*) grown in the presence of both *Aerobacter aerogenes* and *Alcaligenes faecalis* were found to contain two bacteriolytic enzymes considered to be lysozymes.[1509]

The extracellular, bacteriolytic enzymes of *Myxococcus virescens* were separated into three active fractions by gel filtration and further purification was achieved by ion-exchange chromatography.[1858] The molecular weights of the three fractions, as determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, were 4.0×10^4, 3.0×10^4, and 2.0×10^4. Adsorption phenomena experienced when the enzymes were fractionated on Sephadex were attributed to either the strongly basic natures of the enzymes or the formation of enzyme–substrate type complexes. Bacteriolytic enzymes have been shown to be produced by *M. xanthus*.[1511]

Phage P22 induced the formation of a lysozyme in *Salmonella typhimurium* and the enzyme (molecular weight 2×10^4) has been purified by ion-exchange chromatography and gel filtration.[1859] The activity of the enzyme (pH optimum 7—8) depended upon the ionic strength of the medium and was inhibited by such salts as magnesium, sodium, and potassium chlorides. Gram-negative cells better acted as substrates for the lysozyme than Gram-positive cells. The properties of the phage P22 enzyme were compared with those of λ- and T4-lysozymes.

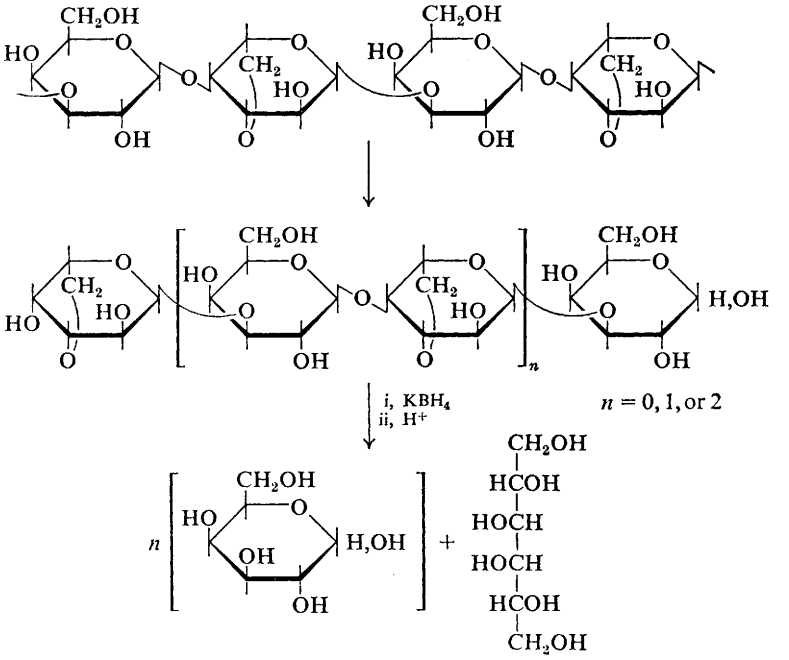
A strain of *Streptomyces griseus* was shown to produce bacteriolytic enzymes, one of which has been purified by ion-exchange chromatography and gel filtration.[1860] This enzyme, which possessed a broad pH optimum centred around pH 7, caused lysis of whole cells of *Bacillus* genus and *Staphylococcus aureus*, but not those of *Micrococcus lysodiekticus*. Liberation of reducing sugars from the cell walls attacked by the enzyme suggested that it is a lysozyme.

Insolubilized derivatives of immunologically active lysozyme peptides have been prepared.[1861]

[1856] E. Harcke, A. Hütterman, and H. Kühlwein, *Archiv Mikrobiol.*, 1972, **85**, 6.
[1857] H. B. Jensen and K. Kleppe, *European J. Biochem.*, 1972, **26**, 305.
[1858] G. Haskå, *Physiol. Plant.*, 1972, **26**, 221.
[1859] G. R. K. Rao and D. P. Burma, *J. Biol. Chem.*, 1971, **246**, 6474.
[1860] T. Yoshimoto and D. Tsuru, *J. Biochem.* (*Japan*), 1972, **72**, 379.
[1861] R. Arnon, E. Maron, M. Sela, and C. B. Anfinsen, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 1450.

Other Polysaccharidases and Oligosaccharidases

Agarases.—Agarase activity has been detected in molluscs of *Littorina* genus.[1862] The crude enzyme preparation hydrolysed agar in an analogous manner to bacterial agarases, giving rise to oligosaccharides (Scheme 12), the chain lengths of which were determined by reduction and hydrolysis.



Scheme 12

Arabinanases.—Studies of the action of an enzyme produced by *Aspergillus niger* on rice-straw arabinoxylan revealed that the substrate was degraded to L-arabinose, D-xylose, xylobiose, and xylotriose.[1863]

Many strains of *Bacillus subtilis* have been found to secrete enzymes that are active against L-arabinose-containing polysaccharides,[1864] but in no case was free L-arabinose detected in the hydrolysates.

Enzyme(s) that degraded a variety of neutral polysaccharides have been obtained from *Corynebacterium diphtheriae*.[1865] Analyses of digests of an

[1862] A. I. Usov, M. D. Martynova, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1970, **194**, 455.
[1863] Y. Tsujisaka, S. Takenishi, and J. Fukumoto, *J. Agric. Chem. Soc. Japan*, 1971, **45**, 253.
[1864] S. Emi, J. Fukumoto, and T. Yamamoto, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1891.
[1865] S. Kotani, T. Kato, T. Matsubara, M. Sakagoshi, and Y. Hirachi, *Biken J.*, 1972, **15**, 1.

arabinogalactan revealed that the enzyme hydrolyses L-arabinosidic linkages by a mechanism involving *endo*-attack, the products being high and low molecular weight fractions rich in D-galactose and L-arabinose, respectively. The enzyme exhibited maximum activity at pH 6 and was inactive against a number of poly- and oligo-saccharides which did not contain L-arabinose.

Alginases and Alginate Lyases.—Extracts of the hepatopancreas of the tunicate *Pyura stolonifera* contained an alginase that was active against alginates rich in L-guluronic acid and D-mannuronic acid.[1500]

Alginase and alginate lyase activities in the digestive systems of over forty species of marine invertebrates from Vermes, Crustacea, Mollusca, Brachiopoda, Echinodermata, and Tunicata phyla have been investigated.[1866] Seasonal variations in the amount of enzymes present were observed.

Amylopectin-1,6-glucosidases.—Extracts of germinated barley were found to contain two de-branching enzymes that were separated from each other and α-amylase by continuous electrophoresis followed by gel filtration.[1726] One of the enzymes, amylopectin-1,6-glucosidase, hydrolysed the outermost inter-chain linkages in amylopectin, but had no action on glycogen or α-limit dextrins. The enzyme exhibited optimum activity at pH 5 and its effect on the β-amylolysis limits of glycogen was examined.

The effect of growth regulators and Cu^{2+} ions on the activation of amylopectin-1,6-glucosidase activity in pea seedlings has been investigated.[1867]

Cellulases.—The cellulases have been reviewed.[1868]

A direct theoretical approach has been described that enables viscosimetric data to be used to determine standard units of cellulase using carboxymethylcellulose as substrate.[1869] Application of the theory showed that, under suitably defined conditions, calculated units are directly proportional to the quantity of enzyme employed.

The facile purification of cellulase by ion-exchange chromatography has been reported.[1870] The single-step procedure takes advantage of an extremely strong, but reversible, binding of the enzyme to DEAE-cellulose and elution with very high salt concentrations. Extraneous proteins were only weakly adsorbed by the column.

In an attempt to identify the role of component C_1 in cellulolytic systems, it has been shown that C_1 hydrolyses native cellulose to cellobiose in a solubilizing reaction that is promoted more effectively by β-glucosidase than by carboxymethylcellulase.[1614] Stimulation of cellulolysis was

[1866] V. V. Favorov and V. E. Vaskovsky, *Comp. Biochem. Physiol.* (*B*), 1971, **38**, 689.
[1867] L. Vlodawsky, E. Harel, and A. M. Mayer, *Physiol. Plant.*, 1971, **25**, 363.
[1868] D. R. Whitaker, in 'The Enzymes', 3rd edn., ed. P. D. Boyer, Academic Press, New York, 1972, p. 273.
[1869] M. A. Hulme, *Arch. Biochem. Biophys.*, 1971, **147**, 49.
[1870] J. J. Marshall, *J. Chromatog.*, 1972, **71**, 367.

increased up to 80% and the only products recognized in the two systems were cellobiose and D-glucose. The ability of β-glucosidase, which has no action on cellulose, to promote saccharification of the substrate by component C_1 suggested that the latter is inhibited by cellobiose. Inhibition was competitive, being dependent upon the relative concentrations of cellulose and cellobiose, and could be overcome by increasing the substrate concentration. Similar effects were observed using carboxymethylcellulose as substrate.

Nojirimycin and D-glucono-1,5-lactone have been shown to be powerful inhibitors of glucosidases, but poor inhibitors of *exo-* and *endo*-glucanases.[1520] These compounds can be used to differentiate between β-glucosidase and cellulase.

Cellulase activity has been detected in the octopus (*Octopus vulgaris*).[1871] Radiographic evidence has been reported for the presence of a digestive cellulase in the sea hare (*Aplysia vaccaria*).[1872] Extracts of the hepatopancreas of the tunicate *Pyura stolonifera* have been shown to contain cellulase activity,[1500] and enzymes from a xylophagic insect (*Rhagium inquisitor*) have been found to be active against carboxymethylcellulose, hydroxyethylcellulose, and hemicellulose.[1719]

Whilst some poly- and oligo-saccharidases are produced in the aleurone cells of barley by a mechanism that is sensitive to exogenous gibberellic acid, cellulase appears to be produced by a non-sensitive pathway.[1627] Foliage from common and Merion Kentucky bluegrasses has been shown to contain cellulase activity, which was increased by infection of the foliage with *Helminthosporium sativum*.[1873] Very low levels of cellulase activity have been found throughout the *Nicotiana glutinosa* plant.[1874]

Cell-free extracts of micro-organisms of buffalo-calf rumen fluid have been prepared and were shown to hydrolyse carboxymethylcellulose and cellobiose.[1631] The cellulase activity possessed a broad pH optimum (pH 5—6) and inhibition studies indicated the participation of thiol groups in enzymic activity. The cellulase was only slightly inhibited by D-glucono-1,4-lactone, and D-glucose and cellobiose were the end-products from digestion of carboxymethylcellulose.

Intracellular cellulase has been induced by antheridiol during standardized vegetative growth in *Achlya ambisexualis*.[1875] The kinetics of inhibition of cellulase from *Aspergillus niger* by dihydroxyphenylalanine–melanin have been studied and it was found that the inhibitor complexed with the enzyme to alter its properties.[1876] Various results pointed to a primary involvement of electrostatic bonding in the formation of the

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[1873] R. R. Muse, H. B. Couch, L. D. Moore, and B. D. Muse, *Canad. J. Microbiol.*, 1972, **18**, 1091.
[1874] A. E. Moore and B. A. Stone, *Planta*, 1972, **104**, 93.
[1875] C. O. Warren and B. H. Sells, *J. Gen. Microbiol.*, 1971, **67**, 367.
[1876] A. T. Bull, *Enzymologia*, 1970, **39**, 333.

complex and it appeared that the inhibitor combined with groups other than those involved in the active site of the enzyme. Investigation of the action of *A. niger* β-glucanase on barley β-glucan led to the classification of a number of polysaccharidases, including a cellulase.[1877]

Cellvibrio fulvus has been shown to produce cellulose-bound and extracellular cellulases.[1878] Addition of cellulose to cultures of the organism enhanced the production of the cellulase, whereas it was suppressed by the presence of D-glucose. The enzyme, which exhibited optimum activity against carboxymethylcellulose at pH 7, was inhibited by mercuric acetate, but not by 4-chloromercuribenzoate–H_4edta. Fractionation of the cellulase preparations yielded many components of different molecular weights, and the activities of these components against cellulose and carboxymethylcellulose differed from one another. Parameters affecting the secretion of cellulase by *Colletotrichum lindemuthianum* grown in culture have been studied.[1517] Using cellulose and birch woodmeal in the growth media, the production of extracellular cellulases by cultures of wood-rotting microorganisms *Bacillus polymyxa*, *Fomes annosus*, *Lenzites trabea*, *Poria monticola*, and *Polystictus versicolor* has been demonstrated.[1879] The effect of substrate availability and other parameters on the production of cellulase was examined.

The production of cellulase by *Ruminococcus albus* and *R. flavefaciens* has been investigated and the degradation of cellulose *in vivo* was inhibited by cellobiose.[1880] Cellulase activity has been detected in exudates and sclerotial extracts from *Sclerotinia sclerotiorum*.[1641] Possible functions of vesicles and crystal-containing microbodies on the production of cellulase by *S. sclerotiorum* have been investigated.[1881]

The enzymic degradation of carboxymethyl-, carboxymethylhydroxyethyl-, hydroxyethyl-, and methyl-celluloses by a cellulase from a *Streptomyces* sp. has been investigated.[1882] Both carboxymethyl- and hydroxyethyl-celluloses appeared to be attacked by the cellulase in a similar fashion and the two groups were equally effective in limiting hydrolysis by this enzyme. Analogous derivatives of lichenin (a soluble β-glucan related to cellulose) were degraded in a manner similar to that of the cellulose derivatives.

Cellulolytic activity has been detected in several marine *Streptomycetes* sp.[1883] Extracellular cellulase from *Trichoderma viride* has been used in experimental studies of a model system for the continuous enzymic saccharification of cellulose embodying continuous removal of D-glucose and feedback of the enzyme.[1884] Cellulose strongly adsorbed the cellulase

[1877] P. Ducroo and R. Delecourt, *Bios*, 1971, **10**, 9.
[1878] B. Berg, B. von Hofsten, and G. Petterson, *J. Appl. Bact.*, 1972, **35**, 201.
[1879] H. Greaves, *Austral. J. Biol. Sci.*, 1971, **24**, 1169.
[1880] M. C. Fusee and J. M. Leatherwood, *Canad. J. Microbiol.*, 1972, **18**, 347.
[1881] D. P. Maxwell, P. H. Williams, and M. D. Maxwell, *Canad. J. Bot.*, 1972, **50**, 1743.
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[1884] T. K. Ghose and J. A. Kostick, *Biotechnol. and Bioeng.*, 1970, **12**, 921.

from *T. viride* at pH 4.0—5.0 (*i.e.* the pH at which the enzyme exhibits optimum activity).[1885] The amount of enzyme adsorbed was sufficient to digest the cellulose and no replenishment of the enzyme was required, even though the liquid phase containing the products was continuously removed. As the cellulose was digested, the released enzyme became readsorbed on to excess or newly added cellulose with retention of its activity. Using these phenomena, adsorbed cellulase has been used to produce continuously syrups containing 5—14% D-glucose either from stirred reactors containing 10—20% cellulose or from cellulose columns.

Chitinases.—Purified lysozymes from human urine, hen egg-white, and fig and papaya plants, and a chitinase from a *Streptomyces* sp. have been examined for chitinase activity on glycol-chitin, chitin-agar, chitosan-agar, and chitopentaose, and for bacteriolytic activities.[1505] The release of reducing sugar from colloidal chitin was also determined. Whereas the animal lysozymes are more active as lysozymes than chitinases, the plant enzymes are more active against chitin, *etc.* and are considered to be true chitinases. The bacterial chitinase was inactive against cell-wall material.

Chitinase has been extracted from ethylene-treated leaves of red kidney bean (*Phaseolus vulgaris*) and has been partially purified.[1886]

The exocellular chitinase system of *Chytriomyces hyalinus* has been studied, and conditions for achieving maximum concentration and yield in the culture fluid have been described.[1887] The activity of the enzyme was determined using a [^{14}C]chitin substrate and was maximal at pH 5.5. Whereas the enzyme was not inhibited by 2-acetamido-2-deoxy-D-glucose and D-glucose, bivalent Cu^{2+} and Cd^{2+} ions caused complete inhibition. The degradation of chitin by cell extracts of *Cytophaga johnsonii* has been investigated and some strains of the organism were found not to exhibit extracellular chitinase activity.[1888]

endo-β-Acetamidodeoxyglucosidase activity has been detected in the culture fluid of *Diplococcus pneumoniae* [1345] and in crude extracts of a soil amoeba (*Hartmanella glebae*) grown in the presence of *Aerobacter aerogenes* and *Alcaligenes faecalis*.[1509]

Chitinase activity has been detected in a purified enzyme fraction from *Myriococcum albomyces*.[1889] Bacteriolytic enzymes produced by *Myxococcus xanthus* apparently contain chitinase [1511] and *endo*-β-acetamidodeoxyglucosidase activities.[1510]

An extracellular, bacteriolytic *endo*-β-acetamidodeoxyglucosidase from *Staphylococcus aureus* degraded colloidal chitin, chitin oligosaccharides, and ethylene glycol–chitin at a pH optimum of 4.5.[1890] Chromatography

[1885] M. Mandels, J. Kostick, and R. Parizek, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 445.
[1886] F. B. Abeles, R. P. Bosshart, L. E. Forrence, and W. H. Habig, *Plant Physiol.*, 1971, **47**, 129.
[1887] P. S. Reisert, *Mycologia*, 1972, **64**, 288.
[1888] N. Sundarraj and J. V. Bhat, *Arkiv. Mikrobiol.*, 1972, **85**, 159.
[1889] H. Okazaki and H. Iizuka, *J. Agric. Chem. Soc. Japan*, 1971, **45**, 461.
[1890] T. Wadström, *Acta Chem. Scand.*, 1971, **25**, 1807.

of the products showed that chitobiose is the principal product and that free 2-acetamido-2-deoxy-D-glucose is not produced. Degradation of *Micrococcus lysodeikticus* cell walls and cell-wall glycan showed that the enzyme (pH optimum 7) yielded exclusively disaccharides having terminal, reducing 2-acetamido-2-deoxy-D-glucose residues. The action pattern of the enzyme was contrasted with that of lysozyme.

Chromatography of crude filtrates from cultures of *Streptomyces albidoflavus* on columns of chitin yielded two distinct fractions of β-acetamidodeoxyglucosidase active against chitin, but which differed in their pH optima and thermal stabilities.[1512]

Chondroitin Sulphate Hydrolases and Chondroitin Sulphate Lyases.—The hydrolysis of chondroitin sulphate proteoglycan from bovine articular cartilage by enzymes from human polymorphonuclear leucocytes was demonstrated by means of viscometry and gel electrophoresis.[1891] The hydrolysis was prevented by plasma trypsin inhibitor.

A method has been described for the assay of glycosaminoglycuronan-degrading activity in serum.[1892] Normal human serum was shown to contain a hyaluronidase active against hyaluronic acid and chondroitin 4- and 6-sulphates, and exhibiting maximum activity at pH 3.7. A second enzyme (pH optimum 7.0) that degraded chondroitin 4-sulphate and dermatan sulphate was detected, but it has little effect on hyaluronic acid and chondroitin 6-sulphate.

Testicular hyaluronidase and hyaluronate lyase from *Staphylococcus aureus* have both been shown to degrade chondroitin sulphate.[1893] The action of the testicular enzyme on this substrate was independent of the degree of sulphation, whereas the bacterial enzyme was more active against the desulphated substrate.[1894] The product resulting from action of the bacterial enzyme was characterized as an unsaturated disaccharide, whereas the testicular enzyme produced higher saturated oligomers.[1895]

Extracts of the hepatopancreas of the tunicate *Pyura stolonifera* were able to degrade chondroitin sulphate.[1500]

Hyaluronidase activity has been assayed in aqueous extracts of venom sacs of species of the Hymenoptera (*Dolichovespula saxonica, D. media, Paravespula germanica, P. vulgaris, Polistes gallicus, Vespa crabro*, and *V. orientalis*) by turbidimetric measurement of the products released after incubation with hyaluronic acid and chondroitin sulphate.[1896] All the extracts depolymerized both substrates, but the activity towards the former was greater.

Chondroitin sulphate lyase ABC from *Proteus vulgaris* has been used in the detection of glycosaminoglycuronans in tissue sections.[866]

[1891] D. M. Abadi and D. G. Mortvedt, *Enzyme*, 1971, **12**, 41.
[1892] M. L. Salkie, *Enzyme*, 1971, **12**, 409.
[1893] H. Mialhe, F. Castaigne, and C. C. Tuong, *Enzyme*, 1971, **12**, 355.
[1894] H. Mialhe, F. Castaigne, and C. C. Tuong, *Enzyme*, 1971, **12**, 362.
[1895] H. Mialhe, F. Castaigne, and C. C. Tuong, *Enzyme*, 1971, **12**, 368.
[1896] D. Allalouf, A. Ber, and J. Ishay, *Comp. Biochem. Physiol.*, 1972, **43B**, 119.

Dermatan Sulphate Hydrolases and Dermatan Sulphate Lyases.—A method for the assay of glycosaminoglycuronan-degrading activity has been used to detect an enzyme (pH optimum 7.0) in human serum that degraded dermatan sulphate and chondroitin sulphate, but which was largely inactive against chondroitin 6-sulphate and hyaluronic acid.[1892]

Two factors purified from normal human urine by means of polyacrylamide gel electrophoresis were found to accelerate the degradation of dermatan sulphate by fibroblasts from a case of Hunter syndrome.[1661] The factors were free from common glycosidase and sulphatase activities.

Dextranases.—A method based on the liberation of a dye complex from Blue Dextran 2000 has been described for the assay of dextranase.[1897] A method for the automated determination of dextranase in homogenates of human small intestinal mucosa involved dialysis into alkaline ferricyanide solution of the reducing sugars liberated on incubation with the substrate.[1522]

A purified α-glucosidase fraction from rabbit muscle exhibited weak dextranase activity.[831]

Hydrolysis of cross-linked dextran by a commercial dextranase was enhanced by the presence of poly(ethylene glycol).[1898] The increase in the K_m value suggested that, in the presence of poly(ethylene glycol), there is increased affinity between the enzyme and the substrate. A method for detecting dextranase-producing micro-organisms has been reported and is based on the use of Blue Dextran.[1899]

The isolation and purification of extracellular dextranases from a soil bacterium gave two enzymes having different pH optima (4.5—5.5 and 4.5—7.0).[1900] The former enzyme hydrolysed dextran to a series of straight-chain oligosaccharides, the smallest of which was isomaltose. The other enzyme did not produce isomaltose even after prolonged incubation with dextran, but traces of isomaltotriose and large amounts of higher oligosaccharides were produced.

Of four hundred strains of organisms from soil, nine strains (including *Aspergillus* and *Penicillium* sp.) were found to produce dextranase in the presence of dextran;[1901] *A. carneus* produced the greatest amounts of dextranase. Dextranase from the latter organism has been purified by repeated ion-exchange chromatography and gel filtration.[1902] The enzyme was separated into two fractions (p*I* values 4.12 and 4.35) by isoelectric focusing; the two forms were homogeneous by disc electrophoresis, possessed identical molecular weights (7.1×10^4) and pH optima (pH 5.0—5.5), and were stable over the pH range 4.5—9.0. Heavy-metal ions such as Cu^{2+},

[1897] T. Y. Koh and B. T. Khouw, *Canad. J. Biochem.*, 1970, **48**, 225.
[1898] M. Ceska, *Experientia*, 1972, **28**, 146.
[1899] F. Mencier, *Ann. Inst. Pasteur*, 1972, **122**, 153.
[1900] G. N. Richards and M. Streamer, *Proc. Austral. Biochem. Soc.*, 1971, **4**, 104.
[1901] D. Tsuru, N. Hiraoka, T. Hirose, and J. Fukumoto, *Agric. and Biol. Chem.* (*Japan*), 1971, **35**, 1727.
[1902] N. Hiraoka, J. Fukumoto, and D. Tsuru, *J. Biochem.* (*Japan*), 1972, **71**, 57.

Hg^{2+}, Fe^{3+}, and Ag^{+} were found to inactivate both enzymes. The substrate specificity of dextranase from *A. carneus* has been investigated using dextran and a series of isomaltodextrins and their derivatives as substrates.[1903] The enzyme readily hydrolysed dextran containing 95% α-(1 → 6)-linkages to give isomaltotriose, isomaltose, a small amount of D-glucose, and traces of higher oligosaccharides. Dextran containing 66% α-(1 → 6)-linkages was hydrolysed only slowly and to a lesser extent. Isomaltodextrins (4—8 units) were cleaved to give isomaltose and isomaltotriose at a rate similar to that observed on hydrolysis of the 95% α-(1 → 6)-linked dextran, whereas isomaltotriose was hydrolysed extremely slowly and isomaltose not at all. Comparison of the products arising from hydrolysis of isomaltodextrins and their reduced derivatives by the enzyme suggested that the enzyme primarily removes an isomaltotriosyl unit and, to a somewhat lesser extent, an isomaltosyl unit from the non-reducing end. Synthetic reactions catalysed by the enzyme were investigated.

A dextranase produced by *Penicillium funiculosum* has been purified to homogeneity by gel electrophoresis and isoelectric focusing.[1904] The enzyme (molecular weight 4.1×10^4; p*I* 4.6) hydrolysed dextran to isomaltose.

Dextrin-1,6-glucosidases.—Dextrin-1,6-glucosidase has been purified from rabbit liver and the preparation was shown to contain both glycosidase and transferase activities.[830] In view of the observations that the enzyme moved as a single band on gel electrophoresis and that the ratio of the two activities was constant throughout purification, it was concluded that one molecule possesses both activities. The enzyme (molecular weight 1.79×10^5; pH optimum 6) was inhibited by tris(hydroxymethyl)-methylamine and imidazole buffers.

Rat-liver dextrin-1,6-glucosidase was inhibited *in vivo* by *X*-rays or mixed gamma–neutron radiation.[842] The effect of glucagon on the dextrin-1,6-glucosidase of rat tissues has been examined.[835]

The hydrolytic action of yeast dextrin-1,6-glucosidase can be distinguished from that of yeast isoamylase.[1905] Whereas the latter enzyme hydrolysed amylopectin by cleaving the outermost inter-chain linkages, with the liberation of linear malto-oligosaccharides having an average DP of fifteen D-glucose residues, the former acted by a combination of transferase and glucosidase activities.

Galactanases.—Infection of *Aerobacter aerogenes* with a phage isolated from raw sewage induced the synthesis of a polysaccharidase that was active against the capsular polysaccharide of the host organism.[1906] The

[1903] D. Tsuru, N. Hiraoka, and J. Fukumoto, *J. Biochem.* (*Japan*), 1972, **71**, 653.
[1904] L. Chaiet, A. J. Kempf, R. Harman, E. Kaczka, R. Weston, K. Nollstadt, and F. J. Wolf, *Appl. Microbiology*, 1970, **20**, 421.
[1905] R. B. Evans and D. J. Manners, *Carbohydrate Res.*, 1971, **20**, 339.
[1906] E. C. Yurewicz, M. A. Ghalambor, D. H. Duckworth, and E. C. Heath, *J. Biol. Chem.*, 1971, **246**, 5607.

enzyme exists in two forms (soluble and phage-bound) with identical catalytic properties. The physicochemical properties of the soluble enzyme (molecular weight 3.79×10^5) were determined after its purification to homogeneity, and its dissociation gave rise to two subunits (molecular weights 6.32 and 3.64×10^4). The enzyme was found to be highly specific and randomly attacked the *O*-α-D-galactosyl-(1 → 3)-D-galactose linkages of the capsular polysaccharide, one susceptible bond occurring in each tetrasaccharide repeating unit of the polymer. The smallest oligosaccharide that acted as a substrate was a dodecasaccharide composed of three tetrasaccharide repeating units. The enzyme exhibited optimum activity at pH 5.2. The depolymerase activity of the phage-bound enzyme was shown to be identical with that of the soluble enzyme. The bound enzyme also yielded subunits on treatment with urea or guanidine hydrochloride and the subunits behaved identically with those from the soluble enzyme on gel filtration.

Many strains of *Bacillus subtilis* have been found to secrete a number of hemicellulolytic enzymes and an arabinogalactanase (molecular weight 3.7×10^4, p*I* 8.4) was obtained in a crystalline state.[1864] The enzyme exhibited optimum activity at pH 6.0 and hydrolysed soybean arabinogalactan to afford galactobiose, as the main product, but no arabinose.

Glucanases.—Since nojirimycin and D-glucono-1,5-lactone are powerful inhibitors of β-glucosidases, but only slightly inhibit *exo*-β-glucanases, they may be used to distinguish between the two types of enzymes.[1520]

The debranching enzymes involved in the metabolism of glycogen have been reviewed.[813]

A homogeneous preparation of a glycosidase from pig spleen has been obtained by means of affinity chromatography on Sephadex.[1620] The enzyme was shown to cleave D-glucosyl disaccharides of a number of types (maltose, nigerose, kojibiose, and isomaltose) and also glycogen, amylose, amylopectin, dextrans, and nigeran. Dextrans containing α-(1 → 6)- and α-(1 → 3)-linkages were completely hydrolysed by the enzyme, whereas the presence of α-(1 → 2)-linkages reduced the susceptibility of the substrate.

A 'glucoamylase' separated from canine serum by gel filtration appeared to possess both glucoamylase and α-glucosidase activities, and to attack α-(1 → 6)- and α-(1 → 4)-linkages.[1621] pH Optima of 6.1 were found for both activities, which were inhibited by tris(hydroxymethyl)methylamine, turanose, and erythritol.

Glucanases of *Helix pomatia* have been purified by affinity chromatography on columns of pachyman.[1512] Extracts of the hepatopancreas of the tunicate *Pyura stolonifera* have been shown to contain a polysaccharidase active against starch.[1500] Polysaccharidases of a xylophagic insect (*Rhagium inquisitor*) were found to be active against a glycomannan.[1719]

Production of *endo*-β-glucanase in the aleurone cells of barley appears to occur by a mechanism that is sensitive to exogenous gibberellic acid.[1627] The starch-degrading enzymes of the leaves of a temperate grass species,

orchard grass (*Dactylis glomerata*), and three tropical grasses, 'Pangola' digit grass (*Digitaria decumbens*), pearl millet (*Pennisetum glaucum*), and sugar cane (*Saccharum officinarum*), have been separated by polyacrylamide gel electrophoresis and their properties were examined.[1728] Densitometer tracings of gels stained with starch–iodine revealed distinctive patterns for each species.

Strains of *Aerobacter aerogenes* and *A. cloacae* have been shown to contain a polysaccharidase, which yielded large amounts of maltohexaose from amylose, amylopectin, and whole starch.[1907] The enzyme was isolated from a strain of *A. aerogenes* and was separated from other starch-hydrolysing activities by precipitation and ion-exchange chromatography. The data supported the theory that the enzyme possesses an *exo*-action pattern.

Culture fluids from *Arthrobacter luteus* contained yeast-cell lytic activity, the action pattern of which was found to be quite different from that of the β-1,3-glucanase activity generally considered to play a major role in the lysis of yeast cell walls.[1908] This particular β-1,3-glucanase has been further studied using pachyman as substrate.[1909]

Aspergillus nidulans has been shown to produce enzymes capable of hydrolysing the alkali-resistant glucan component of the cell wall of the organism.[1910] The production of glucanase and α-1,3-glucanase activities by *A. nidulans* has been followed during cleistothecium development and the latter activity was increased when the availability of D-glucose was depleted.[1738] An *exo*-α-1,3-glucanase has been found to be produced extracellularly by *A. nidulans* and it has been purified by gel filtration.[1911] The enzyme (pH optimum 5.0—6.2) was inactive against nigeran, but completely hydrolysed an α-1,3-glucan from the organism; it is apparently distinct from amylases and laminarinase.

The action of glucanases of *A. niger* upon barley β-glucan indicated that they are β-1,3- and β-1,4-glucanases.[1877] Classifications of the enzymes were reported. An extracellular α-glucanase has been isolated from *A. oryzae* and was purified by ion-exchange chromatography.[1912] From studies with various polysaccharides as substrates, the enzyme appeared to be specific for mango glucan, which contains blocks of nine α-(1 → 3)- and nine α-(1 → 4)-linked D-glucopyranosyl units in a repeating sequence. The α-glucanase exhibited optimum activity at pH 5.5 and 45 °C when mango glucan was used as substrate.

β-1,3-Glucanases have been recognized in cultures of such thermophilic fungi as *Chaetomium thermophilum*, *Myriococcum albomyces*, *Thermomyces*

[1907] K. Kainuma, S. Kobayashi, T. Ito, and S. Suzuki, *F.E.B.S. Letters*, 1972, **26**, 281.
[1908] K. Kitamura, T. Kaneko, and Y. Yamamoto, *Arch. Biochem. Biophys.*, 1971, **145**, 402.
[1909] K. Kitamura, T. Kaneko, and Y. Yamamoto, *J. Gen. Appl. Microbiol.*, 1972, **18**, 57.
[1910] B. J. M. Zonneveld, *Biochim. Biophys. Acta*, 1971, **249**, 506.
[1911] B. J. M. Zonneveld, *Biochim. Biophys. Acta*, 1972, **258**, 541.
[1912] A. K. Kundu and S. Manna, *Indian J. Exptl. Biol.*, 1971, **9**, 75.

ibadanensis, and *T. lanuginosa*.[1889] The enzymes were purified by ion-exchange chromatography and, since they yielded only D-glucose from laminarin and yeast glucan, it is considered that they are *exo*-β-1,3-glucanases. Other properties of the enzyme from *T. lanuginosa* differed from those of the enzymes from the other fungi.

Coniothyrium minitans and *Trichoderma viride* parasitic on *Sclerotinia sclerotiorum* were found to contain an *exo*-β-1,3-glucanase that degraded sclerotan [a β-(1 → 3)-glucan] to D-glucose and gentiobiose.[1913]

Glucanase from *Nicotiana glutinosa* was demonstrated to be an *endo*-acting enzyme highly specific for linear β-(1 → 3)-glucan substrates, which were depolymerized to mixtures of oligosaccharides of DP 2—7.[1914] Laminaribiose and laminaritriose were not hydrolysed by the enzyme, but laminari-tetraose and -pentaose were hydrolysed slowly. The rate of hydrolysis of carboxymethylpachyman decreased with increasing degree of substitution. These findings suggested that the substrate must contain at least three contiguous β-(1 → 3)-D-glucopyranosyl residues for cleavage to occur. A method for the purification of β-glucanase from *Rhizopus arrhizus* has been described and the action pattern of the enzyme on a series of β-glucans was investigated and compared with that of the enzyme from *N. glutinosa*.

An enzyme that degraded yeast glucan and the cell wall of living yeast has been crystallized from the culture filtrate of a *Rhizopus* sp.[1915] The enzyme (molecular weight 1.9×10^4) was separated from laminarinase and luteanase activities by the purification procedure and was shown to be homogeneous. Although the exact bond cleaved by the enzyme was not identified, studies of substrate specificity indicated that the enzyme acts as a debranching enzyme by cleaving glycosidic bonds at the branch points in yeast glucan.

Culture fluids from synchronously dividing cultures of brewers' yeast (*Saccharomyces carlsbergensis*) exhibited cyclical changes in glucanase activity.[1916] On account of this variation, production of the enzyme is considered to be a function of the budding process. The same enzyme was found in autolysates of the yeast and the action of the enzyme on a yeast glucan yielded products larger than tetrasaccharides. The *exo*- and *endo*-β-1,3-glucanases active against laminarin and sodium carboxymethylpachyman have been detected in a budding yeast (*Saccharomyces cerevisiae*);[1917] the enzymes are located in the vesicles.[1918]

Glycosaminoglycuronan Hydrolases.—A method for the computer-assisted documentation and analysis of complex patterns of enzymic activity has

[1913] J. S. D. Bacon, A. H. Gordon, and D. Jones, *Biochem. J.*, 1972, **129**, 27P.
[1914] A. E. Moore and B. A. Stone, *Biochim. Biophys. Acta*, 1972, **258**, 248.
[1915] S. Yamamoto, T. Shiraishi, and S. Nagasaki, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1802.
[1916] I. S. Maddox and J. S. Hough, *J. Inst. Brewing*, 1971, **77**, 44.
[1917] P. Matile, M. Cortat, A. Wiemken, and A. Frey-Wyssling, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 636.
[1918] M. Cortat, P. Matile, and A. Wiemken, *Arch. Mikrobiol.*, 1972, **82**, 189.

been used to examine the glycosaminoglycuronan hydrolase activity of sera from patients with various conditions.[1919] Although no diagnostic patterns were found, there were striking differences in the patterns obtained using different glycosaminoglycuronans as substrates, suggesting that the hydrolase systems of human serum are complex. From further studies of the glycosaminoglycuronan hydrolase activities of human sera from normals and patients, distinctive features emerged for patients with myocardial infarction.[1920]

Heparin Hydrolases, Heparin Lyases, and Heparan Sulphate Lyases.—A study of the degradation of heparin in mouse mastocytoma tissue suggested the presence of a heparin-degrading *endo*-enzyme.[953] Labelling *in vivo* of the heparin with [^{35}S]sulphate showed an initial labelling of large molecules followed by a progressive shift of the radioactivity to lower molecular weight fractions. Incubation *in vitro* of [^{35}S]heparin with a mastocytoma fraction degraded the polysaccharide, whereas labelled chondroitin sulphate was not degraded.

Heparin lyase and heparan sulphate lyase from *Flavobacterium heparinum*, in conjunction with an α-glycuronidase from the same organism, degraded heparan sulphate and heparin to monosaccharides.[1667] Heparin lyase from *F. heparinum* has been used to eliminate the inhibition caused by heparin in routine coagulation assays.[1921]

Hyaluronidases and Hyaluronate Lyases.—Hyaluronidases have been reviewed recently.[1922]

A method has been described for the assay of glycosaminoglycuronan-degrading activity in sera.[1892] Normal human serum has been shown to contain a hyaluronidase active at pH 3.7 against hyaluronic acid, chondroitin 4-sulphate, and chondroitin 6-sulphate, but not against dermatan sulphate. Levels of serum hyaluronidase in cases of aspartylglucosaminuria were increased above normal,[976] whereas decreased levels were detected in sera from cases of Morquio syndrome.[1476]

Hyaluronidase has been detected in bovine and rat brains.[1923] The enzyme (pH optimum 3.7) degraded hyaluronic acid and, at a slower rate, chondroitin sulphate to a mixture of oligosaccharides having reducing, terminal 2-acetamido-2-deoxyhexose units. The enzyme was associated with lysosomal fractions of the tissues. Testicular hyaluronidase and hyaluronate lyase (from *Staphylococcus aureus*) have been investigated as spreading factors and both possessed activity against chondroitin sulphate, particularly the mammalian enzyme.[1893] The bacterial enzyme degraded

[1919] M. K. Salkie and D. McDonald, *Clinica Chim. Acta*, 1972, **37**, 323.
[1920] M. L. Salkie, *Clinica Chim. Acta*, 1972, **37**, 329.
[1921] E. D. Hutt and H. S. Kingdon, *J. Lab. Clinical Med.*, 1972, **79**, 1027.
[1922] K. Meyer, in 'The Enzymes', 3rd edn., ed. P. D. Boyer, Academic Press, New York, 1972, p. 307.
[1923] R. U. Margolis, R. K. Margolis, R. Santella, and D. M. Atherton, *J. Neurochem.*, 1972, **19**, 2325.

hyaluronic acid and chondroitin sulphate to unsaturated disaccharides, whereas the mammalian enzyme degraded the polysaccharides to saturated oligomers.[1895] The action of testicular hyaluronidase on chondroitin sulphate was shown to be independent of the degree of sulphation of the polysaccharide, whereas the bacterial enzyme was more active against desulphated chondroitin sulphate.[1894] Hyaluronidase activity has been detected in the three major zones of rat kidney.[960] The activity of the enzyme in the cortex was increased by treatment with hydrocortisone and it was suggested that the additional enzyme is retained in a compartment separate from the intercellular matrix.

The activity of hyaluronidase in embryonic chick somites and limb buds has been correlated with the results of experiments on culturing the cells in the presence of hyaluronic acid.[999]

Hyaluronidase activity has been assayed in aqueous extracts of venom sacs of a number of Vespinae (Hymenoptera) such as *Dolichovespula saxonica, D. media, Paravespula germanica, P. vulgaris, Polistes gallicus, Vespa orientalis,* and *V. crabro.*[1896] Measurement was achieved by turbidimetry of the products released on incubation with either hyaluronic acid or chondroitin sulphate and by spectrophotometric estimation of the terminal 2-acetamido-2-deoxy-D-glucose residues released from hyaluronic acid. All the extracts depolymerized both hyaluronic acid and chondroitin sulphate, but the latter was degraded to lesser extents and, in this respect, the insect hyaluronidases resemble mammalian testicular hyaluronidase. A number of gel filtration and ion-exchange chromatographic stages were necessary to purify hyaluronidase from bee venom.[1924] The enzyme (molecular weight $3.5—4.0 \times 10^4$) is not the highest molecular weight component of the venom.

Venom from a snake (*Bungarus multicinctus*) has been shown to contain hyaluronate lyase, which could be purified and separated into three species by ion-exchange chromatography.[1925]

Hyaluronidase extracted with dilute acetic acid from gastric mucosa of mackerel (*Scomber japonicus*) has been purified by gel filtration and ion-exchange chromatography.[1926] The final preparation was free from other glycohydrolases and was homogeneous on polyacrylamide gel electrophoresis. Extracts of the hepatopancreas of the tunicate *Pyura stolonifera* contained an enzyme active against hyaluronic acid and chondroitin sulphate.[1500]

Hyaluronidase from *Streptomyces hyalurolyticus* has been used for the identification of glycosaminoglycuronans in tissue sections.[866]

Hyaluronidase has been insolubilized by attachment to agarose and the covalent derivative was active against high molecular weight substrates.[1927]

[1924] C. P. Ivanov, S. Shkenderov, and M. A. Krysteva, *Doklady Bolg. Akad. Nauk,* 1972, **25**, 229.
[1925] C. Y. Lee, S. L. Chang, S. T. Kau, and S. Luh, *J. Chromatog.*, 1972, **72**, 71.
[1926] H. Yamamoto and M. Kitamikado, *Bull. Jap. Soc. Sci. Fish.*, 1971, **37**, 621.
[1927] W. H. Stimson and A. Serafini-Fracassini, *F.E.B.S. Letters,* 1971, **17**, 318.

Inulases.—Two of the three β-fructofuranosidases obtained by ion-exchange chromatography from an extract of dandelion (*Taraxacum officinale*) roots exhibited activity (pH optimum 4.0) against inulin oligosaccharides, but were inactive against sucrose.[1532] An investigation of the mode of action of the enzymes on inulin indicated that they are *exo*-enzymes.[1533]

A strain of *Arthrobacter ureafaciens* has been shown to produce an extracellular enzyme capable of degrading inulin to a difructose dianhydride and small amounts of oligosaccharides.[1928] Attempted identification of the anhydride indicated that it corresponds to di-D-fructofuranose 1,2′:2,3′-dianhydride. It was noted that the products of hydrolysis of inulin differ from those produced by common inulase (E.C. 3.2.1.7).

Isoamylases.—The debranching actions of crystalline isoamylase from *Pseudomonas* SB 15 on amylopectin and glycogen have been studied by determining the increase in reducing power and coloration with iodine of the substrate, and also by gel-filtration analysis of the digests.[1929] Branch points in both substrates were hydrolysed completely using a small enzyme : substrate ratio, whereas crystalline pullulanase from *Aerobacter aerogenes* achieved the same extent of hydrolysis only when higher enzyme : substrate ratios were used. Analyses of the debranched glucans produced after various times of incubation suggested that the isoamylase hydrolyses both inner and outer branched linkages of the substrates, whereas the pullulanase hydrolyses only the outer branched linkages. The two enzymes have been used in structural analysis of amylopectins.[1930]

The action of purified yeast isoamylase on amylopectin, like that of bacterial pullulanase, resulted in hydrolysis of the outermost, inter-chain linkages with the liberation of linear malto-oligosaccharides having an average DP of fifteen D-glucose units.[1905] The hydrolytic action is considered to distinguish yeast isoamylase from yeast amylopectin-1,6-glucosidase, which acts by a combination of transferase and glucosidase activities.

Laminarinases.—The action of nojirimycin and D-glucono-1,5-lactone as inhibitors of laminarinase has been investigated.[1520]

A xylophagic insect (*Rhagium inquisitor*) has been shown to produce a laminarinase.[1719] β-1,3-Glucanases from *Helix pomatia* have been purified by affinity chromatography on columns of pachyman.[1512] Two laminarinases differing in their modes of action were separated; one yielded only D-glucose, whereas the other gave rise to a series of β-(1 → 3)-linked oligosaccharides.

The specificity and action pattern of laminarinases from a marine mollusc *Spisula sachalinensis* have been studied.[1931] The enzymes were

[1928] K. Tanaka, T. Uchiyama, and A. Ito, *Biochim. Biophys. Acta*, 1972, **284**, 248.
[1929] T. Harada, A. Misaki, H. Akai, K. Yokobayashi, and K. Sugimoto, *Biochim. Biophys. Acta*, 1972, **268**, 497.
[1930] H. Akai, K. Yokobayashi, A. Misaki, and T. Harada, *Biochim. Biophys. Acta*, 1971, **252**, 427.
[1931] V. V. Sova and L. A. Elyakova, *Biochim. Biophys. Acta*, 1972, **258**, 219.

shown to be specific for β-(1 → 3)-linkages in glucans, but were inactive against α-(1 → 3)-linkages, and were considered to be *endo*-polysaccharidases. Studies by ^{1}H n.m.r. spectroscopy have shown that the anomeric configuration of the products obtained on hydrolysis of laminarin by the enzymes retained that of the linkages in the substrate.[1932]

The *de novo* synthesis of laminarinase by aleurone cells of barley has been demonstrated.[1933] The *endo*-laminarinase of aleurone cells of barley appears to be produced by a mechanism sensitive to exogenous gibberellic acid.[1627]

A high level of laminarinase has been shown to be present in all parts of *Nicotiana glutinosa* plants.[1874] The activity of the enzyme was found to increase through leaf senescence and the enzyme present in yellow leaves was electrophoretically distinguishable from that in green leaves.

In an assessment of air pollution by ethylene, the effects of ambient ethylene levels on the laminarinase content of kidney bean (*Phaseolus vulgaris*) have been investigated.[1934] Laminarinase has been extracted from ethylene-treated leaves of kidney bean and was partially purified.[1886]

The activities of laminarinase in cleistothecium development of *Aspergillus nidulans* have been determined.[1738] Classification of the action of β-glucanases from *A. niger* on barley β-glucan led to the identification of a laminarinase.[1877]

β-1,3-Glucanases have been recognized in cultures of such thermophilic fungi as *Chaetomium thermophilum*, *Myriococcum albomyces*, *Thermomyces ibadanensis*, and *T. lanuginosa*.[1889] The enzymes were purified by ion-exchange chromatography and, since they yielded only D-glucose from laminarin, are considered to be *exo*-glucanases. A number of properties of the enzyme from *T. lanuginosa* differed from those of the enzymes from the other sources.

Two types of *endo*-β-(1 → 3)-glucanase have been obtained by chromatography from the media of cultures of *Cytophaga johnsonii* grown in the presence of inducers.[1935, 1936] The glucanases were active against laminarin. One type extensively solubilized yeast cell walls to produce oligosaccharides having five or more residues as the principal end-products, whereas lysis by the other type was incomplete.

The mycelium of *Phytophthora palmivora* has been shown to contain enzymes capable of hydrolysing β-(1 → 3)-glucans by endwise removal of D-glucose units;[1937] this laminarinase activity is associated with particular fractions and is not secreted. Laminarin oligosaccharides were also cleaved

[1932] V. V. Isakov, V. V. Sova, V. A. Denisenko, V. G. Sakharovsky, L. A. Elyakova, and A. K. Dzizenko, *Biochim. Biophys. Acta*, 1972, **268**, 184.
[1933] P. A. Bennett and M. J. Chrispeels, *Plant Physiol.*, 1972, **49**, 445.
[1934] F. B. Abeles, L. E. Forrence, and G. R. Leather, *Plant. Physiol.*, 1971, **48**, 504.
[1935] J. S. D. Bacon, A. H. Gordon, and D. M. Webley, *Biochem. J.*, 1970, **117**, 43P.
[1936] J. S. D. Bacon, A. H. Gordon, D. Jones, I. F. Taylor, and D. M. Webley, *Biochem. J.*, 1970, **120**, 67.
[1937] V. Z. Holten and S. Bartnicki-Garcia, *Biochim. Biophys. Acta*, 1972, **276**, 221.

by the enzyme; the rate of hydrolysis was greatest for laminaribiose and reached a minimum for laminaritetraose, but rose again with laminaripentaose and laminarin. Although these findings suggested the co-existence of a β-glucosidase and a β-1,3-glucanase, no separation of the two activities could be achieved. The enzyme (pH optimum 5—6) did not require the addition of metal ions for manifestation of activity and was inhibited by heavy-metal ions, particularly Hg^{2+}.

Fluids from synchronously dividing cultures of brewers' yeast (*Saccharomyces carlsbergensis*) showed cyclical changes in laminarinase activity and this process is considered to be a function of the budding of the organism.[1916] The same enzyme was found in autolysates of the yeast and the enzymes hydrolysed laminarin to D-glucose. Protoplasts of *S. cerevisiae* have been shown to secrete laminarinase and gel filtration of the enzyme revealed that it possesses the same molecular size as the intracellular laminarinase.[1938] Both *exo*- and *endo*-laminarinases have also been isolated from particles of budding *S. cerevisiae*.[1917, 1918] A laminarinase obtained from bakers' yeast by means of ion-exchange chromatography appeared to be an *endo*-hydrolase.[1548] An intracellular laminarinase has been detected in *S. lactis*;[1640] a partially purified preparation of the enzyme also contained β-glucosidase activity, and the activities of the two enzymes were compared.

A β-glucanase having marked specificity for β-(1 → 3)-D-glucosidic linkages was found to be closely associated with the cell wall of the fission yeast *Schizosaccharomyces pombe*.[1939] On account of its *endo*-hydrolytic action pattern and the absence of D-glucose among the initial products of hydrolysis of laminarin, the enzyme is concluded to be a laminarinase.

Limit Dextrinases.—Extracts of germinated barley contained two debranching enzymes, which were separated from each other and from α-amylase by continuous electrophoresis followed by gel filtration.[1726, 1940] One of the enzymes (limit dextrinase) was assayed using pullulan as substrate and the purified enzyme released α-(1 → 6)-linked maltosyl and maltotriosyl units from both oligo- and poly-saccharide substrates. The enzyme readily hydrolysed α-limit dextrins, but had no action on substrates containing α-(1 → 6)-linked D-glucosyl residues, amylopectin, glycogen, and α-limit dextrin from glycogen. The enzyme exhibited maximum activity at pH 5, was selectively activated by Cl^- ions, and synthesized branched oligosaccharides from maltose and various malto-oligosaccharides. The limit dextrinase appears to be produced in the aleurone cells of barley by a mechanism sensitive to exogenous gibberellic acid.[1627]

Luteanases.—Chromatographic fractionation of culture fluids from *Cytophaga johnsonii* grown in the presence of inducers revealed the presence

[1938] P. Biely, V. Farkaš, and S. Bauer, *F.E.B.S. Letters*, 1972, **23**, 153.
[1939] D. R. Barras, *J. Microbiol. Serol.*, 1972, **38**, 65.
[1940] D. J. Manners and D. Yellowlees, *Stärke*, 1971, **23**, 228.

of several β-1,6-glucanases.[1935, 1936] These luteanases could be induced separately from laminarinases by growth of the bacterium in the presence of lutean, and were shown to release mannan-peptides from cell walls.

A β-1,6-glucanase has been obtained from bakers' yeast by means of ion-exchange chromatography and it appeared to be an *endo*-hydrolase.[1548]

Mannanases.—A viscosimetric assay for *endo*-β-mannanase activity has been described using a mannan from *Orchis maculata* as substrate.[1941] Plots of the rate of change of the reciprocal specific viscosity against the concentration of enzyme gave linear relationships.

Polysaccharidases of a xylophagic insect (*Rhagium inquisitor*) have been shown to be active against a glucomannan.[1719]

The β-mannanase activity of wheat and flour has been investigated.[1513] A mannanase has been purified from *Aspergillus niger* by ion-exchange chromatography, gel filtration, isoelectric focusing, and electrophoresis;[1942] studies of substrate specificity showed the enzyme to be an *endo*-β-mannanase. Many strains of *Bacillus subtilis* have been found to secrete enzymes active against a galactomannan.[1864]

An enzyme induced by phage F40 in *Klebsiella* serotype 2 strains hydrolysed polysaccharides prepared from the same organisms.[1943] In each case, the major product was a tetrasaccharide corresponding to the repeating unit of the polysaccharide. Since the terminal, reducing unit in each tetrasaccharide was characterized as D-mannose, the enzyme is considered to be a mannanase capable of hydrolysing β-D-mannopyranosyl-(1 → 4)-D-glucose linkages. The enzyme is highly specific, being inactive against carboxy-reduced type 2 polysaccharide and polysaccharides from a number of other *Klebsiella* strains of different serotype. In contrast, similar phage-induced enzymes from *K. aerogenes* serotype 54 strains hydrolysed both types 2 and 54 polysaccharides, yielding the same products from type 2 as the homologous enzyme.

Fluids from synchronously dividing cultures of brewers' yeast (*Saccharomyces carlsbergensis*) showed cyclical changes in mannanase activity and this variation is considered to have a function in the budding process.[1916] The same enzyme was present in autolysates of the yeast and was shown to yield products larger than tetrasaccharides from bakers' yeast mannan.

Mycodextranases.—The secretion of mycodextranase by *Penicillium melinii* was induced by nigeran; biosynthetic aspects of the induction have been reported.[1944] A microsomal mannosyltransferase utilizing an endogenous acceptor appeared to be related to the induction of the mycodextranase. An α-1,4-glucanase has been purified to homogeneity in high yield from

[1941] J. Tampion, *Enzymologia*, 1972, **42**, 253.
[1942] Y. Tsujisaka, K. Hiyama, S. Takenishi, and J. Fukumoto, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 155.
[1943] I. W. Sutherland, *J. Gen. Microbiol.*, 1972, **70**, 331.
[1944] O. Samuel and J. H. Nordin, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 1376.

culture filtrates of *P. melinii* and was resolved by electrophoresis into fast (*F*) and slow (*S*) forms.[1945] Form *F* constituted 85% of the enzyme's protein and activity. Both forms hydrolysed nigeran and certain nigeran oligosaccharides to give *O*-α-D-glucopyranosyl-(1 → 3)-D-glucose(nigerose) and *O*-α-D-glucopyranosyl-(1 → 3)-*O*-α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 3)-D-glucose as the only end-products. Aspects of the action pattern and catalytic mechanism of form *F* have been examined in detail; it was shown that the enzyme operates by an *endo*-mechanism of the multichain type, with both end-products being formed at a constant rate. The two forms were found to possess a molecular weight of 4×10^4 and to contain carbohydrate (15—16%) consisting of D-glucose and D-mannose only. Treatment with alkaline borohydride selectively removed the carbohydrate moieties, indicating the involvement of L-serine and L-threonine residues in the glycopeptide linkages.

Oligo-1,3-glucosidases.—Nojirimycin and D-glucono-1,5-lactone are powerful inhibitors of glucosidases, but poor inhibitors of *exo*- and *endo*-glucanases and, thus, may be used to differentiate between β-glucosidases and oligo-1,3-glucosidases.[1520]

An oligo-1,3-glucosidase extracted from the leaves of *Nicotiana glutinosa* has been purified to homogeneity by ion-exchange chromatography, gel filtration, and isoelectric focusing.[1946] The enzyme (p*I* 4.87, molecular weight 4.5×10^4) possesses a pH optimum of 5.0 and is inactivated by heat treatment.

The action pattern of an oligo-1,3-glucosidase isolated from a *Basidiomycete* sp. has been investigated using laminarin reduced with sodium borotritide as substrate.[1947] The principal products were the corresponding tetra- and tri-saccharides, and it was concluded that enzymic hydrolysis is a single-chain process.

An oligo-1,3-glucosidase has been obtained from culture filtrates of *Corticum rolfsii* and was purified by ion-exchange chromatography and gel filtration.[1948] The enzyme was shown to possess a pH optimum of 4.5 and a temperature optimum of 55 °C, and was inhibited by Hg^{2+} and Fe^{2+} ions.

Oligo-1,6-glucosidases.—A rapid, thin-layer chromatographic method for the assay of human intestinal glycosidases has been applied to intestinal oligo-1,6-glucosidases in normals and cases of coeliac disease.[1521]

A simplified procedure has been reported for the isolation of a homogeneous β-fructofuranosidase–oligo-1,6-glucosidase complex from rabbit small intestine.[1524] The enzyme (molecular weight 2.2×10^5) was shown to contain galactose (26), glucose (11), mannose (22), fucose (17), 2-amino-2-deoxygalactose (12), and 2-amino-2-deoxyglucose (68 residues per mole). Values for its partial specific volume and hydrophobicity coefficient were

[1945] K. K. Tung, A. Rosenthal, and J. H. Nordin, *J. Biol. Chem.*, 1971, **246**, 6722.
[1946] A. E. Moore and B. A. Stone, *Biochim. Biophys. Acta*, 1972, **258**, 238.
[1947] A. F. Bochkov, V. V. Sova, and S. Kirkwood, *Biochim. Biophys. Acta*, 1972, **258**, 531.
[1948] A. Kaji, T. Ohsaki, and O. Yoshihara, *J. Agric. Chem. Soc. Japan*, 1971, **45**, 278.

obtained. 'Neutral α-oligoglucosidases' from rabbit liver have been purified by ion-exchange chromatography and gel filtration, and the action of the enzymes on α-(1 → 6)-oligosaccharides of DP 2—10 has been studied.[1623] The rate of hydrolysis of the substrates appeared to decrease with an increase in DP and, although that of isomaltodecaose was significant, the enzyme was hardly active against glycogen and dextran.

Oligo-1,6-glucosidase activity of the small intestine of genetically adipose mice has been shown to be greater than that of their siblings of normal weight.[1525]

A β-fructofuranosidase–oligo-1,6-glucosidase complex solubilized from rat intestine has been purified by ion-exchange chromatography.[1526] 4-Nitrophenyl and 6-bromo-2-naphthyl α-D-glucopyranosides functioned as competitive inhibitors of the activities of the complex. The effects of carbohydrates and corticosteroid on an oligo-1,6-glucosidase in the intestines of infant rats,[1530] and those of actinomycin D on the specific activity and intracellular distribution of rat jejunal oligo-1,6-glucosidase,[1529] have been investigated.

Polygalacturonases and Polygalacturonate Lyases.—Polygalacturonase activity has been detected in a xylophagic insect (*Rhagium inquisitor*).[1719] Extracts of the hepatopancreas of the tunicate *Pyura stolonifera* have been shown to contain polygalacturonase activity.[1500]

Polygalacturonase has been found in avocado (*Persea americana*) fruit throughout development.[1949] Some degree of softening of maturing tomato fruit takes place before the mechanism for loss of firmness, involving the sequential actions of pectinesterase and polygalacturonase, is operative to any extent.[1950] It was shown that polygalacturonate lyase is not involved in the early softening process and that it does not play a significant part in the softening of tomato fruit either prior to or during ripening.

Incubation of a mixture of extracellular pectinolytic enzymes from *Aspergillus niger* with insoluble, cross-linked pectic acid, under conditions optimal for polygalacturonase activity, resulted in a selective adsorption of *endo*-polygalacturonase by the insoluble material.[1951] The enzyme could then be liberated using either sodium pectate solution or a solution of pH greater than 6. On the basis of these observations, a chromatographic method for the purification of *endo*-polygalacturonase was developed. Cross-linked pectic acid competitively inhibited the enzyme and it is considered that complex formation is due exclusively to a functional affinity of the binding site of the polygalacturonase for the adsorbent.[1952] The thermal stability of the enzyme was increased on adsorption on to cross-linked pectic acid.

[1949] G. Zauberman and M. Schiffmann-Nadel, *Plant Physiol.*, 1972, **49**, 864.
[1950] R. T. Besford and G. E. Hobson, *Phytochemistry*, 1972, **11**, 2201.
[1951] Ľ. Rexová-Benková and V. Tibenský, *Biochim. Biophys. Acta*, 1972, **268**, 187.
[1952] Ľ. Rexová-Benková, *Biochim. Biophys. Acta*, 1972, **276**, 215.

Polygalacturonate lyase has been purified from the culture medium of *A. sojae* by ion-exchange chromatography and gel filtration.[1953] The enzyme (molecular weight 3.2×10^4) exhibited maximum activity against pectin (68% esterified) at pH 5.5, but for poly(methyl galacturonate) methyl glycoside (98% esterified) the pH optimum was 7.0. Addition of bivalent cations to pectin stimulated the action of the enzyme and shifted the pH optimum to neutrality. The enzymic activity was unaffected by chelating agents, reducing agents, and reagents that converted sulphydryl groups into mercaptides. The gel-filtration characteristics and heat stability of polygalacturonase and polygalacturonate lyase from *A. sojae* have been determined.[1954] The polygalacturonate lyase has been separated by ion-exchange chromatography into adsorbed and non-adsorbed fractions.

A *Bacillus* sp. isolated from water-stored Sitka spruce (*Picea sitchensis*) was found to contain polygalacturonate lyase activity inducible by pectin and its sodium salt.[1747] Polygalacturonate lyase associated with increased permeability of Sitka spruce has been purified from a *Bacillus* sp. by means of ion-exchange chromatography.[1955] The enzyme showed higher activities towards acid-soluble pectin than towards sodium polygalacturonate. Addition of Ca^{2+} ions caused a five-fold increase in activity, whereas Sr^{2+} ions had only a slight activating effect. Most bivalent cations not only inhibited the enzyme's activity in the absence of Ca^{2+} ions, but also suppressed the activating effect of these ions. The enzyme was completely inhibited by H_4edta, but the polygalacturonate lyase showed considerable resistance to inactivation by a range of animal, plant, and bacterial proteases. The action of polygalacturonate lyase from *B. sphaericus* on Vi antigen has been further studied.[681] The enzyme appeared to attack a Vi antigen–protein complex, rather than the free antigen, and did not require an activator when the substrate lacked *O*-acetyl groups.

A partially purified enzyme preparation from the culture fluid of *Bacteroides ruminicola* from sheep rumen was found to act on pectin and polygalacturonic acid to yield unsaturated products.[1956] The enzyme possessed a pH optimum of 8.0—8.5 and was activated by Cu^{2+} ions. Evidence was presented to suggest that the principal enzyme is a polygalacturonate lyase. Polygalacturonate lyase has been detected in strains of *B. ruminicola*, *Streptomyces bovis*, and an unidentified species of rumen bacteria.[1957]

The action pattern of polygalacturonase from *Clostridium felsineum* on flax pectin and the production of the enzyme in filtrates of cultures of the organism have been studied.[1958] Inactivation of the enzyme on cellulose

[1953] S. Ishii and T. Yokotsuka, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 146.
[1954] S. Ishii and Y. Yokotsuka, *J. Agric. Food Chem.*, 1971, **19**, 958.
[1955] O. P. Ward and W. M. Fogarty, *Biochem. J.*, 1972, **128**, 126P.
[1956] M. Wojciechowicz, *Acta Microbiol. Polon., Ser. A*, 1971, **3**, 45.
[1957] M. Wojciechowicz and H. Tomerska, *Acta Microbiol. Polon., Ser. A*, 1971, **3**, 57.
[1958] A. F. Abdel-Fattah, S. S. Mabrouk, and M. Edrees, *J. Gen. Appl. Microbiol.*, 1971, **17**, 421.

paper electrophoresis was attributed to denaturation.[1959] *exo*-Polygalacturonate lyase and pectinesterase from *C. multifermentans* have been purified by gel filtration, when the two activities coincided in a single peak.[1960] The activities were inseparable by ion-exchange chromatography and zonal centrifugation, and it was considered that either the two enzymes form a mutual complex or the same molecule exhibits both activities.

Growth of a fungal plant pathogen (*Colletotrichum lindemuthianum*) in the presence of pectin as the carbon source resulted in selective secretion of *exo*-polygalacturonase,[1516] which was purified by ion-exchange chromatography and gel filtration. Parameters affecting the secretion of the enzyme have been investigated.[1517] Cultures of *C. lindemuthianum* were also induced to secrete an *endo*-polygalacturonase by pectic acid.[1961] The enzyme was purified by various procedures, including affinity chromatography on agarose; this affinity was attributed to the fact that the 3,6-anhydro-D-galactose residues of agarose resemble units in the substrate. The enzyme (molecular weight 7×10^4) was more active against poly(D-galacturonic acid) than pectin and, at a pH optimum of 5, the predominant products from hydrolysis of poly(D-galacturonic acid) were the corresponding di- and tri-saccharides, and small amounts of D-galacturonic acid.

A comparative study of the production of polygalacturonase by eight strains of *Corticum rolfsii* has been reported.[1962] All the enzymes exhibited maximum activity in the pH range 1.5—3.0 and the products of their action on a poly(D-galacturonic acid) identified them as *endo*-polygalacturonases.

The polygalacturonase produced by *Fusarium oxysporum* was separated into two fractions by ion-exchange chromatography;[1963] most of the enzymic activity was not adsorbed by the column. Investigation of its effect upon the viscosity and reducing power of substrate solutions indicated that it is an *endo*-polygalacturonase, although the first detectable product of hydrolysis was D-galacturonic acid. The enzyme was found to contain neutral carbohydrates. The enzyme fraction adsorbed by the ion-exchange column had a lower specific activity and its negative charge was apparently due to complexation with pectin or a component thereof.

Filtrates of cultures of *Helminthosporium sativum*, in which pectin was used as the carbon source, have been shown to contain polygalacturonase and polygalacturonate lyase activities (pH optima 6—7 and 8, respectively).[1873] The activities of the enzymes in the foliage of common and Merion Kentucky bluegrasses infected with, and free from, the organism have been compared. Further studies on the lyase have indicated that it is an *endo*-polygalacturonate lyase.

[1959] A. F. Abdel-Fattah, *Z. Allgem. Mikrobiol.*, 1972, **12**, 3.
[1960] L. Miller and J. D. Macmillan, *J. Bacteriol.*, 1970, **102**, 72.
[1961] P. D. English, A. Maglothin, K. Keegstra, and P. Albersheim, *Plant Physiol.*, 1972, **49**, 293.
[1962] A. Kaji and T. Ohsaki, *J. Agric. Chem. Soc. Japan*, 1971, **45**, 520.
[1963] G. E. Harman and M. E. Corden, *Biochim. Biophys. Acta*, 1972, **264**, 328.

The production of polygalacturonase and polygalacturonate lyase by cultures of *Phoma betae* from the decayed tissues of sugar-beet storage roots has been investigated.[1964] The enzymes were also found in the decayed beet tissue. The production of extracellular pectolytic activity by *P. strasseri* has been studied.[1965] Differential inhibition of pectolytic activity by a range of phenolic compounds suggested that at least two enzymes capable of hydrolysing α-(1 → 4)-linkages of pectic substances are produced by the organism.

Polygalacturonate lyases have been induced in cultures of *Phytophthora palmivora* and *P. parasitica*.[1966] A *Pseudomonas* sp. has been shown to be a better source of polygalacturonate lyase than *Erwinia aroidae*.[1967] The enzymes differed in their characteristics and the former showed maximum activity towards tetramer, followed by trimer, dimer, and polymer substrates. The activity of this enzyme was stimulated by Ca^{2+} ions, but hypoiodite-oxidized and borotritide-reduced, soluble pectic acids were resistant to its action. The end-product of the action of the purified enzyme on oligo- and poly-(D-galacturonic acid) was identified as 4-deoxy-5-keto-D-galacturonic acid.

Polygalacturonase activities have been identified in exudates and sclerotial extracts from *Sclerotinia sclerotiorum*,[1641] and the possible functions of vesicles and crystal-containing microbodies in production of the enzyme have been examined.[1887]

Polygalacturonase from the culture fluids of *Verticillium albo-atrum* has been purified and separated into an *exo-* and an *endo*-polygalacturonase by gel filtration, ion-exchange chromatography, and double isoelectric focusing.[1968] The two fractions were revealed to be homogeneous on polyacrylamide gel electrophoresis, and the *exo*-enzyme (molecular weight 4.4×10^4, p*I* 6.5) possessed pH optima of 4.5 and 5.3 for pectin and its sodium salt, respectively. The *endo*-enzyme (molecular weight 3.45×10^4, p*I* 9.7) possessed corresponding pH optima of 5.0 and 6.0. The enzymes were found to be exceptionally stable on storage in solution.

Pullulanases.—Since limited periodate oxidation of polysaccharide substrates has been shown to prevent attack by *exo-* but not *endo*-acting enzymes, such modified substrates have been advocated to distinguish between pullulanases (*endo*) and *exo*-glucanases.[1454]

The successive and simultaneous actions of pullulanase and α-1,4-glucan: α-1,4-glucan-6-glycosyltransferase on amylose have been examined.[1969] A study of the actions of pullulanase and isoamylase on amylopectins has contributed to the structural analysis of the polysaccharides.[1930]

[1964] W. M. Bugbee, *Canad. J. Bot.*, 1972, **50**, 1705.
[1965] H. A. Melouk and C. E. Horner, *Canad. J. Microbiol.*, 1972, **18**, 1065.
[1966] B. Trique and A. Ravisé, *Compt. rend.*, 1971, **273**, *D*, 1805.
[1967] C. Hatanaka and J. Ozawa, *Agric. and Biol. Chem. (Japan)*, 1971, **35**, 1617.
[1968] H. W. Mussell and B. Strouse, *Canad. J. Biochem.*, 1972, **50**, 625.
[1969] G. S. Drummond, E. E. Smith, and W. J. Whelan, *European J. Biochem.*, 1972, **26**, 168.

The actions of bacterial pullulanase and yeast isoamylase on amylopectin have been compared.[1905] The outermost, inter-chain linkages were hydrolysed with the liberation of linear malto-oligosaccharides having an average DP of fifteen D-glucose units.

The synthesis of pullulanase by a number of strains of *Aerobacter aerogenes* has been studied and precise conditions for optimum production of the enzyme have been reported.[1970] A method has been described for the preparation from *A. aerogenes* of pullulanase free from α-amylase.[1971] Two forms (molecular weights $\lesssim 5 \times 10^5$ and $1.08—1.45 \times 10^5$) of the enzyme were detected and the smaller form was crystallized. The action of the crystalline enzyme was investigated and its rate of action on branched substrates was most rapid when the outer chains had been shortened by prior treatment with α- or β-amylase. Under comparable conditions, the enzyme was inactive against liver glycogen. The action of the crystalline pullulanase from *A. aerogenes* has also been compared with that of isoamylase and significant differences between the two enzymes were apparent.[1929] Whereas the latter enzyme hydrolysed all the branch points of glycogen, the former hydrolysed relatively few. Pullulanase from *A. aerogenes* has been shown by ultracentrifugation and polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate to be a single, polypeptide-chain molecule (molecular weight 1.43×10^5).[1972] Previous reports indicating a lower molecular weight and a subunit structure were explained on the assumption of partial proteolytic degradation of the enzyme. Shape factors for the enzyme were calculated from hydrodynamic data and indicated an oblate, rotational ellipsoid.

An active, insoluble form of pullulanase has been prepared by covalent attachment of the enzyme to an acrylic polymer.[1973]

Trehalases.—Nojirimycin and D-glucono-1,5-lactone have been studied for their inhibitory action on trehalase activity.[1520]

A method for the automated determination of trehalase in homogenates of human small intestinal mucosa is based on dialysis of the reducing sugars released during incubation into alkaline ferricyanide solution.[1522] The trehalase activities in jejunal biopsy specimens from American and South Indian control subjects and patients with tropical sprue have been compared.[1523] The levels and characteristics of human serum trehalase in cases of hepatic diseases have been investigated.[1974]

A membrane fraction of rabbit renal cortex representing brush border membrane together with its associated external coat contained high trehalase activity.[1622] Unlike the α-glucosidase activity of the material, the trehalase was not liberated by treatment with papain.

[1970] A. C. R. Dean, S. C. Gray, G. C. Hope, and I. M. Johnson, *Biochem. J.*, 1972, **126**, 15P.
[1971] C. Mercier, B. M. Frantz, and W. J. Whelan, *European J. Biochem.*, 1972, **26**, 1.
[1972] B. Eisele, I. R. Rasched, and K. Wallenfels, *European J. Biochem.*, 1972, **26**, 62.
[1973] K. Mårtensson and K. Mosbach, *Biotechnol. and Bioeng.*, 1972, **14**, 715.
[1974] J. F. Demelier, M. Massari, J. E. Courtois, and J. Labat, *Clinica Chim. Acta*, 1971, **35**, 335.

The substrate specificities of the trehalases of cockchafer and *Pseudomonas* sp. have been investigated by testing their activity against 6-deoxy-αα-trehalose, 2-*O*-tolyl-*p*-sulphonyl-αα-trehalose, α-D-glucopyranosyl-2,3-anhydro-α-D-mannopyranoside, and 3-azido- and 3-amino-3-deoxy-α-D-altropyranosyl-α-D-glucopyranoside.[1975]

Trehalase has been released from cell-wall preparations of *Neurospora crassa* by the action of snail-gut juice, laminarinase, or chitinase.[1544] The enzyme was not liberated by cellulase, trypsin, and detergents, and it appears that disruption of covalent bonds is necessary for release of the enzyme.

The intracellular trehalase of *Pseudomonas fluorescens* has been extracted and purified.[1976] The enzyme (pH optimum 6.45) was shown to be active against αα-trehalose and 6-deoxy-αα-trehalose only, not to be inhibited by a number of disaccharides, and to be inhibited by K^+ and NH_4^+ ions.

Xylanases.—Sixty-four strains of xylan-decomposing bacteria from Japanese coastal waters, marine sediments, and seaweeds have been investigated and thirty-seven were found to contain both β-(1 → 3)- and β-(1 → 4)-xylan-degrading activities.[1977]

Three hemicellulases produced from *Aspergillus niger* have been purified and were found to hydrolyse xylans and arabinoxylans only.[1863] A study of the mode of action of these enzymes on rice-straw arabinoxylan revealed that two of them produced D-xylose, xylobiose, and xylo-oligosaccharides containing one L-arabinose residue, whereas the third produced L-arabinose, D-xylose, xylobiose, xylotriose, and other xylo-oligosaccharides.

Culturing of a *Bacillus* sp. isolated from water-stored Sitka spruce (*Picea sitchensis*) in the presence of xylan or cellobiose was shown to induce the production of a xylanase.[1747] Inclusion of either cellobiose or xylan in the growth medium of *B. polymyxa* resulted in higher yields of extracellular xylanase.[1978] The purified enzyme exhibited optimum activity at pH 6.5 and a temperature of 45 °C, and was inactivated by Fe^{2+}, Hg^{2+}, and Cu^{2+} ions. Many strains of *B. subtilis* have been found to secrete enzymes that are active against arabinoxylan.[1864] *B. subtilis* has been shown to form a xylanase complex, the production of which does not depend on induction by either xylan or D-xylose.[1979] The enzyme possessed no xylosidase activities.

Carbohydrate Oxidases

Galactose Oxidases.—The stereochemistry of dehydrogenation of the primary alcohol group of D-galactose by galactose oxidase has been

[1975] E. R. Guilloux, J. Defaye, R. H. Bell, and D. Horton, *Carbohydrate Res.*, 1971, **20**, 421.
[1976] E. R. Guilloux, M. A. Arcila, J. E. Courtois, and V. Moumrikoff, *Biochimie*, 1971, **53**, 853.
[1977] H. Fujisawa and M. Murakami, *Bull. Jap. Soc. Sci. Fish.*, 1971, **37**, 119.
[1978] W. M. Fogarty and P. J. Griffin, *Biochem. J.*, 1972, **128**, 125P.
[1979] H. Lyr, *Z. Allgem. Mikrobiol.*, 1972, **12**, 135.

determined.[1980] Using D-[6-^{2}H]galactose and methyl β-D-[6-^{2}H]galactopyranoside, it was established that the reaction involves removal of the 6-*pro-S* hydrogen atom. This conclusion was based on product analysis and the relative effect of the deuterium isotope on the oxidation rates of substrates having different *R*:*S* deuteration patterns. The absolute configurations at C-6 of the substrates were determined by selective chemical transformations to products of known configuration.

Glucose Oxidases.—Use of insolubilized concanavalin A for the purification of glucose oxidase has been reported.[691] The action of nojirimycin and D-glucono-1,5-lactone on glucose oxidase activity has been investigated.[1520]

A method has been described for the isolation of glucose oxidase from *Aspergillus niger* by means of ion-exchange chromatography;[1981] the method is advantageous in that the product contains no detectable catalase or amylase activities. The glycoprotein glucose oxidase (molecular weight 1.6×10^5) from *A. niger* was not reduced in size by guanidine hydrochloride, whereas treatment with 2-mercaptoethanol reduced the two disulphide bonds of the enzyme to yield two smaller species (molecular weights 8.0×10^4).[1982] This and additional data are consistent with a model for glucose oxidase comprising two molecular chains of equal size covalently linked together. The interaction of D-glucal with *A. niger* glucose oxidase has been investigated [1983] and multiple inhibition analysis of the enzyme by D-glucal and halide ions was performed.[1984] The enzyme appeared to be competitively inhibited by chloride ions with respect to the substrate '2-deoxy-D-glucose'.[1985]

Electrochemical measurements have been made with glucose oxidase immobilized in polyacrylamide gel [1986] and the use in an automated assay of glucose oxidase immobilized on nylon has been described.[1987] The pH–activity profiles of glucose oxidase and glucoamylase simultaneously immobilized on Sepharose have been studied.[1778]

Peptidases

Bromelain.—A new technique of linkage analysis has been applied to asparagine oligosaccharides from pineapple bromelain.[652] Alkaline borohydride treatment of the oligosaccharides resulted in complete cleavage of the glycopeptide bond, with the concomitant production of terminal 2-amino-2-deoxy-D-glucitol. Periodate oxidation, borohydride reduction, and hydrolysis then gave rise to 2-amino-2-deoxy-D-xylitol, which is indicative of the presence of a 4-substituted 2-amino-2-deoxy-D-glucose

[1980] A. Maradufu, G. M. Cree, and A. S. Perlin, *Canad. J. Chem.*, 1971, **49**, 3429.
[1981] J. Kucera and J. Hanus, *J. Appl. Chem. Biotechnol.*, 1972, **21**, 347.
[1982] J. J. O'Malley and J. L. Weaver, *Biochemistry*, 1972, **11**, 3527.
[1983] M. J. Rogers and K. G. Brandt, *Biochemistry*, 1971, **10**, 4624.
[1984] M. J. Rogers and K. G. Brandt, *Biochemistry*, 1971, **10**, 4636.
[1985] M. J. Rogers and K. G. Brandt, *Biochemistry*, 1971, **10**, 4630.
[1986] L. B. Wingard, C. C. Liu, and N. L. Nagda, *Biotechnol. and Bioeng.*, 1971, **13**, 629.
[1987] D. J. Inman and W. E. Hornby, *Biochem. J.*, 1972, **129**, 255.

residue. The penultimate 2-amino-2-deoxy-D-glucose residue was found by the same technique to be substituted at O-4 and methylation analysis confirmed this conclusion. On the basis of these results, it was proposed that the glycopeptide involves structure (101) and that this is a common

$$\beta\text{-D-Man}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{NAc-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{NAc} \rightarrow \text{Asn}$$
(101)

structural unit in asparagine oligosaccharides from chicken ovalbumin, *Aspergillus oryzae* α-amylase, and bromelain.

Oxytocinase.—Treatment of oxytocinase (cystine aminopeptidase) with neuraminidase resulted in complete removal of the sialic acid residues.[1988] The K_m value of the modified enzyme increased in the pH interval 6—8 and a change in its properties was observed at pH values where conformational changes are normally observed with oxytocinase.

Thrombins.—Two major classes of thrombin (molecular weights 3.9 and 2.8×10^4) have been resolved by ion-exchange chromatography [1989] and molecular weights were reported for the component chains identified. Human thrombin apparently consists of three chains (molecular weights 6, 6, and 15×10^3) with different charges.[1990]

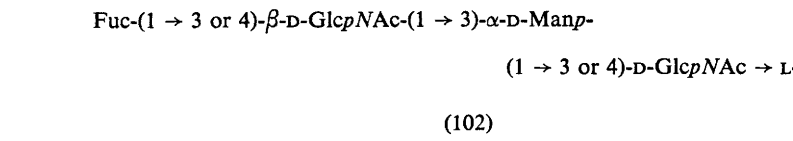
Purification of bovine thrombin by affinity chromatography on a derivative of agarose containing 4-chlorobenzylamido-ligands has been described.[1991] Thrombin was eluted from the adsorbent with benzamide solution and was recovered by gel filtration. Bovine thrombin was found to possess a molecular weight of 3.41×10^4 and the *S*-carboxymethylated derivative to lack clotting and esterase activities.[1992] The different effects of reaction with tetranitromethane on the proteinase and esterase activities of bovine thrombin have been investigated.[1993] The conversion of prothrombin into thrombin in biological anion and proteolytic activation systems has been explored using a water-insoluble derivative of native bovine prothrombin.[1241] Further studies on the mechanism of production of bovine thrombin from prothrombin have been reported.[1237]

Ribonucleases and Deoxyribonucleases

The D-mannopyranosyl residue of an L-asparaginyl glycopeptide obtained from ribonuclease, *viz.* *O*-β-D-mannopyranosyl-(1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine, was liberated by β-mannosidases, but not by α-mannosidases.[655] Three tryptic glycopeptide fractions (I—III) representative of

[1988] T. Barth, L. Yman, B. Kulling, and J. Barthova, *Coll. Czech. Chem. Comm.*, 1971, **36**, 2316.
[1989] K. G. Mann, C. M. Heldebrant, and D. N. Fass, *J. Biol. Chem.*, 1971, **246**, 5994.
[1990] T. M. Chulkova and V. N. Orekhovich, *Clinica Chim. Acta*, 1971, **34**, 493.
[1991] A. R. Thompson and E. W. Davie, *Biochim. Biophys. Acta*, 1971, **250**, 210.
[1992] I. P. Baskova, S. M. Strukova, V. L. Glotova, V. Y. Chernyak, B. A. Kudryashov, and V. P. Korzhenko, *Biokhimiya*, 1971, **36**, 1245.
[1993] R. L. Lundblad and J. H. Harrison, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 1344.

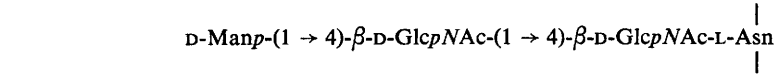
the carbohydrate moieties attached to L-asparagines-21, -34, and -76, respectively, in porcine pancreatic ribonuclease have been subjected to degradation by glycosidases and periodate.[1994] Of the six D-mannose residues in fraction II, four were found to be α-linked and two to be β-linked. These types of residue occur to mutual exclusion in separate side-chains attached to a 2-acetamido-2-deoxy-D-glucose disaccharide core-unit that is linked glycosidically to L-asparagine. The successive actions of glycosidases on fractions I and III yielded heptasaccharide-glycopeptides, the carbohydrate moieties of which were identical and comprised D-mannose (3), fucose (1), and 2-amino-2-deoxy-D-glucose (3 units). Further degradation of the heptasaccharide-glycopeptides yielded tetrasaccharide-glycopeptides possessing the structure (102). The heterogeneity of fractions I and III was



(102)

attributed to variations in the extent to which the common heptasaccharide core moiety is substituted with side-chains containing β-(1 → 6)-linked 2-acetamido-2-deoxy-D-glucose units and terminating in neuraminyl-galactosyl units. The results suggested that differentiation of the two types of heterosaccharide side-chains during biosynthesis of the enzyme occurs as the second carbohydrate residue is attached to a 2-acetamido-1-{(*N*-β-L-aspartyl)amino}2-deoxy-β-D-glucopyranosylamine unit in the peptide chain.

An amino-acid analyser adapted to monitor the extent and type of digestion of L-asparaginyl glycopeptides by glycosidases has been used in structural studies of bovine deoxyribonuclease A.[654] A glycopeptide arising from pronasic digestion of this enzyme was shown to be similar to that derived from ribonuclease and ovalbumin, and the carbohydrate moieties of these glycopeptides appear to have the structure (103).



(103)

Miscellaneous Enzymes

***N*-Acetylneuraminate Lyases.**—Extracellular levels of *N*-acetylneuraminate lyase produced by *Pasteurella multocida* were shown to be greater than intracellular levels.[1677]

Carbohydrate Isomerases.—An automated procedure for determining glucose isomerase activity in extracts of micro-organisms is based on

[1994] I. Kabasawa and C. H. W. Hirs, *J. Biol. Chem.*, 1972, **247**, 1610.

colorimetric determination of D-fructose by a cysteine–carbazole–sulphuric acid reagent.[1995]

Glucose isomerase has been insolubilized by reaction with a diazotized arylamino-derivative of glass.[1996]

Ceruloplasmins.—The serum levels of ceruloplasmin in normal children and adults have been determined [1155] and the corresponding serum levels for normals, pathological controls, and cases of malignancy have been compared.[1156] The electrophoretic mobility of human ceruloplasmin has been measured in twenty types of polyacrylamide gels.[1186] Evidence indicating a generalized role for the terminal sialic acid residues of circulating glycoproteins has been reported, since on injection into rats, desialylized human ceruloplasmin was promptly removed from circulation and was recovered in the liver.[1096]

Polymorphism of ceruloplasmin of astrakhan sheep has been investigated.[1997]

α-Glucan Phosphorylases.—The enigmatic hot-springs alga *Cyanidium caldarium* has been shown to possess one type of α-glucan phosphorylase (glycogen phosphorylase), which appears to be identical with that of blue-green (*Oscillatoria princeps*) and red (*Rhodymenia pertusa*) algae.[1998] The enzyme is a glycoprotein and is capable of initiating polyglucoside synthesis from D-glucose 1-phosphate without the addition of primer molecules.

Gluconolactonases.—*Escherichia coli* has been shown to contain a gluconolactonase.[1999] An assay for the enzyme was developed using a spectral-change pH indicator during hydrolysis of D-glucono-1,4-lactone. The enzyme has been purified and its substrate specificity investigated; it was inactivated by H_4edta, but not by 4-chloromercuribenzoate.

The presence of gluconolactonase has been recognized in *Saccharomyces cerevisiae* and extraction, purification, and characterization of the enzyme have been described.[2000]

Glycopeptide Linkage Hydrolases.—An enzyme that hydrolysed synthetic 2-acetamido-1-{(*N*-β-L-aspartyl)amino}-2-deoxy-β-D-glucopyranosylamine has been extracted from porcine kidney and purified to physicochemical homogeneity.[2001] Besides the principal enzyme molecule (molecular weight 7×10^4), two other forms of the enzyme were shown to be present. Analysis of the products of enzyme action at alkaline pH revealed that 2-acetamido-1-amino-1,2-dideoxy-D-glucose is formed, indicating that

[1995] N. E. Lloyd, K. Khaleeluddin, and W. R. Lamm, *Cereal Chem.*, 1972, **49**, 544.
[1996] G. W. Strandberg and K. L. Smiley, *Biotechnol. and Bioeng.*, 1972, **14**, 509.
[1997] N. K. Mekhtiev, I. P. Shalimov, and M. A. Risk, *Doklady Akad. Nauk S.S.S.R.*, 1971, **198**, 457.
[1998] J. F. Fredrick, *Phytochemistry*, 1972, **11**, 3259.
[1999] F. Hucho and K. Wallenfels, *Biochim. Biophys. Acta*, 1972, **276**, 176.
[2000] A. Constantinides, S. J. Myles, and W. R. Vieth, *Enzymologia*, 1972, **43**, 121.
[2001] M. Kohno and I. Yamashina, *Biochim. Biophys. Acta*, 1972, **258**, 600.

the enzyme is an amidase, namely β-acetamidodeoxyglucosylamine-L-asparagine amidase.

An enzyme preparation from *Bacillus cereus* appeared to contain an *N*-acetylmuramyl-L-alanine amidase.[2002] This assignment was supported by the fact that fragments containing muramic acid and 2-amino-2-deoxy-D-glucose were isolated from *B. cereus* and *Micrococcus lysodeikticus* cell walls after digestion with the enzyme. The insoluble cell walls appeared to be the only substrate and attempts to prepare soluble substrates were unsuccessful. The purification of *N*-acetylmuramyl-L-alanine amidase from *B. megaterium* has been described [2003] and the *N*-acetylmuramyl-L-alanine amidase of *Escherichia coli* has been studied.[2004]

α-Lactalbumins.—The interaction of α-lactalbumin with tris(hydroxymethyl)methylamine and other cationic hydroxyamines has been investigated.[2005] Relative effects of primary and tertiary structures on helix formation in α-lactalbumin have been assessed and compared with analogous effects for lysozyme.[2006] The disulphide groups of α-lactalbumin appeared to be more accessible for reduction than those of lysozyme [1817] and crystal-structure data have been obtained for 'enzyme–substrate' complexes of lysozyme and α-lactalbumin.[1783]

Human-milk α-lactalbumin has been purified.[1793] Diffusion and sedimentation experiments showed that the hydrodynamic shapes and molecular weights of α-lactalbumin and human-milk and urinary lysozymes are strikingly similar. Other physicochemical properties of the molecules were compared and it appeared that α-lactalbumin displays less α-helix than lysozyme. C.d. spectral data have been obtained for human, bovine, camel, and guinea-pig α-lactalbumins, and these data have been compared with those for lysozymes from a variety of sources.[1800]

The α-lactalbumin component of bovine lactose synthetase has been shown to form an equimolar complex with a second component of the enzyme, *viz.* a galactosyltransferase (A protein).[2007] However, the complex was stable only in the presence of one of the enzyme's substrates such as UDP-D-galactose or 2-acetamido-2-deoxy-D-glucose. The effect of derivatization of α-lactalbumin on complex formation was investigated. The extrinsic fluorescence of a bound dye {2-(4-toluidinyl)naphthalene 6-sulphonate} has been utilized to study the hydrophobic sites on the surfaces of bovine α-lactalbumin and lysozyme;[1822] the binding site on α-lactalbumin was found to be more hydrophobic. The effects of modifying bovine α-lactalbumin on its function and conformation have been investigated,[2008] and the immunological reactivities of bovine α-lactalbumin and several lysozymes have been compared.[1847]

[2002] S. Csuzi, *Acta Biochim. Biophys. Acad. Sci. Hung.*, 1970, **5**, 375.
[2003] L. Chan and L. Glaser, *J. Biol. Chem.*, 1972, **247**, 5391.
[2004] Y. Van Heijenoort and J. Van Heijenoort, *F.E.B.S. Letters*, 1971, **15**, 137.
[2005] A. B. Rawitch and M. Gleason, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 590.
[2006] J. Hermans and D. Puett, *Biopolymers*, 1971, **10**, 895.
[2007] W. A. Klee and C. B. Klee, *J. Biol. Chem.*, 1972, **247**, 2336.
[2008] T. E. Barman and W. Bagshaw, *Biochim. Biophys. Acta*, 1972, **278**, 491.

The *in vitro* synthesis of α-lactalbumin by microsomes and bound polyribosomes from the mammary gland of lactating sheep has been studied.[1149]

α-Lactalbumin covalently coupled to acrylamide copolymers has been used in a specific assay for serum anti-α-lactalbumin.[1195] Other covalent, water-insoluble derivatives of α-lactalbumin have been used as affinity-chromatography matrices.[2009–2012]

Lactamases.—A crystalline preparation of β-lactamase II from *Bacillus cereus* has been separated into two fractions by gel filtration in a complex buffer system containing Zn^{2+} ions;[2013] separation caused partial loss of activity that was restored on recombination of the fractions. One of the fractions was inactive enzymically, but contained carbohydrate including 2-amino-2-deoxygalactose (8) and 2-amino-2-deoxyglucose (18 residues per molecular weight of 1.3×10^4).

Lactose Synthetases.—The galactosyltransferase (A protein) component of lactose synthetase has been purified by affinity chromatography on insoluble derivatives of α-lactalbumin.[2009–2012] The galactosyltransferase was specifically bound to the matrices in buffers containing either D-glucose [2009, 2011] or 2-acetamido-2-deoxy-D-glucose,[2010] and could be eluted by omitting these carbohydrates from the buffers. The product was easily inactivated, but could be stabilized with 2-acetamido-2-deoxy-D-glucose or bovine serum albumin.[2011] The transferase (molecular weight 4.2×10^4) contained 12.3% carbohydrate comprising neutral sugars (19), 2-amino-2-deoxygalactose (3), 2-amino-2-deoxyglucose (3), and sialic acid (3 residues per mole). The enzyme was shown to catalyse the transfer of D-galactosyl units from UDP-D-galactose to 2-acetamido-2-deoxy-D-glucose to form *O*-β-D-galactopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose (*N*-acetyl-lactosamine); in the presence of α-lactalbumin, this reaction was partially inhibited, the transfer to D-glucose to form *O*-β-D-galactopyranosyl-(1 → 4)-D-glucose (lactose) being favoured.[2010]

The galactosyltransferase component of bovine lactose synthetase has been purified from skim milk until free from contaminating protein.[2007] The molecule (molecular weight 4.4×10^4) was shown to be composed of a single 3.2S chain containing 5% neutral carbohydrate and sialic acid (2 residues per mole). The enzyme behaved on polyacrylamide gel electrophoresis as a family of related components, all of which were enzymically active and contained carbohydrate. The galactosyltransferase formed a 1:1 complex with the other component (α-lactalbumin) of lactose synthetase, but this complex was stable only in the presence of such substrates as UDP-D-galactose and 2-acetamido-2-deoxy-D-glucose.

[2009] I. P. Trayer, P. Mattock, and R. L. Hill, *Fed. Proc.*, 1970, **29**, 597.
[2010] P. Andrews, *F.E.B.S. Letters*, 1970, **9**, 297.
[2011] I. P. Trayer and R. L. Hill, *J. Biol. Chem.*, 1971, **246**, 6666.
[2012] I. P. Trayer and K. W. Olsen, *Biochem. J.*, 1972, **127**, 9P.
[2013] S. Kuwabara and P. H. Lloyd, *Biochem. J.*, 1971, **124**, 215.

Lipases.—Porcine pancreatic lipase has been purified to homogeneity by gel filtration, ion-exchange chromatography, and fractionation on insolubilized concanavalin A.[2014] Two isoenzymes were separated by polyacrylamide gel electrophoresis and both contained carbohydrate comprised of mannose (3.8) and 2-acetamido-2-deoxyglucose (2.9 moles per mole).

Long-chain fatty acids were liberated from bacterial lipopolysaccharides by extracts from amoebae of *Dictyostelium discoideum*.[2015]

Luciferases.—A purified form of a low molecular weight luciferase has been obtained from the sea pansy (*Renilla reniformis*).[2016] Experiments with dissociating reagents suggested the existence of a single subunit (molecular weight 1.2×10^4) containing 2-amino-2-deoxyglucose (2 residues). The native enzyme exists as an equilibrium mixture of this subunit and higher molecular weight species, and gel-filtration studies indicated that the active species is either a dimer or a trimer.

Neuraminate Transforming Enzyme.—A factor has been detected in animal tissues that caused sialyl residues of sialoglycoproteins to react as free sialic acid in the Warren assay.[1380] The active, particulate fraction from homogenized rabbit-uterine endometrium and myometrium was devoid of neuraminidase. The factor has also been detected in other rabbit tissues and in Cowper's gland of boar. Investigation of the properties of the enzyme showed that it did not liberate free neuraminic acid from the substrate and it appeared to render the C-4—C-5 bond of neuraminic acid susceptible to oxidation by periodate. (This enzyme may be an acylase, since deacetylation of *N*-acetylneuraminic acid would yield a periodate-susceptible β-amino-alcohol.)

5′-Nucleotidases.—During the isolation of glycoproteins from porcine lymphocyte plasma membranes, 5′-nucleotidase activity was found consistently to be associated with carbohydrate and the possibility exists that the enzyme is a glycoprotein.[736]

Pectinesterases.—Pectinesterase activity of the avocado (*Persea americana*) fruit has been determined at various stages of development.[1949] Changes in the pectinesterase activity in four varieties of Iraqi date have been followed during maturation.[2017] Four forms of pectinesterase have been identified in extracts of tomatoes by ion-exchange chromatography[2018] and their presence in a number of varieties of tomatoes at different stages of ripening have been investigated.

The behaviour of pectinesterase from *Aspergillus sojae* on gel filtration and heat treatment has been examined.[1954] Pectinesterase activity has been

[2014] C. W. Garner and L. C. Smith, *J. Biol. Chem.*, 1972, **247**, 561.
[2015] V. N. Nigam, D. Malchow, and E. T. Rietschel, *Z. physiol. Chem.*, 1970, **351**, 1123.
[2016] Y. D. Karkhanis and M. J. Cormier, *Biochemistry*, 1971, **10**, 317.
[2017] H. A. Al-Jasim and K. S. Al-Delaimy, *J. Sci. Food Agric.*, 1972, **23**, 915.
[2018] R. Pressey and J. K. Avants, *Phytochemistry*, 1972, **11**, 3139.

detected in strains of *Bacteroides ruminicola* and in another unidentified species of rumen bacteria.[1957]

Conditions for the determination of pectinesterase in acidic culture media of *Clostridium felsineum* have been investigated.[1958] Inactivation of pectinesterase from this organism on cellulose paper electrophoresis was attributed to denaturation.[1959] Pectinesterase has been purified from *C. multifermentans* by means of gel filtration, but the activity was found to coincide with an *exo*-polygalacturonate lyase.[1960] This coincidence was also maintained after ion-exchange chromatography and zonal centrifugation, and it was concluded that either the two enzymes are complexed or both activities are manifested by a single molecule. The pectinesterase activity could be selectively removed by heat treatment.

Pectinesterase has been purified from culture filtrates of *Fusarium oxysporum* by ion-exchange chromatography and gel filtration.[2019] The action pattern of the enzyme (molecular weight 3.5×10^4) was shown to be similar to that of tomato pectinesterase.

Extracts of the foliage from common and Merion Kentucky bluegrasses infected with *Helminthosporium sativum* exhibited pectinesterase activity (pH optimum 7).[1873]

Phosphatases.—The acid phosphatase of human prostate has been separated into two isoenzymes by gel filtration and ion-exchange chromatography.[2020] The two isoenzymes differed in the contents of *N*-acetylneuraminic acid (6.4 and 9.3 moles per mole) and treatment with neuraminidase resulted in the formation of molecular species with a number of common physicochemical properties. The activity of the desialylized molecules was investigated.

The biosynthesis of a glycoprotein acid phosphatase by protoplasts of a *Saccharomyces* sp. was inhibited by '2-deoxy-D-glucose'.[1551]

Investigation of the effect of desialization on the properties of a variant alkaline phosphatase in human hepatoma led to the conclusion that the neuraminic acid units are responsible for the speed of anodal migration of the enzyme, but not for other properties.[2021] Desialization of liver alkaline phosphatase, which contains a greater amount of neuraminic acid, caused it to become heat labile and it seems that neuraminic acid residues are involved in maintenance of the heat stability of the enzyme.

The isolation and purification of alkaline phosphatase from rat intestine has been described.[2022] Three isoenzymes separated from the enzyme differed in their substrate specificities and each consisted of a set of glycoproteins.

Polyadenosine Diphosphate–Ribose Glycohydrolase.—A polyadenosine diphosphate-D-ribose glycohydrolase has been discovered in, and was

[2019] L. Miller and J. D. Macmillan, *Biochemistry*, 1971, **10**, 570.
[2020] E. Dziembor, J. Gryszkiewicz, and W. Ostrowski, *Acta Biochim. Polon.*, 1971, **18**, 419.
[2021] K. Higashino, M. Hashinotsume, and Y. Yamamura, *Clinica Chim. Acta*, 1972, **40**, 305.
[2022] P. K. Saini and J. Done, *Biochim. Biophys. Acta*, 1972, **258**, 147.

partially purified from, rat liver nuclei.[2023] The enzyme was able to catalyse hydrolytic cleavage of D-ribosyl-(1 → 2)-D-ribose bonds between neighbouring adenosine diphosphate–D-ribose units, but not that of the linkage bonding the polymer to protein. The activity was specifically inhibited by adenosine 3′,5′-cyclic phosphate.

Sulphamidases.—Treatment of various rat tissues with Triton X-100 released a sulphamidase that was active against heparin containing [^{35}S]sulphamido-groups.[2024] The sulphamidase activity was separated from two aryl sulphatases also present in the tissues by isoelectric focusing. It was concluded that sulphamidase activity is widely distributed and is manifested by a molecule different from those with sulphatase activities.

Sulphatases.—Procedures for the determination of cerebroside sulphatase (cerebroside sulphate sulphohydrolase, sulphatide sulphohydrolase) have been based on the release of radioactive sulphate from cerebroside [^{35}S]sulphate.[2025, 2026]

Sulphatases released from rat tissues by Triton X-100 have been separated into two fractions by isoelectric focusing.[2024] The enzymes (aryl sulphatases) were active against nitrocatechol sulphate, but differed from a sulphamidase obtained from the same source.

A chondrosulphatase has been purified from liver extracts of the squid (*Ommastrephes sloani pacifus*) and characterized.[2027] Unlike the chondrosulphatases of *Proteus vulgaris*, which attack only disaccharide sulphates, the squid enzyme is capable of desulphating chondroitin sulphates (principally at O-4) without prior depolymerization. Chondroitin 4-sulphate was hydrolysed at a rate greater than chondroitin 4,6-disulphate, chondroitin 6-sulphate was desulphated to a slight extent, but the enzyme was inactive against dermatan sulphate, keratan sulphate, and heparin. The enzyme was also active against 2-acetamido-2-deoxy-3-*O*-(4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose 4-sulphate, but the isomeric 6-sulphate was hydrolysed only slightly.

A sulphatase from *Flavobacterium heparinum*, which was active against heparin, has been separated from heparin lyase and α-glycuronidase present in the same organism.[1657]

Thioglucosidases.—The effect of the glucosidase inhibitors nojirimycin and D-glucono-1,5-lactone on thioglucosidase (myrosinase) has been investigated.[1520]

[2023] K. Ueda, J. Oka, S. Narumiya, N. Miyakawa, and O. Hayaishi, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 516.
[2024] Y. Friedman and C. Arsenis, *Biochem. Biophys. Res. Comm.*, 1972, **48**, 1133.
[2025] M. T. Porter, A. L. Fluharty, S. D. de la Flor, and H. Kihara, *Biochim. Biophys. Acta*, 1972, **258**, 769.
[2026] A. K. Percy, D. F. Farrell, and M. M. Kaback, *J. Neurochem.*, 1972, **19**, 233.
[2027] K. Atsumi, Y. Kawai, N. Seno, and K. Anno, *Biochem. J.*, 1972, **128**, 983.

Index of Enzymes Referred to in Chapter 6*

Trivial name	*Systematic name*	*E.C. No.*	*Page*
α-Acetamidodeoxy-galactosidase	α-2-acetamido-2-deoxy-D-galactoside acetamidodeoxygalactohydrolase		395
β-Acetamidodeoxy-galactosidase	β-2-acetamido-2-deoxy-D-galactoside acetamidodeoxygalactohydrolase		395
α-Acetamidodeoxy-glucosidase	α-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase		395
β-Acetamidodeoxy-glucosidase	β-2-acetamido-2-deoxy-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.30	395
β-Acetamidodeoxy-glucosylamine-asparagine amidase			485
β-Acetamidodeoxy-hexosidase	β-2-acetamido-2-deoxyhexoside acetamidodeoxyhexohydrolase		395
N-Acetylmuramyl-alanine amidase			485
N-Acetylneuraminate lyase	*N*-acetylneuraminate pyruvate-lyase	4.1.3.3	483
Acid phosphatase	orthophosphoric monoester phosphohydrolase	3.1.3.2	488
Agarase			457
Alginase	poly-β-1,4-mannuronide glycano-hydrolase	3.2.1.16	458
Alginate lyase	poly-β-1,4-mannuronide lyase	4.2.99.4	458
Alkaline phosphatase	orthophosphoric monoester phosphorylase	3.1.3.1	488
Amidase			484
α-Amylase	α-1,4-glucan 4-glucanohydrolase	3.2.1.1	430
β-Amylase	β-1,4-glucan maltohydrolase	3.2.1.2	440
Amylopectin-1,6-glucosidase	amylopectin 6-glucanohydrolase	3.2.1.9	458
Arabinanase			457
α-Arabinofuranosidase			401
α-Arabinopyranosidase			401
α-Arabinosidase			401
Arylsulphatase	aryl-sulphate sulphohydrolase	3.1.6.1	489
Bromelain		3.4.4.24	481
Carbohydrate isomerases			483
Cellulase	β-1,4-glucan 4-glucanohydrolase	3.2.1.4	458
Cerebroside sulphatase			489
Ceruloplasmin			484
Chitinase	poly-β-1,4-2-acetamido-2-deoxy-D-glucoside glycanohydrolase	3.2.1.14	461
Chondroitin sulphate hydrolase	chondroitin sulphate glycano-hydrolase	3.2.1.34	462
Chondroitin sulphate lyase	chondroitin sulphate lyase	4.2.99.6	462
Chondrosulphatase	chondroitin sulphate sulpho-hydrolase	3.1.6.4	489
Cystine aminopeptidase			482

* See Introduction (Part II, Chapter 1).

Trivial name	*Systematic name*	*E.C. No.*	*Page*
Deoxyribonuclease	deoxyribonuclease oligonucleotidohydrolase	3.1.4.5	482
Dermatan sulphate hydrolase			463
Dermatan sulphate lyase			463
Dextranase	α-1,6-glucan 6-glucanohydrolase	3.2.1.11	463
Dextrin-1,6-glucosidase	dextrin 6-glucanohydrolase	3.2.1.33	464
β-Fructofuranosidase	β-D-fructofuranoside fructohydrolase	3.2.1.26	402
α-Fucosidase	α-L-fucoside fucohydrolase		405
β-Fucosidase	β-D-fucoside fucohydrolase	3.2.1.38	405
β-Fucosidase	β-L-fucoside fucohydrolase		405
Galactanase			464
Galactose oxidase	D-galactose:oxygen oxidoreductase	1.1.3.9	480
α-Galactosidase	α-D-galactoside galactohydrolase	3.2.1.22	406
β-Galactosidase	β-D-galactoside galactohydrolase	3.2.1.23	406
α-Glucan phosphorylase	α-1,4-glucan:orthophosphate glucosyltransferase	2.4.1.1	484
Glucanase			465
Glucoamylase	α-1,4-glucan glucohydrolase	3.2.1.3	442
Gluconolactonase	D-glucono-δ-lactone hydrolase	3.1.1.17	484
Glucose isomerase			483
Glucose oxidase	β-D-glucose:oxygen oxidoreductase	1.1.3.4	481
α-Glucosidase	α-D-glucoside glucohydrolase	3.2.1.20	414
β-Glucosidase	β-D-glucoside glucohydrolase	3.2.1.21	414
α-Glucuronidase	α-D-glucuronide glucuronohydrolase		420
β-Glucuronidase	β-D-glucuronide glucuronohydrolase	3.2.1.31	420
Glycopeptide linkage hydrolase			484
Glycosaminoglycuronan hydrolase			467
α-Glycuronidase			422
Heparan sulphate lyase			468
Heparin hydrolase			468
Heparin lyase			468
Hyaluronate lyase	hyaluronate lyase	4.2.99.1	468
Hyaluronidase	hyaluronate glycanohydrolase	3.2.1.35	468
α-Iduronidase	α-L-iduronide iduronohydrolase		423
Inulase	β-2,1-fructan fructanohydrolase	3.2.1.7	470
Isoamylase			470
α-Lactalbumin			485
β-Lactamase			486
Lactose synthetase			486
Laminarinase	β-1,3(4)-glucan glucanohydrolase	3.2.1.6	470
Limit dextrinase			472
Lipase	glycerol-ester hydrolase	3.1.1.3	487
Luciferase			487
Luteanase			472
Lysozyme	mucopeptide *N*-acetylmuramyl-hydrolase	3.2.1.17	444
Mannanase			473
α-Mannosidase	α-D-mannoside mannohydrolase	3.2.1.24	423
β-Mannosidase	β-D-mannoside mannohydrolase	3.2.1.25	423
Mycodextranase			473

Trivial name	*Systematic name*	*E.C. No.*	*Page*
Neuraminate transforming enzyme			487
Neuraminidase	mucopolysaccharide *N*-acetylneuraminyl hydrolase	3.2.1.18	426
5′-Nucleotidase	5′-ribonucleotide phosphohydrolase	3.1.3.5	487
Oligo-1,3-glucosidase	β-1,3-glucan glucanohydrolase	3.2.1.39	474
Oligo-1,6-glucosidase	oligodextrin 6-glucanohydrolase	3.2.1.10	474
Oxytocinase			482
Pectinesterase	pectin pectyl hydrolase	3.1.1.11	487
Peptidase			481
Phosphatase			488
Polyadenosine diphosphate-riboglycohydrolase			488
Polygalacturonase	poly-α-1,4-galacturonide glycanohydrolase	3.2.1.15	475
Polygalacturonate lyase	poly-α-1,4-D-galacturonide lyase	4.2.99.3	475
Pullulanase			478
Sialidase*			426
Sulphamidase			489
Sulphatase			489
Sulphatide sulphatase			489
Thioglucosidase	thioglucoside glucohydrolase	3.2.3.1	489
α-L-*threo*-Hex-4-enopyranosyluronidase			422
Thrombin		3.4.4.13	482
Trehalase	αα′-glucoside 1-glucohydrolase	3.2.1.28	479
Xylanase	β-1,3-xylan xylanohydrolase	3.2.1.32	480
Xylanase	β-1,4-xylan xylanohydrolase	3.2.1.8	480
α-Xylosidase	α-D-xyloside xylohydrolase		429
β-Xylosidase	β-D-xyloside xylohydrolase	3.2.1.37	429

* Sialidase is used generally to include neuraminidase and unspecified neuraminate glycohydrolases.

7
Glycolipids and Gangliosides

A number of reviews have appeared covering various aspects of glycolipids and gangliosides as follows: the occurrence, structure, and nomenclature of glycolipids from animals, plants, and micro-organisms;[2028] the isolation, separation, and synthesis of cerebrosides;[2029] the isolation, separation, and identification of gangliosides, together with tabulation of the coding systems used for abbreviation of ganglioside structures;[2030] the structures and functions of glycolipids as surface components of mammalian cells;[635] the morphology of glycolipids in the coats of mammalian cells;[633] and the distribution and structure of glycerylphosphoryldiglucosyldiglyceride in bacteria.[2031] Further reviews recently published have dealt with biosynthetic aspects of glycolipids and gangliosides as follows: biosynthesis of glycolipids (particularly sphingolipids and glycerolipids) from glycopyranosyl esters of nucleoside pyrophosphates ('sugar nucleotides');[659] recent advances in the biosynthesis of glycophospholipids;[812] the biosynthetic and metabolic regulation of sphingolipids not containing sialic acid;[2032] and the role of glycolipids in the biosynthesis of bacterial-cell envelopes.[2033] Some observations on the relationship between the biosyntheses of glycolipids and glycoproteins have been recorded.[662]

A system reported for the separation of neutral glycosphingolipids involved high-pressure liquid chromatography of the compounds as their benzoylated derivatives.[2034] Serum mono-, di-, tri-, and tetra-glycosylceramides were shown to be quickly and completely resolved by the system. A combination of t.l.c. (for the separation of glycolipids from animal tissues) and the orcinol reaction (carried out in the presence of silica gel) makes prior separation of the glycolipids by column chromatography unnecessary.[2035] The method is applicable to the determination of cerebrosides, sulphatides, and monogalactosyldiglyceride in 50 mg of

[2028] J. M. McKibbin, in 'The Carbohydrates', ed. W. Pigman and D. Horton, Academic Press, New York, 1972, Vol. IIB, p. 711.
[2029] H. M. Flowers, *Methods Carbohydrate Chem.*, 1972, **6**, 459.
[2030] L. Svennerholm, *Methods Carbohydrate Chem.*, 1972, **6**, 464.
[2031] N. Shaw and A. Stead, *F.E.B.S. Letters*, 1972, **21**, 249.
[2032] P. Morell and P. Braun, *J. Lipid Res.*, 1972, **13**, 293.
[2033] L. Rothfield and D. Romeo, *Bacteriol. Rev.*, 1972, **35**, 14.
[2034] J. E. Evans and R. H. McCluer, *Biochim. Biophys. Acta*, 1972, **270**, 565.
[2035] N. Neskovic, L. Sarlieve, J. L. Nussbaum, D. Kostic, and P. Mandel, *Clinica Chim. Acta*, 1972, **38**, 147.

brain tissue. A modified procedure has been established for the determination of glycolipids in liver samples[2035] and procedures have been described for the determination of gangliosides, glycoproteins, and glycosaminoglycans in brain tissues.[646] These procedures have been applied to human normal and pathological samples.

A quantitative gas–liquid chromatographic method has been developed for the analysis of carbohydrates.[642] The method involved the conversion of free or bound monosaccharides into methyl glycosides and analysis of the glycosides as their trifluoroacetyl derivatives. The method has been applied to glycolipids with the result that all the carbohydrate units known to occur could be determined satisfactorily.

The mass spectra of the trimethylsilyl derivatives of a series of homologous cerebrosides, having a β-D-galactopyranose unit linked to different ceramides, have been recorded.[2036] Molecular ions were observed for all the compounds tested and the composition of a number of fragments was established by high-resolution analysis.

The biological characterization of diumycin, a phosphorus-containing glycolipid antibiotic, has been reported.[2037]

Neuraminidase has been shown to act on the protagon–tetanus toxin complex to liberate toxin and sialic acid.[2038] Since the amounts of sialic acid and toxin released were found to be directly related, it was concluded that the bond between the tetanus toxin and its receptor involves a ganglioside sialic acid group.

Animal Glycolipids and Gangliosides

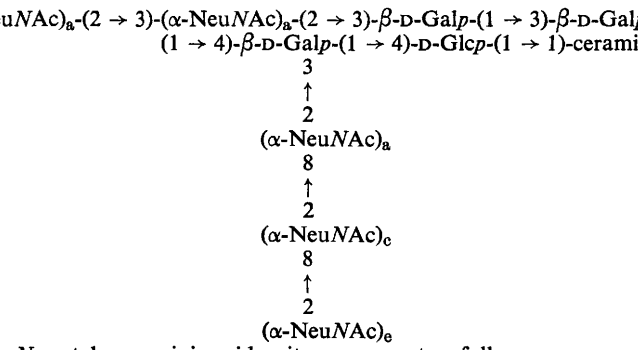
The chemical, metabolic, and organizational differences in glycolipids of normal and tumorous cell surfaces have been outlined.[2039] The contents of sialic acid and ganglioside of some tumours have been investigated.[2040]

The glycolipid composition of tissues, *etc.* from a patient with a neurovisceral lipid-storage disease, characterized by a lactosylceramide galactosyl hydrolase deficiency, have been determined.[2041] Abnormally high levels of lactosylceramide were detected in the tissues. The involvement of cerebroside sulphates in the tissues of cases of cerebral sphingolipidoses, metachromatic leukodystrophy, and infantile amaurotic idiocy has been studied.[1460, 1466] The levels of ganglioside and trihexosylceramide in tissues from three variants of Tays–Sachs disease were found to be abnormal.[1458] The pattern of gangliosides in specimens from an untreated case of galacto-

[2036] K. A. Karlsson, I. Pascher, B. E. Samuelsson, and G. O. Steen, *Chem. and Phys. Lipids*, 1972, **9**, 230.
[2037] E. Meyers, D. S. Sulsarchyk, F. E. Pansy, H. I. Basch, W. H. Trejo, and P. J. DeBaeke, *J. Antibiotics*, 1972, **25**, 163.
[2038] G. N. Kryzhanovsky and O. P. Sakharova, *Biull. eksp. Biol. Med.*, 1972, **73**, 38.
[2039] S. Hakamori, *Z. physiol. Chem.*, 1971, **352**, 522.
[2040] E. V. Diatlovitskaia, A. M. Novikov, and L. D. Bergelson, *Doklady Akad. Nauk S.S.S.R.*, 1971, **197**, 966.
[2041] G. Dawson, *J. Lipid Res.*, 1972, **13**, 207.

semia in an adult was found to resemble the normal adult pattern rather than that for a child.[2042]

All the *N*-acetylneuraminic acid units of the brain gangliosides (104), excepting the one linked to an inner D-galactose residue, were found to be



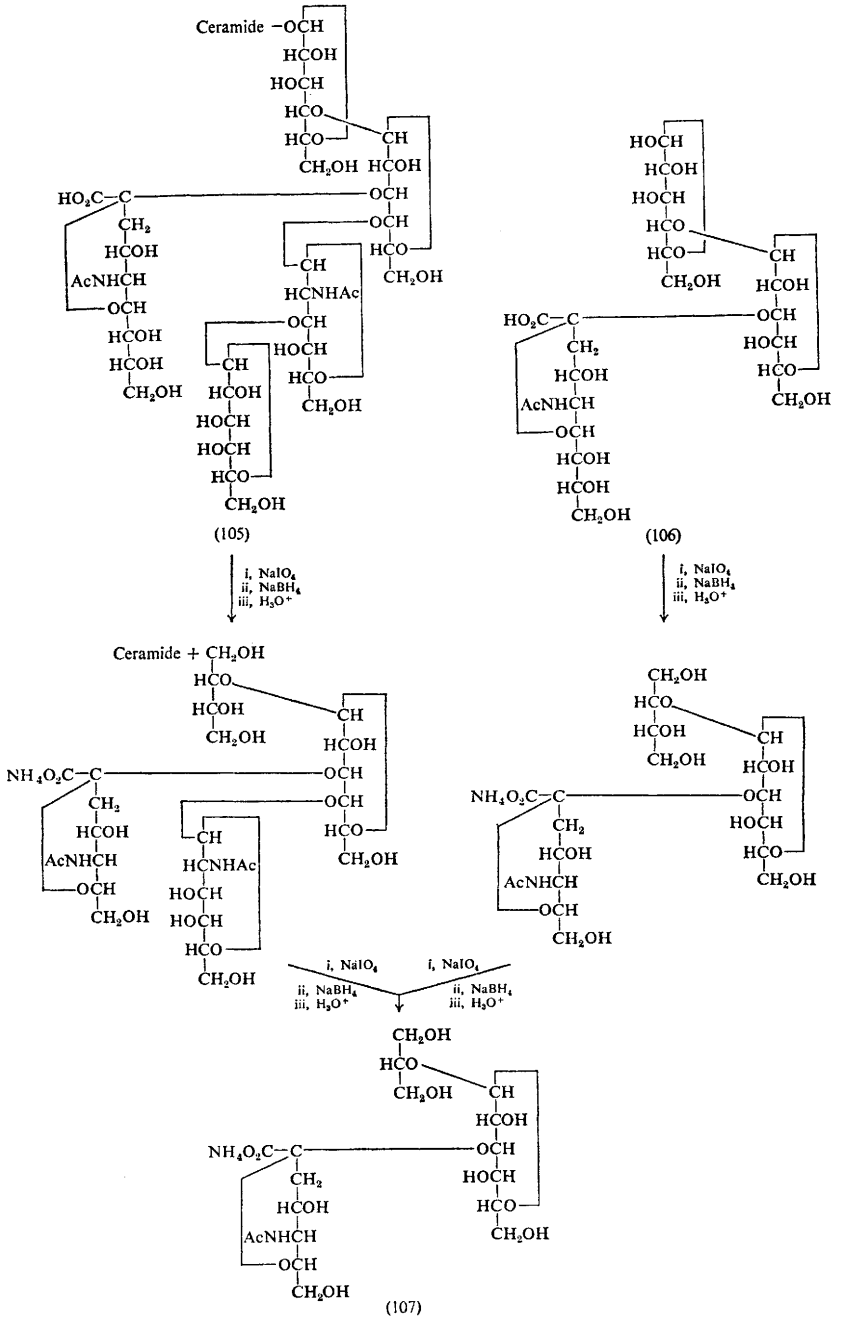
N-acetylneuraminic acid units are present as follows:
monosialoganglioside: a
disialoganglioside: a + b or a + c
trisialoganglioside: a + b + c or a + c + e
tetrasialoganglioside: a + b + c + d or a + b + c + e

(104)

susceptible to neuraminidases and it is assumed that these units are linked α-ketosidically to the rest of the molecule.[2043] That the *N*-acetylneuraminic acid unit resistant to enzymic hydrolysis is also α-ketosidically linked has been deduced from parallel treatments of the monosialoganglioside (105) and *O*-(*N*-acetyl-α-neuraminosyl)-(2 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucose (106) with two cycles of periodate, borohydride, and acid (Scheme 13). The same product, 5-acetamido-3,5-dideoxy-α-L-*arabino*-2-heptulosidonic acid-(2 → 3)-*O*-β-D-galactopyranosyl-(1 → 2)-glycerol (107) was obtained from both compounds.

Gangliosides have been isolated from developing human brain and from brains of cases of amaurotic idiocy.[2044] Neutral glycolipids have also been isolated from the pathological specimens.[2045] The ganglioside patterns showed characteristic distributions, an *N*-acetylneuraminosyl-trisaccharide and an *N*-acetylneuraminosyl-tetrasaccharide being the major components in cases of Tay–Sachs type and Jatzkewitz–Sandhoff type, respectively. Grey matter from these cases showed an abnormal ceramide oligohexoside pattern, *N*-acetylneuraminic acid-free derivatives of the previous two gangliosides occurring as major components in the respective cases. Glucocerebroside and ceramide dihexoside were also recognized in grey

[2042] L. A. Witting, C. Haberland, and E. G. Brunngraber, *Clinica Chim. Acta*, 1972, **37**, 387.
[2043] R. T. C. Huang and E. Klenk, *Z. physiol. Chem.*, 1972, **353**, 679.
[2044] M. Naoi and E. Klenk, *Z. physiol. Chem.*, 1972, **353**, 1677.
[2045] M. Naoi and E. Klenk, *Z. physiol. Chem.*, 1972, **353**, 1684.



Scheme 13

matter, but the abnormal distribution of the ceramide oligohexosides was less marked in white matter. The gangliosides and other sphingolipids of the brain in different variants of amaurotic idiocy have been studied.[2046] The ganglioside distributions in normal brain and in that of cases of late infantile amaurotic idiocy have been determined; the cerebroside contents of grey and white matter from the latter were found to be low.[893]

Tissues from a patient purported to have Tay–Sachs disease were found to have abnormally high concentrations of the ganglioside (108) in the brain, asialo-(108) in the brain and liver, and globoside (109) in the liver, kidney, and spleen.[2047] The case was considered subsequently to be one of G_{M_2}-gangliosidosis with globoside accumulation.

D-Gal*N*Ac-(1 → 4)-D-Gal-(1 → 4)-D-Glc-ceramide
3
↑
2
Neu*N*Ac
(108)

D-Gal*N*Ac-(1 → 3)-D-Gal-(1 → 4)-D-Gal-(1 → 4)-D-Glc-ceramide
(109)

The sequence of incorporation of monosaccharides in the biosynthesis of the branched oligosaccharide of Tay–Sachs ganglioside has been investigated.[2048] The metabolism of Tay–Sachs ganglioside has been investigated by means of catabolic studies with lysosomal enzymes from normal and Tay–Sachs brain tissues.[1464] An attempt has been made to explain the molecular mechanism of Tay–Sachs disease by an examination of the interactions between bovine brain gangliosides and phosphatidylcholine (lecithin) as a model for ganglioside–lipid interactions.[2049]

Gangliosides can be quantitatively isolated from human, bovine, and rabbit retinae by extraction with chloroform–methanol, phase partition, and chromatography on silica gel.[2050] The ganglioside patterns of the retinae from the three species were found to be very similar and the ganglioside *N*-acetylneuraminic acid was found to be 12% of the total brain *N*-acetylneuraminic acid.

The concentrations of seven glycosphingolipids found in normal human skin fibroblasts have been determined for fibroblasts cultured from patients with Fabry's disease, lactosylceramidosis, Gaucher's disease, G_{M_1}-gangliosidosis type I, G_{M_2}-gangliosidosis type I (Tay–Sachs disease), globoid cell leukodystrophy, metachromatic leukodystrophy, Hurler syndrome, fucosidosis, and G_{M_2}-gangliosidosis type II.[1469]

[2046] E. Klenk, M. Naoi, and D. Basu, *Z. physiol. Chem.*, 1970, **351**, 1311.
[2047] P. D. Snyder, W. Krivit, and C. C. Sweeley, *J. Lipid Res.*, 1972, **13**, 128.
[2048] H. J. F. Maccioni, A. Arce, and R. Caputto, *F.E.B.S. Letters*, 1972, **23**, 136.
[2049] R. Lester, M. W. Hill, and A. D. Bangham, *Nature*, 1972, **236**, 32.
[2050] M. Holm, J. E. Månsson, M. T. Vanier, and L. Svennerholm, *Biochim. Biophys. Acta*, 1972, **280**, 356.

The concentration and types of gangliosides present in normal human heart have been determined.[2051] Similar determinations for specimens from cases of Tay–Sachs disease showed that the ganglioside abnormality is similar to that established for brain tissue in such cases.

The phenol–sulphuric acid reaction gave results comparable with the anthrone reaction for the measurement of gangliosides and cerebrosides in normal human spleen.[2052] Quantitative determination of the glycosphingolipids isolated from spleens of normals and cases of Gaucher's disease showed that the glucosylceramide, sialyl-lactosylceramide, and trihexosylceramide of the pathological tissue were greater than normal.[2053] Differences were also recognized between the fatty-acid groups of the glycolipids from the two tissue types. Fractionation of glycolipoprotein material with discrete allo-antigenic activity derived from human spleen cells yielded high, intermediate, and low molecular weight materials, which were shown to contain carbohydrate (35, 16, and 29%, respectively) comprised of xylose, galactose, glucose, mannose, fucose, 2-acetamido-2-deoxygalactose, 2-acetamido-2-deoxyglucose, and sialic acid.[1016]

Two glycolipids found to accumulate in the spinal ganglia of a patient with Fabry's disease were identified as (110) and (111).[2054] Only one of these glycolipids (110), however, was found to accumulate in the sympathetic ganglia of the patient.

α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-ceramide
(110)

α-D-Gal*p*-(1 → 4)-D-Gal*p*-(1 → 1)-ceramide
(111)

Galactosylgalactosylglucosylceramide has been extracted and purified from pooled normal human kidney tissues and also from the kidney of a case of Fabry's disease.[1581] ^{1}H N.m.r. signals attributable to the anomeric protons were consistent with the following structural features: a β-D-glucopyranosyl residue linked to ceramide, an internal β-D-galactopyranosyl residue, and a terminal α-D-galactopyranosyl residue. The terminal D-galactosyl unit was labelled by oxidation with galactose oxidase and reduction with sodium borotritide, whereafter the radioactivity was partially released by an α-galactosidase, thereby confirming the α-configuration of the terminal linkage. α-Galactosidase has also been shown to liberate the terminal D-galactose unit from a digalactosylceramide that accumulates in Fabry's kidney.[2055] The penultimate unit was released by

[2051] R. Rodriguez-Torres, L. Schneck, and W. Kleinberg, *Bull. New York Acad. Med.*, 1971, **47**, 717.
[2052] H. G. J. Worth, *Clinica Chim. Acta*, 1971, **34**, 443.
[2053] T. T. Kuske and A. Rosenberg, *J. Lab. Clinical Med.*, 1972, **80**, 523.
[2054] T. Miyatake and T. Ariga, *J. Neurochem.*, 1972, **19**, 1911.
[2055] Y. T. Li, S. C. Li, and G. Dawson, *Biochim. Biophys. Acta*, 1972, **260**, 88.

β-galactosidase and the structure (112) was proposed for the whole molecule. Thus, both the glycolipids accumulating in Fabry's disease possess terminal α-D-galactosyl residues.

α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 1)-ceramide
(112)

The concentrations of gangliosides in plasma from children have been determined and elevated levels of *N*-acetylneuraminic acid were found in cases of gangliosidoses.[2056] No significant differences were found between the sexes or different age groups in this respect. Gangliosides from human, bovine, and rabbit plasma have been purified by solvent partition, ion-exchange and silicic acid chromatography, and were then quantified by g.l.c.[2057] Whereas the sialic acid of the total human ganglioside fraction was found to contain *N*-acetylneuraminic acid only, the corresponding rabbit and bovine fractions also contained *N*-glycollylneuraminic acid (1% and 50% of sialic acid, respectively). Based on the sialic acid content, the molar ratios of hematosides to 2-amino-2-deoxyhexose-containing gangliosides were found to be 2:1, 2:3, and 3:2 for the human, bovine, and rabbit fractions, respectively. It has been shown that the α-galactosidase activity of the plasma from patients with Fabry's disease measured on artificial substrates is different from that determined using digalactosylglucosylceramide as substrate.[1568]

Purified Lp(a) lipoglycoprotein from human plasma has been shown to contain neutral carbohydrate (10.8), 2-amino-2-deoxyhexose (8.4), and sialic acid (6.6%);[1232] the carbohydrate content is greater than that of other lipoglycoproteins of plasma. The lipoglycoprotein can be dissociated into three components by electrophoresis; the immunological, electrophoretic, and flotation behaviours of these components indicated that one of them contained a low-density lipoglycoprotein and another albumin. The low-density material was shown to contain neutral carbohydrate (5.5), 2-amino-2-deoxyhexose (2.8), and sialic acid (1.0%).

Two systems have been developed for the separation of glycosphingolipids from total lipid extracts of erythrocytes.[2058] One enabled separation of the ceramide hexosides according to the number of glycose units in the chain, while the other separated complex glycosphingolipids. The red cells of human, horse, cow, sheep, pig, cat, rabbit, and guinea-pig showed no species variation in the qualitative composition of the ceramide hexosides, but marked variations were observed in the concentrations and compositions of complex glycosphingolipids. The terminal D-galactosyl residue of trihexosylceramides from human erythrocytes, hamster BHK fibroblasts, and a globoside of human erythrocytes were hydrolysed

[2056] G. Dacremont, *Clinica Chim. Acta*, 1972, **37**, 449.
[2057] R. K. Yu and R. W. Leden, *J. Lipid Res.*, 1972, **13**, 680.
[2058] K. Eberlein and G. Gercken, *J. Chromatog.*, 1971, **61**, 285.

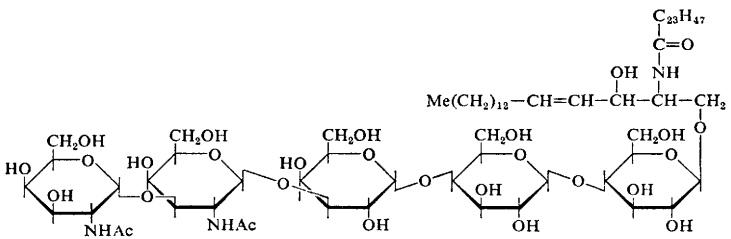
completely by an α-galactosidase, the products being D-galactose and lactosylceramide in each case.[1583] The sequence and anomeric linkages of the carbohydrate units were determined using glycosidases and the presence of α-anomeric protons was supported by ^1H n.m.r. data. These results and those from methylation analyses indicated the trihexosylceramide to have the structure (110) and the globoside to have the structure (113).

β-D-Gal*p*NAc-(1 → 3)-α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-ceramide
(113)

A comparison has been made of the levels of ganglioside in leucocytes from non-leukaemic individuals (group 1), patients with chronic myelogenous leukaemia (group 2), and patients with chronic lymphocytic leukaemia (group 3).[2059] The levels were high in group 2 and low in group 3. The gangliosides present in myeloid cells from groups 1 and 2 are of the slow-migrating type comparable with the major gangliosides of human brain. Traces of ganglioside present in the lymphocytes from group 3 were identified as hematosides.

The glycosphingolipids of human blood platelets have been isolated, identified, and quantitated.[1479] Identification and quantitation of the gangliosides showed that they represent 0.5% of the platelet lipids and account for 6% of the total neuraminic acid contribution of the lipids.[2060]

The structure of the Forssman antigen (glycolipid hapten) of horse spleen has been revised to (114) on the bases of methylation studies and previously published structural data.[2061]



(114)

A method reported for the fractionation of cerebrosides of bovine brain into classes with different compositions of fatty acid depends on benzoylation in the crude state followed by column chromatography on silicic acid and Hyflo Super-Cel.[2062] The titration characteristics of bovine brain gangliosides and the reactions of these compounds with cations in

[2059] J. Hildebrand, P. A. Stryckmans, and J. Vanhouche, *Biochim. Biophys. Acta*, 1972, **260**, 272.
[2060] A. J. Marcus, H. L. Ullman, and L. B. Safier, *J. Clinical Invest.*, 1972, **51**, 2602.
[2061] B. Siddiqui and S. Hakomori, *J. Biol. Chem.*, 1971, **246**, 5766.
[2062] A. J. Acher and J. N. Kanfer, *J. Lipid Res.*, 1972, **13**, 139.

aqueous systems have been studied.[2063] When free from calcium ions, all the sialic acid groups can be titrated to half-neutralization at pH 4. In the presence of metal chlorides, the titrations indicated that some of the acid groups are bound to metal ions, the effect being particularly pronounced with calcium chloride. The reactions with metal cations and some amines were investigated by measuring the cation–proton exchange occurring at pH 4. In calcium chloride solution of concentration 1 mmol l^{-1}, this exchange was found to be greater than in solutions of univalent metal chlorides of concentration 50 mmol l^{-1}. The amines exchanged in a similar way to the alkali metals. Further analysis of the titration data indicated that a configurational change may occur during the course of protonation. The effects of egg lecithin on the titration curves and metal–ganglioside interactions were also reported.

Endogenous and added gangliosides have been confirmed as substrates for calf brain sialidase and the release of sialic acid was enhanced in the presence of Triton X-100.[1021] The results of these studies were related to the turnover of gangliosides in the brain. Sialyl residues of bovine brain gangliosides were hydrolysed by sialidase from *Vibrio cholerae* irrespective of whether the substrate was in disperse or micellar form.[1682]

The antigenic identity between bovine nervous tissues and treponema has been shown to be due to the presence in an extract from *Treponema reiteri* of a galactodiglyceride hapten 2,3-di-*O*-acyl-1-*O*-β-D-galactopyranosyl-D-glycerol,[2064] which showed a cross-reaction with cerebrosides. Bovine cerebral tissues were shown to contain a glycolipid that liberated a 1-*O*-β-D-galactosylglycerol on deacylation.

The interactions of bovine brain gangliosides with phosphatidylcholine in aqueous dispersions have been investigated.[2065] Detailed studies of the surface potentials of mixtures of the mono- and di-sialogangliosides showed that the gangliosides are stable in the monolayer and produce a charge that is concordant with the number of charged groups in the surface. A variety of bovine gangliosides interacted with botulinum toxin to inactivate it.[2066]

A mixture of glycosphingolipids has been obtained from bovine blood-group J substance and this, together with other data, suggested that the blood-group substance occurs in serum as a glycolipoprotein.[772] A component of the J-hapten of cattle blood-group J-glycolipid from an alkaline hydrolysate of the parent glycolipid was shown by compositional analysis and partial acidic hydrolysis to be a digalactosylglucosylceramide.[2067] The parent glycolipid also contains phosphorus and glycerol. The structure of the parent J-active glycolipid has been investigated and

[2063] M. B. Abramson, R. K. Yu, and V. Zaby, *Biochim. Biophys. Acta*, 1972, **280**, 365.
[2064] P. Dupouey, *J. Immunol.*, 1972, **109**, 146.
[2065] M. W. Hill and R. Lester, *Biochim. Biophys. Acta*, 1972, **282**, 18.
[2066] L. L. Simpson and M. M. Rapport, *J. Neurochem.*, 1971, **18**, 1341.
[2067] B. L. Slomiany and M. I. Horowitz, *Immunochemistry*, 1971, **8**, 719.

deacylation by mild alkali liberated the *O*-acyl-linked fatty acids.[2068] Studies with galactose oxidase showed that the D-galactose units in the intact and deacylated molecules are not oxidized. On the basis of these and further studies, the partial structure (115) was proposed for the blood-group J-glycolipid.

ceramide-D-Glc-D-Gal-D-Gal-glycerol-fatty acids
phosphate
(115)

In an investigation of the biosynthesis of cerebroside sulphate in sheep brain, it has been shown that the endogenous lipid acceptor, which accepts sulphate from adenosine 3′-phosphate-5′-sulphatophosphate to form cerebroside sulphate, is galactosylcerebroside.[2069] This precursor has been shown to occur in a protein-bound form and the relations between the various compounds involved in the biosynthetic pathway were established. Forssman hapten has been isolated from ovine erythrocytes by a combination of fractional crystallization, solvent extraction, and chromatography; the homogeneous preparation was found to contain galactose, glucose, and 2-amino-2-deoxygalactose (molar ratio, 2 : 1 : 1), and dihydrosphingosine fatty acids.[2070]

The ganglioside content of nerve-cell perikaryon isolated from porcine brain stem has been determined.[2071] The effect of development of the animal on the gangliosides of porcine brain has been investigated.[2072] The oligosaccharide moieties of the ceramide trihexoside and globoside fractions isolated from porcine spleen, mouse Nakahara–Fukuoka sarcoma, and rat kidney were liberated by treatment with ozone and alkali.[2073] Reduction, enzymic hydrolysis, and ^{1}H n.m.r. spectroscopy indicated that the ceramide trihexoside and globoside possess the structures (116) and (117), respectively.

α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-D-Glc-(1 → 1)-ceramide
(116)

β-D-Glc*pN*Ac-(1 → 3)-α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-D-Glc-(1 → 1)-ceramide
(117)

The separation of the glycolipids of canine whole small-intestinal mucosa by t.l.c. into three different groups according to the content of carbohydrate has been described.[2074] The three groups corresponded to

[2068] B. L. Slomiany and M. I. Horowitz, *Immunochemistry*, 1972, **9**, 1067.
[2069] V. R. Bhandari and B. K. Bachhawat, *Indian J. Biochem. Biophys.*, 1972, **9**, 72.
[2070] M. F. Mallette and R. L. Rush, *Immunochemistry*, 1972, **9**, 809.
[2071] Y. Tamai, S. Matsukawa, and M. Satake, *J. Biochem.* (*Japan*), 1971, **69**, 235.
[2072] J. W. T. Dickerson, A. Merat, and E. M. Widdowson, *Biochem. J.*, 1971, **125**, 40P.
[2073] J. Kamanami and T. Tsuji, *Chem. and Phys. Lipids*, 1971, **7**, 49.
[2074] E. L. Smith and J. M. McKibbin, *Analyt. Biochem.*, 1972, **45**, 608.

gangliosides, neutral glycolipids not containing fucose, and neutral glycolipids containing fucose. Resolution and analyses of components of the latter category showed that the carbohydrate compositions varied with individual dogs, *viz.* galactose, glucose, fucose, and 2-amino-2-deoxyglucose (molar ratio 2—3 : 1 : 1—2 : 1).

The detection and determination of gangliosides occurring in mammalian sympathetic ganglia of cats and rats have been described.[2075] Using radioactive precursors, the metabolism *in vitro* of the gangliosides was studied.

Studies have been carried out *in vivo* on the biosynthesis and metabolism of gangliosides of the optic pathway in rabbits; [^{3}H]acetate and 2-amino-2-deoxy-D-[1-^{14}C]glucose were used as precursors.[2076] Glycolipid components of isolated brush borders of rabbit small intestine have been shown to contain galactose, glucose, 2-amino-2-deoxygalactose, 2-amino-2-deoxyglucose, and sialic acid.[1039] The biosynthesis of these glycolipids was also examined. A galactosyltransferase from rabbit bone marrow was found to catalyse the transfer of D-galactose from UDP-D-galactose to *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 3)-*O*-β-D-galactopyranosyl-(1 → 4)-D-glucosyl-(1 → 1)-ceramide.[2077]

Evidence has been presented for the occurrence of a glycolipid containing 2-amino-2-deoxyglucose in hamster fibroblast membranes and endoplasmic reticulum.[1041] The glycolipid was labelled by incorporation of radioactive precursors and its electrophoretic properties were examined. Further studies have been carried out on the synthesis of glycolipids by NIL 2 line hamster cells.[2078] The glycolipid compositions of various clones isolated from this line were determined by g.l.c. The biosynthesis of glycolipids in normal and hamster sarcoma virus- or polyoma virus-transformed cells was investigated by assessment of the incorporation of [^{14}C]palmitate. Reasons have been offered for the depression of synthesis of galactosylgalactosylglucosylceramide and 2-acetamido-2-deoxyglucosyl-galactosylgalactosylglucosylceramide in NIL 2 line hamster cells that is brought about by virus transformation.[2079]

A proportion of the label arising from intracerebral injection of mice with radioactive 2-amino-2-deoxy-D-glucose has been found previously to migrate as a macromolecular component on polyacrylamide gel electrophoresis, but to be extractable with chloroform–methanol.[2080] However, further studies have shown the component to be a mixture of gangliosides that also acts as a macromolecular form on gel filtration in the presence of sodium dodecyl sulphate. The incorporation of [1-^{14}C]acetate into

[2075] J. U. Harris and J. D. Klingman, *J. Neurochem.*, 1972, **19**, 1267.
[2076] M. Holm, *J. Neurochem.*, 1972, **19**, 623.
[2077] M. Basu and S. Basu, *J. Biol. Chem.*, 1972, **247**, 1489.
[2078] H. Sakiyama, S. K. Gross, and P. W. Robbins, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 872.
[2079] P. W. Robbins and I. MacPherson, *Nature*, 1971, **229**, 569.
[2080] G. R. Dutton and S. H. Barondes, *J. Neurochem.*, 1972, **19**, 559.

cerebrosides of the developing mouse brain [2081] and the effect of 4-methoxymethylpyridoxine on the gangliosides in mouse brain have been examined.[2082]

Variations of the galactolipids of cerebral microsomes of mice have been determined as a function of age.[2083] From an investigation of the biosynthesis of cerebrosides containing 2-hydroxy-acids in mouse brain microsomes, in which deuterium-labelled precursors were employed, it was concluded that the galactosyl- and glucosyl-ceramides are synthesized *via* the ceramide pathway.[2084]

The brain of the jimpy mouse has little ability to synthesize galactosyldiglyceride.[1574] The low concentration of this compound was attributed to low activity of a galactosyltransferase rather than to high activity of a degradative enzyme. Whereas normal brain contains the glycolipid in both myelin and microsomal fractions, it is restricted to the latter fraction in the jimpy brain. The apparent inability of the tissues to transfer glycolipid from the site of synthesis (microsomes) to the site of deposition (myelin) is correlated, therefore, with the absence of myelin. From a comparison of the incorporation *in vivo* of uniformly labelled D-[^{14}C]glucose into the galactolipids of the brains of control and jimpy mutant mice, it was concluded that the biosynthetic pattern for the mutant mice does not accord with a primary defect in the biosynthesis of cerebrosides and sulphatides.[2085]

The biosynthesis of glycolipids in the early prereplicative phase of isoprotenerol-stimulated salivary glands of mice has been shown to be greater than normal.[1381]

The sequence of methanolysis, *N*-acetylation, and trimethylsilylation followed by g.l.c. has been applied to the quantitative identification of small amounts of gangliosides present in cell lines from mice.[2086] The distribution of gangliosides in established mouse cell lines in culture was investigated before and after transformation of the cells with SV 40 virus. A drastically reduced content of disialotetrasaccharide ganglioside resulted from the viral transformation in certain cases. The activities of glycosyltransferases involved in the biosynthesis of gangliosides have been measured in two established mouse cell lines and in SV 40 and polyoma virus-transformed variants thereof.[2087] The activity of UDP-2-acetamido-2-deoxy-D-galactose:hematoside 2-acetamido-2-deoxy-D-galactosyltransferase was found to be markedly reduced in the transformed cells.

Gangliosides of normal embryonic mouse fibroblasts and of SV 40 and mouse leukaemia virus-transformed mouse cells have been investi-

[2081] I. Kanazawa, N. Ueta, and T. Yamakawa, *J. Neurochem.*, 1972, **19**, 1483.
[2082] G. D. Cherayil and A. E. Cyrus, *J. Neurochem.*, 1972, **19**, 1215.
[2083] J. M. Bourre, S. Pollet, O. Daudu, and N. Baumann, *Compt. rend.*, 1972, **274**, *D*, 2100.
[2084] S. Hammarström and B. Samuelsson, *J. Biol. Chem.*, 1972, **247**, 1001.
[2085] M. J. Druse and E. L. Hogan, *J. Neurochem.*, 1972, **19**, 2435.
[2086] I. Dijong, P. T. Mora, and R. O. Brady, *Biochemistry*, 1971, **10**, 4039.
[2087] P. H. Fishman, V. W. McFarland, P. T. Mora, and R. O. Brady, *Biochem. Biophys. Res. Comm.*, 1972, **48**, 48.

gated.[2088] In particular, the hematoside content was found to be affected by the transformations. Neutral glycosphingolipids and gangliosides of normal 3T3 mouse fibroblasts and of polyoma and SV 40 virus-transformed clonal isolates have been examined.[2089] The normal cells were found to contain mono- and di-hexosylceramides and a complex pattern of gangliosides, whereas less complex patterns were found for the polyoma virus-transformed cells. A variety of patterns was observed for the SV 40-transformed cells. Possible explanations of these observations were discussed and the biosynthesis of the glycolipids was investigated by incorporation of 2-amino-2-deoxy-D-[1-^{14}C]glucose.

Variations of the ganglioside content of rat brain with development of the animal have been studied.[2090] Biosynthetic and metabolic relationships between the four major gangliosides of rat brain have been investigated *in vivo* using [^{3}H]acetate and 2-amino-2-deoxy-D-[1-^{14}C]glucose as precursors.[2091] Labelling *in vivo* of rat brain gangliosides with uniformly labelled D-[^{14}C]glucose and 2-amino-2-deoxy-D-[6-^{3}H]glucose demonstrated that the relative rates of labelling of the various gangliosides differ with age.[2092] A scheme for the biosynthesis of the gangliosides was presented. Naturally occurring galactosyl(2-acetamido-2-deoxygalactosyl)[*N*-acetylneuraminosyl]galactosylglucosylceramide and galactosyl(2-acetamido-2-deoxygalactosyl)[di-*N*-acetylneuraminosyl]galactosylglucosylceramide, from which the terminal D-galactose residues had been removed enzymically, acted as substrates for the transfer of D-galactose from UDP-D-galactose by a particulate subcellular fraction from rat brain.[2093] The results obtained in this comparative study suggested that the agalacto-compounds are intermediates in the biosynthetic pathways to the two parent compounds and that the transfer of D-galactose to these intermediates is catalysed by one enzyme.

Inhibition of a rat brain enzyme, which forms galactosylceramide from UDP-D-galactose and hydroxyceramide, has been tested using various synthetic substances resembling hydroxyceramide.[2094] Strong inhibition was obtained with *N*-bromoacetyl DL-*erythro*-3-phenyl-2-aminopropane-1,3-diol. The biosynthesis of cerebroside sulphate and its inhibition by detergent have been studied in rat brain.[2095]

A sensitive, specific, gas-chromatographic assay has been used to determine the concentrations of monogalactosyldiglyceride in fractions of brains from rats of various ages.[2096] In adult brain, most of the glycolipid

[2088] H. Diringer, G. Ströbel, and M. A. Koch, *Z. physiol. Chem.*, 1972, **353**, 1769.
[2089] G. Yogeeswaran, R. Sheinin, J. R. Wherrett, and R. K. Murray, *J. Biol. Chem.*, 1972, **247**, 5146.
[2090] J. W. T. Dickerson and A. Merat, *Biochem. J.*, 1971, **125**, 40P.
[2091] M. Holm and L. Svennerholm, *J. Neurochem.*, 1972, **19**, 609.
[2092] H. J. Maccioni, A. Arce, and R. Caputto, *Biochem. J.*, 1971, **125**, 1131.
[2093] F. A. Cumar, J. F. Tallman, and R. O. Brady, *J. Biol. Chem.*, 1972, **247**, 2322.
[2094] R. C. Arora and N. S. Radin, *Biochim. Biophys. Acta*, 1972, **270**, 254.
[2095] B. K. Bachhawat, S. Kokrady, and S. Saxena, *Biochem. J.*, 1972, **128**, 8P.
[2096] T. Inoue, D. S. Deshmukh, and R. A. Pieringer, *J. Biol. Chem.*, 1971, **246**, 5688.

was found to be associated with the myelin and the enzyme responsible for biosynthesis of the glycolipid to reside almost exclusively in the microsomal fraction. The data indicated that a temporal relationship exists between the site of synthesis (microsomes) and that of deposition (myelin) of monogalactosyldiglyceride. The formation of lipid-linked monosaccharides in rat brain and liver microsomes has been investigated and stimulators of this process have been identified.[2097]

The galactosylceramides of enriched neuronal and glial fractions from rat brain have been examined.[2098] The labelling of the mitochondrial and synaptosomal ganglioside fractions of rat brain injected with 2-amino-2-deoxy-D-[6-^{3}H]glucose was stimulated by the influences of light and sound.[2099]

An enzyme that catalysed the transfer of D-galactose from UDP-D-galactose to 2-acetamido-2-deoxygalactosyl[*N*-acetylneuraminosyl]galactosylglucosylceramide has been found in extraneural tissues of adult and young rats.[2100] The incorporation of radioactive D-galactose into the ganglioside was stimulated by the addition of exogenous ganglioside of the same type. The radioactive product was identified as a galactosyl derivative of the substrate ganglioside.

Evidence has been presented for the transfer of 2-acetamido-2-deoxy-D-glucose from UDP-2-acetamido-2-deoxy-D-[1-^{14}C]glucose to lipids by cell-free preparations of rat and hen livers.[1071] CDP-choline was found to stimulate the production of glycolipid. The lability of the glycolipid to alkali and acid was investigated and it was shown to contain radioactive 2-acetamido-2-deoxy-D-glucose. A study of the simultaneous incorporation of labelled precursor into lipids and proteins in the microsomes showed that peak incorporation into the two types of substrates occurred after 5 and 30 min, respectively. It was suggested that the incorporation of 2-acetamido-2-deoxy-D-glucose into protein may be mediated through a glycolipid intermediate.

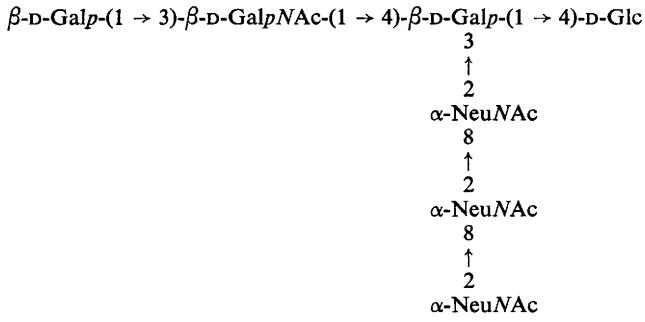
Sialidase and ganglioside substrates have been localized in the cellular structure of rat liver.[1673] Treatment with ammonia of the product formed by incubation of dolichol monophosphate-D-glucose with rat liver microsomes yielded a water-soluble, negatively charged compound (molecular weight 3550);[2101] the negative charge could be removed enzymically with phosphatase. Acidic hydrolysis of the original compound, followed by acetolysis, yielded a number of oligosaccharides and it was concluded that this compound is dolichol joined through either a phosphate or pyrophosphate linkage to an oligosaccharide containing approximately twenty monosaccharide residues.

[2097] W. Jankowski and T. Chojnacki, *Biochim. Biophys. Acta*, 1972, **260**, 93.
[2098] S. Raghavan and J. N. Kanfer, *J. Biol. Chem.*, 1972, **247**, 1055.
[2099] A. H. R. Maccioni, M. S. Giménez, and R. Caputto, *J. Neurochem.*, 1971, **18**, 2363.
[2100] M. C. M. Yip, *Biochim. Biophys. Acta*, 1972, **273**, 374.
[2101] N. H. Behrens, A. J. Parodi, and L. F. Leloir, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 2857.

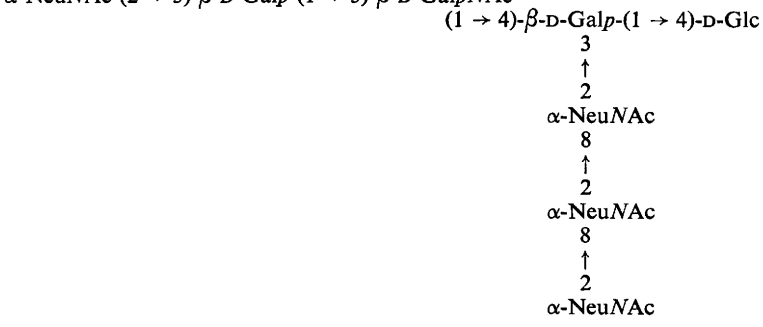
The levels of mono-, di-, and tri-sialogangliosides in the brain and eyes of chicken have been followed during embryonic development.[1497] The glycolipids of chicken embryonic cells and those transformed by a temperature-sensitive mutant of Rous sarcoma virus have been compared by radioactive incorporation studies.[1428]

Investigation of changes in the patterns of neutral glycosylceramides in duck erythrocytes during infection with the avian malarial parasite *Plasmodium laphurae* has shown that, although there is no change in the qualitative pattern, the levels of certain glycolipids are markedly decreased.[2102] A material isolated from the salt gland of the herring gull has been identified by mass spectrometry as a xylose-containing cerebroside.[2103] A xylopyranose ring appeared to be involved in the molecule.

The carbohydrate moieties of three gangliosides isolated from cod fish brain, which contain three, four, and five sialic acid residues, respectively, have been characterized as (118)—(120).[1686] Gangliosides have been purified from the brain of the common goldfish (*Carrassius auratus*).[2104]



(118)



(119)

[2102] J. McLaughlin and R. B. McGhee, *Life Sci., Pt. II*, 1972, **11**, 397.
[2103] K.-A. Karlsson, B. E. Samuelsson, and G. O. Steen, *J. Lipid Res.*, 1972, **13**, 169.
[2104] R. H. McCluer and B. W. Agranoff, *J. Neurochem.*, 1972, **19**, 2307.

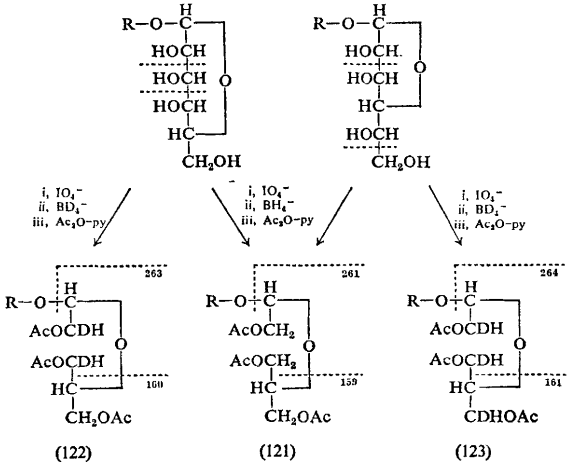
α-Neu*N*Ac-(2 → 8)-α-Neu*N*Ac-(2 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*N Ac-(1 → 4)-β-D-Gal*p*-(1 → 4)-D-Glc

3
↑
2
α-Neu*N*Ac
8
↑
2
α-Neu*N*Ac
8
↑
2
α-Neu*N*Ac

(120)

Incorporation of D-[1-^{3}H]galactose into these gangliosides and the effects of puromycin on the incorporation have been studied.

Cerebroside has been isolated from the sea star (*Asterias rubens*) [2105] and the glycolipid was shown to contain glucose, uncommon long-chain bases, and saturated 2-hydroxy-fatty acids.[2106] In order to identify the ring size of the carbohydrate unit, a new method was developed (Scheme 14). Whereas periodate oxidation followed by reduction with sodium borohydride, *etc.* of hexo-furanoside and -pyranoside gives the same product (121), periodate oxidation followed by reduction with sodium borodeuteride yielded products [(122) and (123)] that are distinguishable by mass spectrometry. The hexose unit of the cerebroside under investigation was found to involve a pyranose ring.



(122) (121) (123)

Scheme 14

[2105] L. R. Björkman, K.-A. Karlsson, and K. Nilsson, *Comp. Biochem. Physiol.*, 1972, **43B**, 409.

[2106] L. R. Björkman, K.-A. Karlsson, I. Pascher, and B. E. Samuelsson, *Biochim. Biophys. Acta*, 1972, **270**, 260.

Compositional studies have been carried out on carotenoid-containing lipoglycoproteins of a number of aquatic invertebrates, particularly those of the ovaries of *Homarus gammarus*, *Cancer pagurus*, and *Pecten maximus*, and eggs of *Plesionika edwardsi*.[1436]

Plant Glycolipids

The problems associated with isolation of glycolipids from plant tissues, owing to the co-existence of pigments, phospholipids, and isoprenoids, *etc.*, have been overcome by chromatography on columns of silicic acid eluted with chloroform–acetone.[2107] With the aid of this technique, monogalactosyl-, digalactosyl-, and sulphoquinovosyl-diglycerides have been isolated from alfalfa leaves. The biosynthesis of galactolipids in photoautotrophic chloroplasts of *Euglena gracilis* has been studied.[2108]

Glycolipoproteins isolated from fruit envelopes and leaves of the grapevine have been shown to contain 33% carbohydrate, comprised of galactose, glucose, and arabinose, as well as 50% lipid.[672] Using [1-^{14}C]acetate as precursor, the biosynthesis of the galactolipids by young leaves of a pea plant (*Pisum sativum*) has been studied.[2109]

The biosynthesis of monogalactosyldiglyceride by chloroplasts from certain Gramineae, *e.g.*, meadow fescue (*Festuca elatior*), phalaris (*Phalaris arundinacea* × *tuberosa*), ryegrass (*Lolium perenne*), Yorkshire fog (*Holcus lanatus*), and from spinach (*Spinacea oleracea*) has been investigated by studying the ability of the chloroplasts to transfer D-galactose from UDP-D-[^{14}C]galactose to synthetic diglycerides.[2110] The mono- and di-galactolipids of spinach subchloroplast fragments have been determined [2111] and the incorporation of fatty acids into monogalactosyldiglycerides by cell-free preparations of spinach leaves has been demonstrated.[2112]

From compositional studies of lipids obtained from wheat at various stages of growth, it appeared that the biosynthesis of digalactosyldiglycerides from monogalactosyldiglycerides occurs in a selective manner.[2113] D-Galactose is transferred from UDP-D-galactose preferentially to monogalactosyldiglyceride molecules containing little polyunsaturated acyl moieties. Galactolipids were suggested to play a significant role in the biosynthesis of linoleic and linolenic acids.

Algal Glycolipids

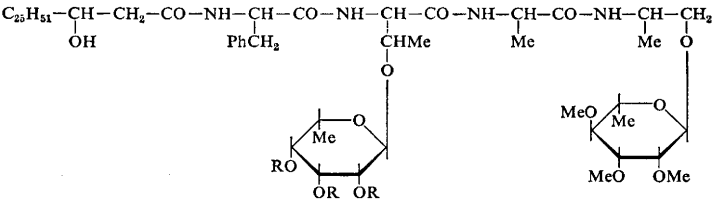
Monogalactoside and monoglucoside derivatives of long-chain, polyhydroxy-compounds have been identified in an unsaponifiable fraction

[2107] S. Ito and Y. Fujino, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 319.
[2108] M. F. Lin and S. B. Chang, *Phytochemistry*, 1971, **10**, 1543.
[2109] A. Trémolières and P. Mazliak, *Compt. rend.*, 1972, **275**, *D*, 1883.
[2110] T. R. Eccleshall and J. C. Hawke, *Phytochemistry*, 1971, **10**, 3035.
[2111] J. F. G. M. Wintermans, *Biochim. Biophys. Acta*, 1971, **248**, 530.
[2112] S. S. Bajwa and P. S. Sastry, *Biochem. J.*, 1972, **128**, 44P.
[2113] H. Bolling and A. W. El Bayâ, *Chem. and Phys. Lipids*, 1972, **8**, 102.

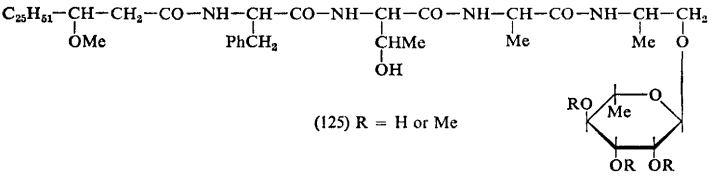
from the alga *Anabaena cylindrica*.[2114] The involvement of lecithin and monogalactosyldiglyceride in the biosynthesis of linoleate by green and blue-green algae (*Chlorella vulgaris*) has been investigated.[2115] The occurrence of monogalactosyldiglyceride (2,3-di-*O*-acyl-1-*O*-β-D-galactopyranosyl-D-glycerol), digalactosyldiglyceride [2,3-di-*O*-acyl-1-*O*-β-(6-*O*-α-D-galactopyranosyl-D-galactopyranosyl)-D-glycerol], and the corresponding trigalactosyldiglyceride in genera of the Chlorophycae algae, namely *Codium fragile*, *Enteromorpha intestinalis*, *Cladophora rupestris*, *Urospora*, *Monostroma gravellei*, *Mougeotia*, *Ulva lactuca*, and *Acetabularia crenulata*, has been investigated.[2116] Investigation of the fatty-acid components revealed that deacylated variants occur.

Bacterial Glycolipids

Two glycolipids tentatively identified as glucuronsyldiglyceride and a glycosylphosphatidylglycerol have been found in an unidentified halogenotolerant and moderately halogenophilic bacterium from evaporation ponds of the Dead Sea.[2117]



(124) R = H or Ac



(125) R = H or Me

The presence of mycosides has been recognized in bacteria isolated from cows during an attack of farcy;[2118] their structures were determined as (124) and (125).

[2114] T. A. Bryce, D. Welti, A. E. Walsby, and B. W. Nichols, *Phytochemistry*, 1972, **11**, 295.
[2115] R. S. Appleby, R. Safford, and B. W. Nichols, *Biochim. Biophys. Acta*, 1971, **248**, 205.
[2116] P. Brush and E. Percival, *Phytochemistry*, 1972, **11**, 1847.
[2117] E. Peleg and A. Tietz, *F.E.B.S. Letters*, 1971, **15**, 309.
[2118] G. Laneelle, J. Asselineau, and G. Chamoiseau, *F.E.B.S. Letters*, 1971, **19**, 109.

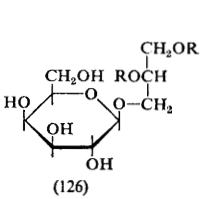
Deacylation of a D-glucose-containing phospholipid from *Acholeplasma laidlawii* gave a single, water-soluble phosphate ester that was distinguishable from synthetic glucosylphosphorylglycerols.[2119] Alkaline hydrolysis of the glycolipid gave a mixture of fatty acids, glycerol 2-phosphate, *sn*-glycerol 3-phosphate, and *O*-α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl-(1 → 1)-D-glycerol. Periodate oxidation showed that *sn*-glycerol 3-phosphate esterifies the 6-hydroxy-group of one of the D-glucose residues in the diglucosyldiglyceride. From the data obtained, it was concluded that the structure previously proposed for the glycolipid is incorrect; in fact, the compound is a new type of bacterial glycolipid possessing the structure 3-{*sn*-glycerol-3-phosphoryl-6′-(*O*-α-D-glucopyranosyl-(1 → 2)-*O*-α-D-glucopyranosyl)}-*sn*-1,2-diglyceride. The location of the fatty-acid residues was also established.

A novel type of fungal glycolipid has been obtained in pure form from *Aspergillus niger* by column chromatography on Fluorosil and t.l.c. of an acetone extract.[2120] From i.r. and mass spectral data, together with evidence of its alkaline stability, it was established that the compound is a monoglucoside of a *trans*-2-hydroxyoctadecanoic acid. The glycolipid appears to have an important role in maintaining the structure and the function of fungal membranes.

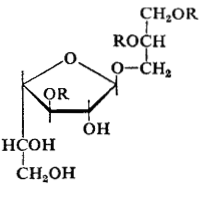
When *Bacillus megaterium* was harvested in the stationary phase of growth from a medium in which the pH was gradually lowered from 7.2 to 5.0 during growth, two glycophospholipid isomers were found to be present.[2121] The isomers were characterized by a number of chemical and enzymic procedures as 3-*sn*-phosphatidyl-1′-{3′-(2-amino-2-deoxy-β-D-glucopyranosyl)}-*sn*-glycerol and 3-*sn*-phosphatidyl-1′-{2′-(2-amino-2-deoxy-β-D-glucopyranosyl)}-*sn*-glycerol; these structures were also confirmed by synthesis. Accumulation of the latter isomer was favoured by a low environmental pH. In addition to the two glycolipids, a compound containing 2-amino-2-deoxyglucose, glycerol, and fatty acids (1 : 1 : 2), but no phosphate, has been isolated from the organism.[2122] The new glycolipid constituted 5% of the total lipid 2-amino-2-deoxyglucose. Partial acidic or alkaline hydrolysis yielded 1-*O*-(2-acetamido-2-deoxy-β-D-glucosyl)glycerol, while deamination with nitrous acid yielded a diglyceride and 2,5-anhydro-D-mannose. The glycolipid was identified finally as 1-*O*-(2-acetamido-2-deoxy-β-D-glucosyl)-2,3-diglyceride. Variation of the diglucosyldiglyceride content of *B. subtilis* with changes in the conditions of growth has been investigated.[2123]

[2119] N. Shaw, P. F. Smith, and H. M. Verheij, *Biochem. J.*, 1972, **129**, 167.
[2120] R. A. Laine, P. F. S. Griffin, C. C. Sweeley, and P. J. Brennan, *Biochemistry*, 1972, **11**, 2267.
[2121] J. A. F. Opdenkamp, H. M. Verheij, and L. L. M. van Deenen, *Bioorganic Chem.*, 1972, **1**, 174.
[2122] P. J. R. Phizackerley, J. C. MacDougall, and R. A. Moore, *Biochem. J.*, 1972, **126**, 499.
[2123] D. E. Minnikin, H. Abdolrahimzadeh, and J. Baddiley, *F.E.B.S. Letters*, 1972, **27**, 16.

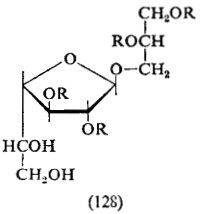
The structures of six major galactosyldiglycerides from *Bifidobacterium bifidum* have been established as (126)—(131) by chemical analysis and from the results of chemical and enzymic degradations.[2124] It was noted



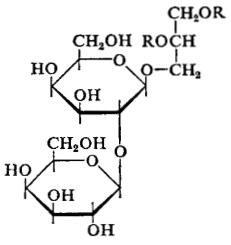
(126)



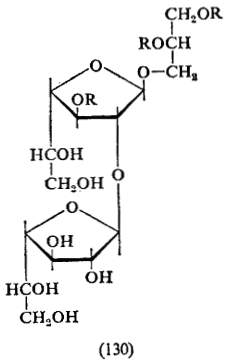
(127)



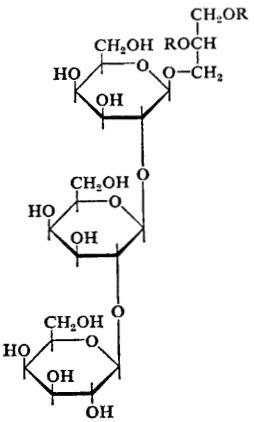
(128)



(129)



(130)



R = fatty-acid groups

(131)

that all are β-D-galactosides of 1′,2′-di-*O*-acyl-*sn*-glycerol having β-(1 → 2)-linkages between the D-galactose residues and that mono- and di-acylated sugar rings have the furanose form, whereas those in which the carbohydrates are not acylated are generally in the pyranose form.

Glucosyl- and acetyl-transferases involved in the biosynthesis of glycolipids of *Candida bogoriensis* have been studied.[2125] A glycolipid extract

[2124] J. H. Veerkamp, *Biochim. Biophys. Acta*, 1972, **273**, 359.
[2125] T. W. Esders and R. J. Light, *J. Biol. Chem.*, 1972, **247**, 1375.

has been obtained from a heptose-deficient mutant of *Escherichia coli* with di-isobutyl ketone–acetic acid–water.[2126] A glycolipid purified from the extract by ion-exchange chromatography was found to contain 2-amino-2-deoxyglucose, phosphate, 2-keto-3-deoxyoctonate, acetate, and fatty acids (1 : 2 : 2 : 1.7 : 5), as well as another amino-sugar that appeared to be 4-amino-4-deoxyarabinose.

The major glycolipid component of an extreme thermophile *Flavobacterium thermophilum* has been extracted and it was shown to contain D-galactose, D-glucose, 2-amino-2-deoxy-D-glucose, and glycerol (molar ratio 2 : 1 : 1 : 1).[2127] On the basis of methylation studies, one of two possible structures [(132) or (133)] was proposed for the glycolipid.

D-Gal*f*-(1 → 2)-D-Gal*p*-(1 → 6)-D-Glc*N*-(1 → 2)-D-Glc*p*-diglyceride
|
fatty
acid

(132)

D-Gal*f*-(1 → 2)-D-Gal*p*-(1 → 2)-D-Glc*p*-(1 → 6)-D-Glc*N*-diglyceride
|
fatty
acid

(133)

A series of esters of αα-trehalose and phleic acids (polyunsaturated acids, principally hexatriaconta-4,8,12,16,20-pentaenoic acid) has been isolated from *Mycobacterium phlei*.[2128] The compounds differ in the number of esterified hydroxy-groups and the principal member of the series has been obtained in a pure form by chromatography. Compositional analysis and i.r. and ^{1}H n.m.r. spectroscopy of this compound (molecular weight 4400) indicated that it is an octaphleate of αα-trehalose. The effect of αα-trehalose 6,6′-dimycolate from *M. tuberculosis* on mouse liver microsomes has been studied *in vivo*.[2129]

Serologically active glycolipids of *Mycoplasma pneumoniae* have been purified by combined chromatography on DEAE-cellulose and silicic acid.[2130] The glycolipid haptens were found to become immunogenic when bound to membrane proteins of *Acholeplasma laidlawii* by reaggregation. Several *Nocardia* sp. have been shown to produce large quantities of glycolipids containing αα-trehalose when cultured in D-glucose-rich media.[2131] One of the glycolipids was identified as a trehalose dimycolate.

[2126] S. A. Rooney and H. Goldfine, *J. Bacteriol.*, 1972, **111**, 531.
[2127] M. Oshima and T. Yamakawa, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 185.
[2128] C. P. Asselineau, H. L. Montrozier, J. C. Promé, A. M. Savagnac, and M. Welby, *European J. Biochem.*, 1972, **28**, 102.
[2129] K. Fukuyama, J. Tani, and M. Kato, *J. Biochem.* (*Japan*), 1971, **69**, 511.
[2130] S. Razin, B. Prescott, W. D. James, G. Caldes, J. Valdesuso, and R. M. Chanock, *Infection and Immunity*, 1971, **3**, 420.
[2131] I. Yano, Y. Furukawa, and M. Kusunose, *Jap. J. Gen. Appl. Microbiol.*, 1971, **17**, 329.

A lipopolysaccharide–protein complex extracted from the cell envelopes of *Pseudomonas aeruginosa* has been spread on aqueous surfaces as a monolayer.[685] The surface pressure, viscosity, and potential were measured as a function of the surface area. The film of the complex was formed principally from protein, which was stiffened and stabilized by lipopolysaccharide attached to the lower surface. The phosphoglucolipid from *P. diminuta* has been identified as a phosphatidylglucosyldiglyceride, *viz.* a tetra-acyl derivative of 3-*O*-(6′-*sn*-glycero-3″-phosphoryl)-α-D-glucopyranosyl-*sn*-glycerol.[2132] UDP-D-[^{14}C]glucuronic acid has been incorporated into a lipid of *P. diminuta* by a particulate fraction.[2133] The glycolipid formed was characterized as a glucuronosyldiglyceride by methanolysis and methylation studies.

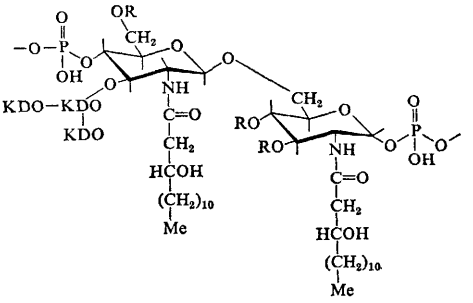
Seven inositol-containing phospholipids occurring in *Saccharomyces cerevisiae* have been resolved by two-dimensional chromatography on silicic acid-impregnated paper.[2134] Four of the glycolipids were found to be stable to mild alkali and methanolysis, and one of them has been identified as a mannosyldi(inositolphosphoryl)ceramide. Using radioactive '2-deoxy-D-glucose' as precursor, the labelling of the glycolipids was investigated during culture of the organism. The occurrence of glycolipids in the cell-envelope fractions of bakers' yeast (*S. cerevisiae*) has been investigated.[2135] A complex glycosphingolipid containing mannose, inositol, and phosphorus was also isolated from the cell envelope.

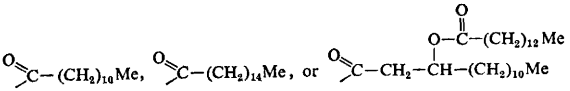
Various methods for the stepwise liberation of fatty acids have been used to ascertain the distribution of acyl groups on the backbone of the lipid A component of *Salmonella minnesota* lipopolysaccharide.[2136] A structure (134) was proposed for the disaccharide unit of the lipid A component and a similar structure appears to exist in lipopolysaccharides of other *Salmonella* sp.

The consequences of glycerol deprivation on the biosynthesis of membrane components of a glycerol auxotroph of *Staphylococcus aureus* have been investigated.[2137] An immediate decrease was observed in the rates of synthesis of monoglucosyl- and diglucosyl-diglycerides, whereas there was conservation of the complex glucolipids.

The biosynthesis of the phosphatidyldiglucosyldiglyceride of *Streptococcus faecalis* from diglucosyldiglyceride and either phosphatidyl- or diphosphatidyl-glycerol has been examined.[2138] The phosphatidyl group was transferred from either of the latter two compounds to diglucosyldiglyceride in a reaction catalysed by a disrupted membrane preparation from the organism.

[2132] S. G. Wilkinson and M. E. Bell, *Biochim. Biophys. Acta*, 1971, **248**, 293.
[2133] J. M. Shaw and R. A. Pieringer, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1201.
[2134] S. Steiner and R. L. Lester, *J. Bacteriol.*, 1972, **109**, 81.
[2135] T. Nurminen and H. Suomalainen, *Biochem. J.*, 1971, **125**, 963.
[2136] E. T. Rietschel, H. Gottert, O. Lüderitz, and O. Westphal, *European J. Biochem.*, 1972, **28**, 166.
[2137] P. H. Ray, T. T. Lillich, and D. C. White, *J. Bacteriol.*, 1972, **112**, 413.
[2138] R. A. Pieringer, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 502.



(134) R = 

with one mole of each per unit; KDO = 2-keto-3-deoxyoctonate

An extract from *Treponema reiteri* has been shown to contain a galactosyl-diglyceride hapten, *viz.* 2,3-di-*O*-acyl-1-*O*-β-D-galactopyranosyl-D glycerol.[2064] The hapten showed a cross-reaction with cerebrosides and bovine nervous tissues.

The envelope of Semliki Forest virus, a group A arbovirus, has been found to contain a glucose-containing glycolipid.[690]

8

Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, and Glycolipids

Synthesis of Polysaccharides, Oligosaccharides, Glycoproteins, Glycolipids, and Gangliosides

Polysaccharides.—The enzymic synthesis of amylose and comb- or star-shaped branched structure glucans has been reported [1455] and the degree of branching in the product could be controlled.

α-D-Glucopyranosyl fluoride has been shown to parallel sucrose in serving as an effective substrate for dextransucrase.[2139] A soluble dextran can be formed from the fluoro-compound by the action of the dextransucrase from *Leuconostoc mesenteroides*, whereas water-soluble and -insoluble dextrans were produced by the action of the enzyme from *Streptococcus* sp.

The synthesis of an α-(1 → 6)-linked 2,3,4-tri-*O*-methylglucan using 1,6-anhydro-2,3,4-tri-*O*-methyl-β-D-glucopyranose and chlorosulphonic acid in an atmosphere of nitrogen has been reported.[2140] The polysaccharide was found to be microcrystalline and its i.r. spectrum was identical with that of linear, methylated dextrans. The structure of the polysaccharide was studied further and the fully methylated compound was shown to possess a molecular weight of 2.9×10^4.

Use of the sedimentation velocity parameter in the determination of the molecular weight distribution of synthetic, stereoregular, tribenzylated α-(1 → 6)-glucans has been investigated.[2141] Moderate to marked dependencies on pressure and concentration, accompanied by diffusion, were observed with two polymeric samples of low and medium polydispersity. Corrections for these effects were applied in a sequential manner, each effect being treated independently from the other. Values for the degrees of polydispersity obtained from the corrected sedimentation coefficients compared favourably with estimates obtained by independent methods.

Whereas phosphorus pentoxide–DMSO caused predominantly oxidation of carbohydrates at 60 °C, the same reagent has been shown principally

[2139] D. S. Genghof and E. J. Heare, *Proc. Soc. Exp. Biol. Med.*, 1972, **140**, 1298.
[2140] A. Klemer and C. Apostolides, *Carbohydrate Res.*, 1972, **22**, 432.
[2141] J. P. Merle and A. Sarko, *Macromolecules*, 1972, **5**, 132.

to catalyse a polymerization reaction at temperatures below 35 °C.[2142] A series of synthetic polysaccharides were prepared from D-glucose, lactose, maltose, cellobiose, D-glucose with α-D-glucose 1-phosphate, 2-acetamido-2-deoxy-D-glucose, L-arabinose, D-xylose, D-xylose with xylan, 2-acetamido-2-deoxy-D-glucose with D-glucose, D-glucose with D-glucofuranurono-6,3-lactone, and 2-acetamido-2-deoxy-D-glucose with D-glucofuranurono-6,3-lactone in up to 48% yield by the action of phosphorus pentoxide–DMSO at 10—25 °C. The polysaccharides produced possessed $S_{20,w}$ values of 0.68—1.34 and DPs of 15.3—4.7 monosaccharide units per chain on the basis of reducing-end-group assays. However, it was noted that additional reducing groups might have arisen during the reaction. Methylation analysis of one of the synthetic glucans revealed that it contained α-(1 → 4)- and α-(1 → 6)-D-glucosidic linkages in the main chain, which was also branched in various ways. The synthetic polysaccharides were found to contain 1.3—20.9% of phosphorus.

Examination of a chemically synthesized α-(1 → 6)-D-mannopyran, obtained by the phosphorus pentafluoride-catalysed polymerization of 2,3,4-tri-*O*-benzyl-β-D-mannopyranose, by degradation with an α-mannanase from an *Arthrobacter* sp., revealed that flaws occur in the regularity of the polysaccharide at a frequency of less than one residue per hundred.[2143] A synthetic mannan consisting of α-(1 → 6)-linked D-mannopyranose units, which resembled the basic chain of the dermatophyte galactomannans in having a molecular weight of 4×10^4, has been shown to induce humoral antibodies in mice.[2144] The polysaccharide also induced a delayed cutaneous reactivity in guinea-pigs and conferred reactivity to a dermatophyte polysaccharide.

A β-(1 → 4)-linked mannan has been produced enzymically by the action of a mannose polymerase, derived from the hypocotyls of *Phaseolus aureus*, on GDP-D-mannose.[2145] The only other carbohydrate that appeared to be polymerized by the enzyme was D-glucose from GDP-D-glucose.

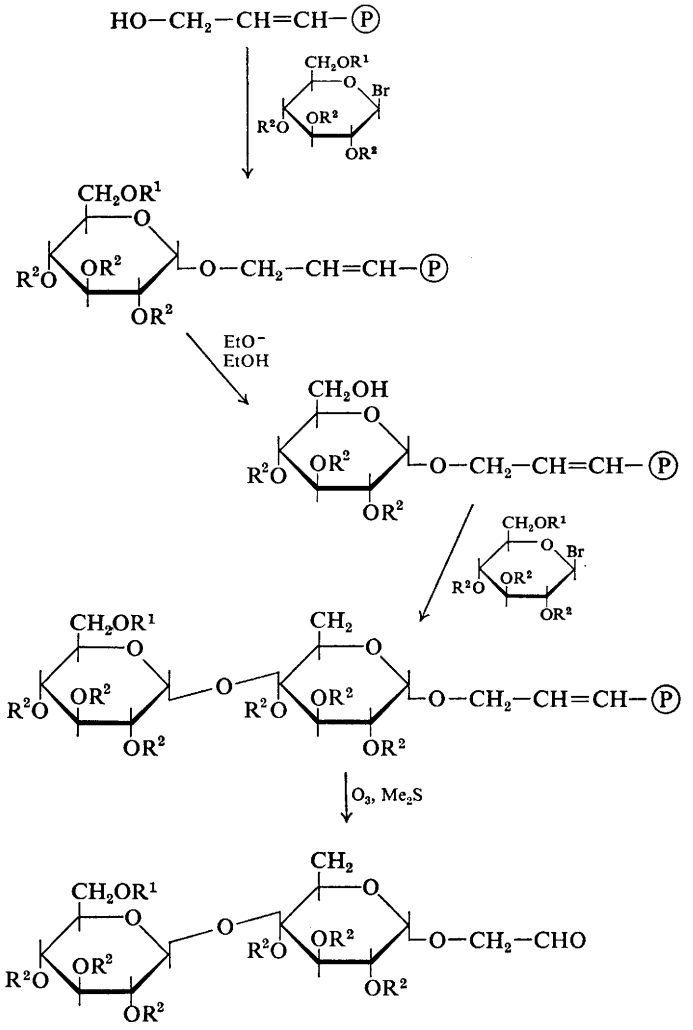
In an investigation of the thermal reactions of α-D-xylopyranose and methyl, 4-methoxyphenyl, phenyl, 4-phenylphenyl, 4-chlorophenyl, and 4-nitrophenyl β-D-xylopyranosides, polymerization of the glycosyl moieties was observed, particularly when zinc chloride was added as a catalyst.[2146] The polymerization reactions occurred at much lower temperatures than decomposition of the polymers formed.

Oligosaccharides.—Poly-{4-(propen-1-yl-3-ol)styrene}, a polystyrene resin containing allyl alcohol functional groups, has been prepared and used as the matrix in the solid-phase synthesis of oligosaccharides.[2147] Synthesis of

[2142] S. Hirano, N. Kashimura, N. Kosaka, and K. Onodera, *Polymer*, 1972, **13**, 190.
[2143] J. S. Tkacz, J. O. Lampen, and C. Schuerch, *Carbohydrate Res.*, 1972, **21**, 465.
[2144] S. F. Grappel, *Experientia*, 1971, **27**, 329.
[2145] J. S. Heller and C. L. Villemez, *Biochem. J.*, 1972, **128**, 243.
[2146] F. Shafizadeh, G. D. McGinnis, R. A. Susott, and H. W. Tatton, *J. Org. Chem.*, 1971, **36**, 2813.
[2147] J. M. Frechet and C. Schuerch, *J. Amer. Chem. Soc.*, 1971, **93**, 492.

oligosaccharides using this matrix has been tested with an appropriately substituted glycosyl halide and is illustrated by the preparation of a disaccharide (Scheme 15).

D-Fructose oligosaccharides have been synthesized enzymically with the aid of a β-fructofuranosidase from *Claviceps purpurea*.[1540] Gentio-triose,



$R^1 = -\overset{O}{\overset{\|}{C}}-C_6H_4NO_2\text{-}p$,

R^2 = Bn, Ⓟ = polymer main chain

Scheme 15

-tetraose, -pentaose, and -hexaose have been synthesized by Koenigs–Knorr condensations of glycosyl bromides of acetylated oligosaccharides with preformed oligosaccharide blocks bearing unprotected primary hydroxy-groups at the non-reducing terminals[2148] (Table 2). Incidental acetyl migrations occurring during the reaction were discussed.

O-α-D-Glucopyranosyl-(1 → 4)-[*O*-(α-D-glucopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 6)]-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucose (6^2-α-maltosylmaltotriose) has been synthesized enzymically from cyclohexaamylose and panose with the aid of a transglycosylase from *Bacillus macerans*;[1749] this particular oligosaccharide was isolated from the mixture of products obtained. 1-*O*-α-D-Glucopyranosyl-L-sorbose has been shown to be the main product synthesized by a transglucosidation reaction using an α-glucosidase from Brewer's yeast;[1638] 3- and 4-*O*-α-D-glucopyranosyl-L-sorbose were also formed by the enzyme.

Mannodextrins comprising β-(1 → 4)-linked D-mannopyranose units have been formed by hydrolysis of ivory-nut mannan with fuming hydrochloric acid at 0 °C and separation of the oligosaccharides on charcoal–Celite.[2149]

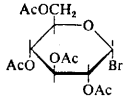
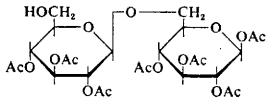
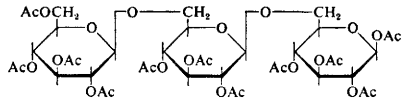
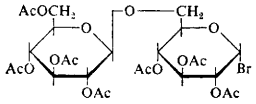
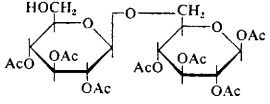
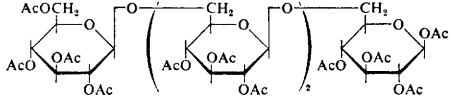
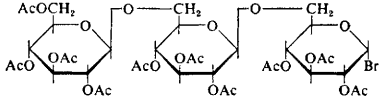
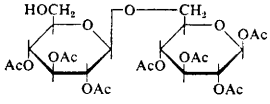
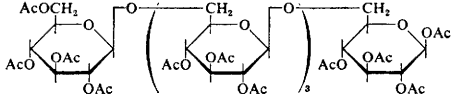
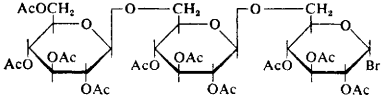
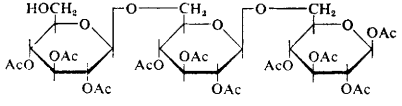
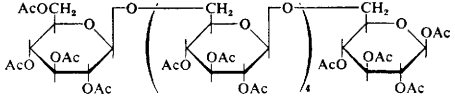
Glycoproteins.—A pseudo-glycoprotein has been prepared by conjugation of bovine serum albumin with diazotized 4-aminophenyl β-panoside.[2150] The product was used as an artificial antigen. Pseudo-glycoproteins have also been prepared by the coupling of diazotized aminophenyl β-lactosides to a histidine derivative of an acyl azide of Bio-Gel[2151] and to polyacrylamide.[2152] The products obtained were used for the separation of cells by affinity chromatography and for the isolation of lymphocytes that specifically bind a lactoside hapten, respectively.

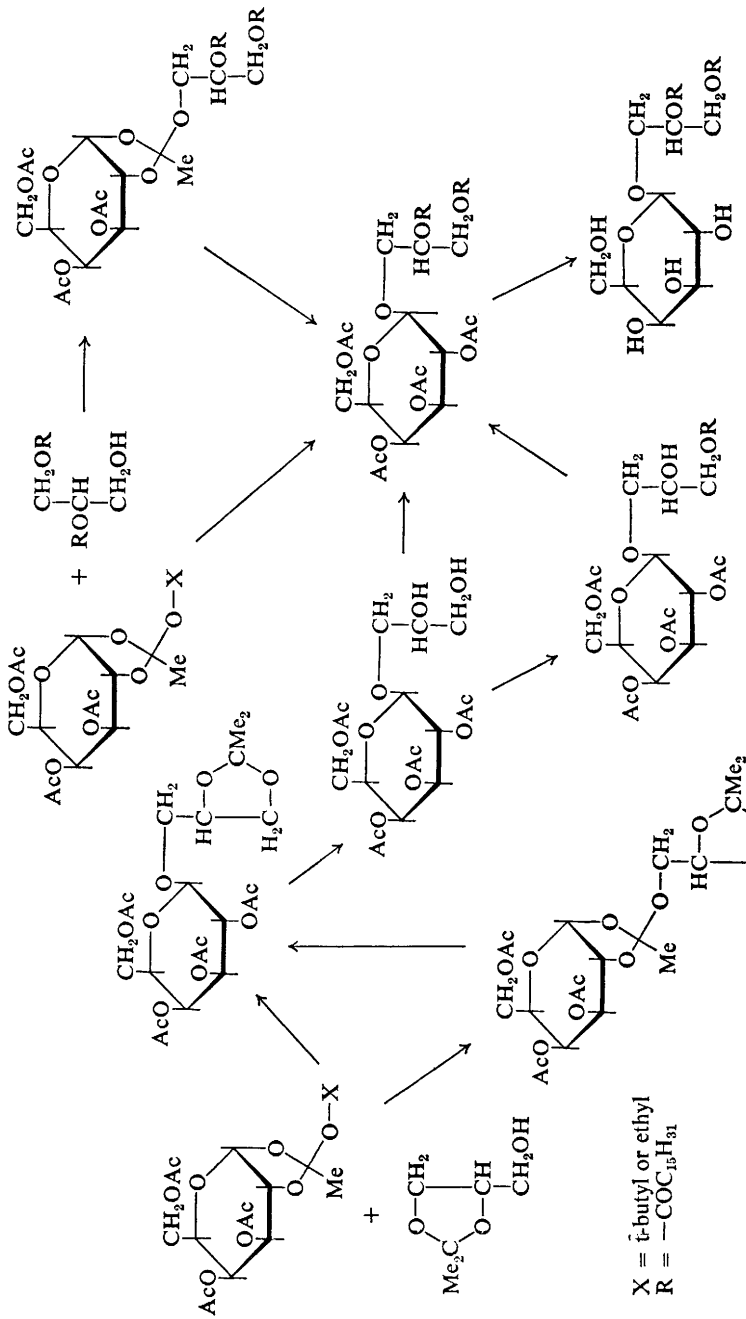
Glycolipids and Gangliosides.—Methodology for the synthesis of cerebrosides[1990] and a semi-synthetic method for the preparation of glucocerebrosides have been reported.[2153]

Methods for the directed synthesis of various acylglycosyldiglycerides have been published[2154] and routes for the synthesis of D-galactosylglycerides have been described (Scheme 16).[2155] In the presence of glycerol as acceptor, β-galactosidase from *Escherichia coli* catalysed the conversion of D-galactal into glyceryl 2-deoxy-β-D-*lyxo*-hexoside.[1589] The corresponding glyceryl 2-deoxy-β-D-*arabino*-hexoside was formed by the action of β-glucosidase from sweet almond oil on D-glucal and glycerol.

[2148] K. Takiura, S. Honda, T. Endo, and K. Kakehi, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 438.
[2149] W. Brown and K. Chitumbo, *J. Chromatog.*, 1972, **66**, 370.
[2150] R. S. Martineau, P. Z. Allen, I. J. Goldstein, and R. N. Iyer, *Immunochemistry*, 1971, **8**, 705.
[2151] L. Wofsy, J. Kimura, and P. Truffa-Bachi, *J. Immunol.*, 1971, **107**, 725.
[2152] C. Henry, J. Kimura, and L. Wofsy, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 34.
[2153] A. Y. Veinberg, S. Y. Mel'nik, G. I. Roslovtseva, and G. I. Samokhvalov, *Zhur. obshchei Khim.*, 1970, **40**, 2745.
[2154] A. I. Bashkatova, N. N. Senyushkina, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1972, **8**, 208.
[2155] A. I. Bashkatova, G. V. Smirnova, V. N. Volynskaia, V. I. Shvets, and R. P. Evstigneeva, *Doklady Akad. Nauk S.S.S.R.*, 1971, **201**, 1231.

Table 2 *Synthesis of gentio-oligosaccharides by block condensation*

Reactants		*Product*	*Yield/%*
Bromide	*Alcohol*		
			43
			31
			31
			19



Scheme 16

3-*sn*-Phosphatidyl-1′-{3′-(2-amino-2-deoxy-β-D-glucopyranosyl)}-*sn*-glycerol and 3-*sn*-phosphatidyl-1′-{2′-(2-amino-2-deoxy-β-D-glucopyranosyl)}-*sn*-glycerol have been synthesized.[2121] A cell-free particulate system from *Mycobacterium smegmatis* has been shown to synthesize D-[^{14}C]mannosyl-phosphoryl-lipid and radioactive *myo*-inositol oligomannosides from GDP-D-[^{14}C]mannose.[2156]

Modification of Polysaccharides and Oligosaccharides, and Uses of Modified Polysaccharides and Oligosaccharides

The methylation of polysaccharides and oligosaccharides for structural analysis has been described and detailed attention has been accorded to the analysis of the products of hydrolysis of methylated carbohydrates by g.l.c. and mass spectrometry.[2157] Methodology has been described for the sulphation of polysaccharides.[2158]

From an investigation of the periodate oxidation of a range of polysaccharides, it was concluded that inter-residue hemiacetal formation is a general feature of this type of oxidation.[2159] It was also apparent that the relative abundance of inter- and intra-residue cyclic structures in any instance is fairly predictable from established stabilities based upon ring size and type. Reduction of the hemiacetals with sodium borohydride, after completion of the initial rapid phase of the oxidation, permitted the smooth consumption of further periodate at a rate close to the initial rate and also allowed attainment of the correct Malapradian-oxidation limit. Methodology has been described for the oxidation of polysaccharides with lead tetra-acetate in DMSO.[2160]

The formation of an insoluble complex between water-soluble DEAE-dextran and acidic polysaccharides has proved to be useful in detecting acidic polysaccharides in gels.[2161] The reaction was particularly applicable to electrophoretic and Ouchterlony-type diffusions, and the converse procedure (using dextran sulphate as precipitant) was effective for the detection of basic polysaccharides.

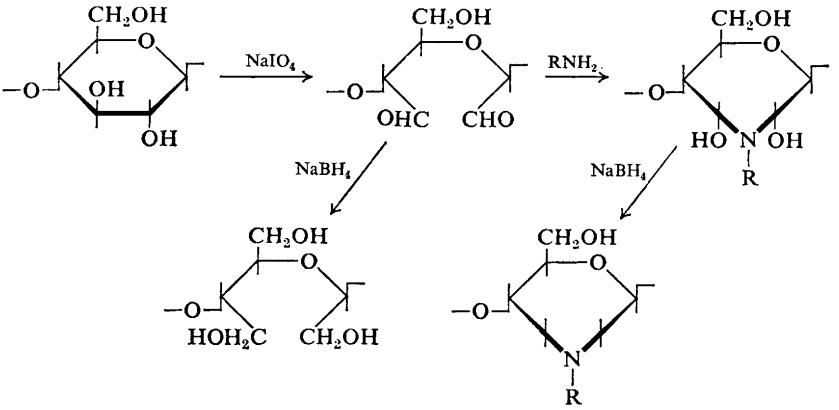
Critical features of the Morgan assay of hydroxyethyl and hydroxypropyl substituents in polysaccharides have been examined and conditions for obtaining accurate and reproducible values have been described.[2162] Compounds suitable for a standardization of the procedure have been reported.

The molecular weights and sulphate contents of reaction products obtained on treatment of a range of polysaccharides with concentrated sulphuric acid at 0 °C for 2 h have been determined.[2163]

[2156] K. Takayama and E. L. Armstrong, *F.E.B.S. Letters*, 1971, **18**, 67.
[2157] H. Björndal, C. G. Hellerqvist, B. Lindberg, and S. Svensson, *Angew. Chem. Internat. Edn.*, 1970, **9**, 610.
[2158] R. L. Whistler, *Methods Carbohydrate Chem.*, 1972, **6**, 426.
[2159] M. F. Ishak and T. Painter, *Acta Chem. Scand.*, 1971, **25**, 3875.
[2160] C. T. Bishop, *Methods Carbohydrate Chem.*, 1972, **6**, 350.
[2161] W. F. Dodman, *Analyt. Biochem.*, 1972, **46**, 668.
[2162] A. N. de Belder, A. Persson, and S. Makstrom, *Stärke*, 1972, **24**, 361.
[2163] K. Nagasawa, Y. Tohira, Y. Inoue, and N. Tanoura, *Carbohydrate Res.*, 1971, **18**,95.

Derivatives of polysaccharides have continued to be popular as matrices for the covalent insolubilization of enzymes and many reviews of immobilized enzymes have appeared.[638, 1449, 2164–2168] The reviews have included sections on enzymes attached to polysaccharides and some have provided classified lists of references. Methodology has also been reported for the attachment of proteolytic enzymes to polysaccharides [2169] and for the attachment of enzymes to cellulose.[2170] Polysaccharide derivatives have been used extensively as matrices for the preparation of materials for affinity chromatography in which a ligand exhibiting an affinity for the species to be purified is attached to the matrix. Reviews of affinity chromatography have included details on the applications of polysaccharides in this area.[1446, 2164, 2167, 2171–2174]

Polysaccharides oxidized to their dialdehyde derivatives with periodate ion have been used for insolubilization of proteinaceous molecules by reaction between the aldehydic groups and side-chain amino-groups of the protein chain (Scheme 17).[1196] The carbinolamine-type linkages formed can be stabilized by reduction to tertiary amines with sodium borohydride and the reduction also served to remove unreacted aldehyde groups.



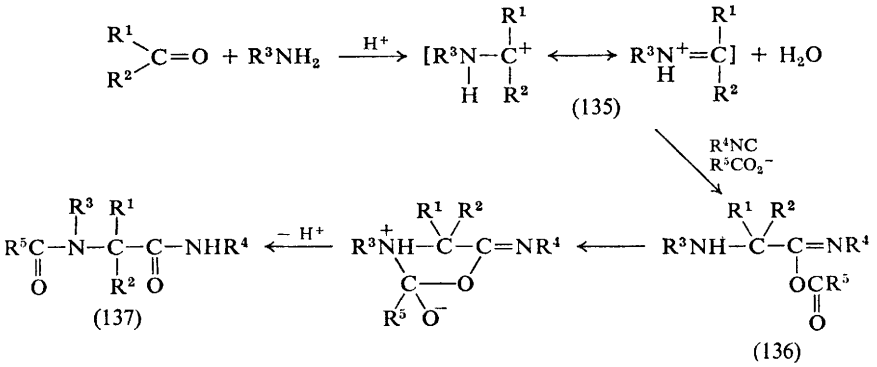
R = rest of proteinaceous molecule

Scheme 17

[2164] F. Friedberg, *Chromatog. Rev.*, 1971, **14**, 121.
[2165] J. Gryszkiewicz, *Folia Biol.*, 1971, **19**, 119.
[2166] G. J. H. Melrose, *Rev. Pure Appl. Chem.*, 1971, **21**, 83.
[2167] H. D. Orth and W. Brümmer, *Angew. Chem. Internat. Edn.*, 1972, **11**, 249.
[2168] Anonymous, *Chem. Eng. News*, 1969, **47**, No. 40, p. 44.
[2169] L. Goldstein, *Methods Enzymol.*, 1970, **19**, 935.
[2170] E. M. Crook, K. Brocklehurst, and C. W. Wharton, *Methods Enzymol.*, 1970, **19**, 963.
[2171] P. Cuatrecasas, in 'Biochemical Aspects of Reactions on Solid Supports', ed. G. R. Stark, Academic Press, New York, 1971, p. 79.
[2172] P. Cuatrecasas and C. B. Anfinsen, *Methods Enzymol.*, 1971, **22**, 345.
[2173] I. Parikh and P. Cuatrecasas, *Vox Sanguinis*, 1972, **23**, 141.
[2174] M. Wilchek, *Biochem. J.*, 1972, **127**, 7P.

The derivatization of polysaccharides with cyanogen bromide to give, supposedly, cyclic imidocarbonate derivatives has been used extensively as a means of activating the carbohydrate for the covalent attachment of enzymes, affinity ligands, and immunologically reactive species, *etc.* In order to define more closely the reaction of polysaccharides with cyanogen bromide, model studies have been carried out using methyl 4,6-*O*-benzylidene-α-D-glucopyranoside.[2175] The main component of the mixture of products was identified as methyl 4,6-*O*-benzylidene-α-D-glucopyranoside 2,3-imidocarbonate and small amounts of the corresponding 2,3-carbonate and 2- and 3-carbamates were also formed.

An amide linkage between acidic polysaccharides and the amino-groups of enzymes can be formed (Scheme 18) by the reaction of aldehydes or



Scheme 18

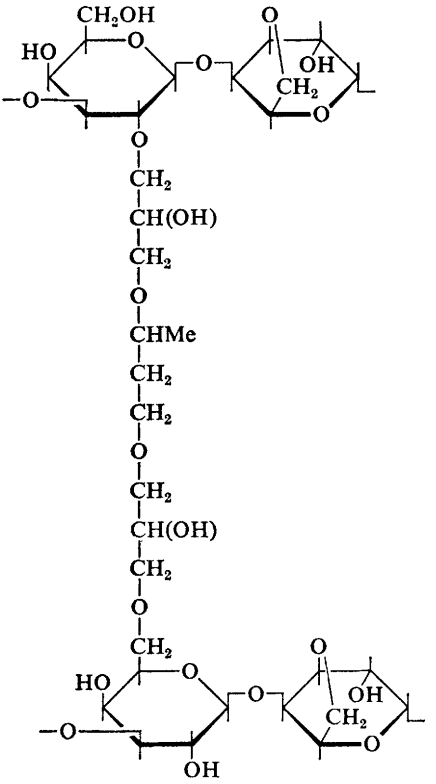
ketones with amines to give an immonium ion (135), which may be treated with isocyanide (R^4NC) to give an intermediate that reacted with carboxylate ions.[2176] The product (136) rearranged to give a stable amide (137). Clearly, in this reaction, the polysaccharide matrix can be either an aldehyde (*e.g.* certain periodate-oxidized polysaccharides) or a derivative bearing one of the other groups involved in the reaction, whereas carboxy- or amino-groups in the enzyme can be employed.

Agar, Agaropectin, and Agarose.—It has been proposed that the accepted structures of agaropectin and agarose are oversimplifications of the true situation and that agarose occurs only to very small extents in agar.[2177] Most molecules in agar carry carboxylate and/or sulphate groups and are not suitable for the preparation, by separation alone, of an agarose having the requisite properties for electrophoresis and enzyme insolubilization. Charge-free agar has been produced, therefore, by alkaline desulphation in the presence of sodium borohydride followed by reduction, with lithium

[2175] L. Ahrgren, L. Kågedal, and S. Åkerström, *Acta Chem. Scand.*, 1972, **26**, 285.
[2176] R. Axén, P. Vretblad, and J. Porath, *Acta Chem. Scand.*, 1971, **25**, 1129.
[2177] T. Låås, *J. Chromatog.*, 1972, **66**, 347.

aluminium hydride, of the carboxy-groups. The charge-free agar was used successfully for the electrophoresis of very basic proteins for which commercially available agars and agaroses are unsuitable.

Epichlorohydrin-cross-linked, desulphated agar has been produced by treatment of swollen agar or agarose with epichlorohydrin in alkaline sodium borohydride.[2178] Subsequent autoclaving of the product with alkaline borohydride at 120 °C gave a desulphated material. The reduced form of this material was produced by acetylation with acetic anhydride, reduction with lithium aluminium hydride, and alkaline deacetylation. Bisepoxide-cross-linked, desulphated agarose (138) was obtained by reaction of Sepharose (see later) with 1,3-bis-(2,3-epoxypropoxy)butane in



(138)

alkaline borohydride and subsequent autoclaving. The properties of these materials were investigated with regard to their suitability as chromatographic media and they were found to be superior to those of agar and agarose by possessing lower adsorption capacities.

[2178] J. Porath, J.-C. Janson, and T. Låås, *J. Chromatog.*, 1971, **60**, 167.

By using various concentrations of acrylamide monomer and agarose, a series of composite gels with various degrees of cross-linking has been prepared in bead form by polymerization of the acrylamide.[2179] The molecular sieve properties of these gels were studied; the gels were found to possess a good degree of selectivity on protein mixtures and uniform mechanical properties due to the agarose matrix. The very low tendency of these gels to shrink allowed high elution rates. Agar has been used as a binder in the t.l.c. of amino-acids on silica gel.[2180]

Sepharose (a commercially available macroporous agarose) has been converted into an aldehydo-derivative by periodate oxidation and the oxidized product has been used for the insolubilization of proteinaceous materials (particularly bovine serum albumin) by reaction between the

Table 3 *Use of Sepharose cyclic imidocarbonate for the preparation of active water-insoluble enzymes*

Enzyme	*E.C. No.*	*Ref.*
D-Amino-acid oxidase[a, b]	1.4.3.3	2181
Aminopeptidase	3.4.1.2/3	2182, 2183
Chymotrypsin	3.4.4.5/6	2182, 2184, 2185, 2186
Glucoamylase	3.2.1.3	1778[c]
Glucose oxidase	1.1.3.4	1778[c]
Guanyloribonuclease	2.7.7.26	2187
Lipoxygenase	1.13.1.13	2188
Pancreatopeptidase E	3.4.4.7	2189
Pepsin[d]	3.4.4.1	2190
Prolidase	3.4.3.7	2182
Proteinase (bacterial)		2191
Renin	3.4.4.15	1182
Ribonucleic acid nucleotidyltransferase		2192
Trypsin	3.4.4.4	2193, 2194, 2186

[a] See (140A), p. 536; [b] reaction involving carbodi-imide; [c] mixed enzyme derivative; [d] see text, p. 531.

[2179] E. Boschetti, R. Tixier, and J. Uriel, *Biochimie*, 1972, **54**, 439.
[2180] M. Jellinek, *J. Chromatog.*, 1972, **69**, 402.
[2181] S. C. Tu and D. B. McCormick, *Separation Sci.*, 1972, **7**, 403.
[2182] H. P. J. Bennett, D. F. Elliott, P. J. Lowry, and C. McMartin, *Biochem. J.*, 1971, **125**, 80P.
[2183] D. H. Calam and H. J. Thomas, *Biochim. Biophys. Acta*, 1972, **276**, 328.
[2184] V. Kasche, H. Lundqvist, R. Bergman, and R. Axén, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 615.
[2185] D. Gabel, I. Z. Steinberg, and E. Katchlaski, *Biochemistry*, 1971, **10**, 4661.
[2186] R. Axén and S. Ernback, *European J. Biochem.*, 1971, **18**, 351.
[2187] J. C. Lee, *Biochim. Biophys. Acta*, 1971, **235**, 435.
[2188] S. Grossman, M. Trop, P. Budowski, M. Perl, and A. Pinsky, *Biochem. J.*, 1972, **127**, 909.
[2189] L. Sundberg and T. Kristiansen, *F.E.B.S. Letters*, 1972, **22**, 175.
[2190] P. Vretblad and R. Axén, *F.E.B.S. Letters*, 1971, **18**, 254.
[2191] D. Gabel and B. von Hofsten, *European J. Biochem.*, 1970, **15**, 410.
[2192] S. Litvak, L. Tarrago-Litvak, D. S. Carré, and F. Chapeville, *European J. Biochem.*, 1971, **24**, 249.
[2193] D. Gabel, P. Vretblad, R. Axén, and J. Porath, *Biochim. Biophys. Acta*, 1970, **214**, 561.
[2194] D. Gabel and J. Porath, *Biochem. J.*, 1972, **127**, 13P.

aldehydic groups of the polysaccharide and side-chain amino-groups of the protein.[1196] The carbinolamine linkages formed were stabilized by reduction to alkylamines with sodium borohydride and the reduction also served to remove unreacted aldehyde groups. The bovine serum albumin adduct was used as an immunoadsorbent.

The cyclic imidocarbonate derivative of Sepharose, prepared by reaction of the polysaccharide with cyanogen bromide, has continued to be used extensively for the insolubilization of enzymes (Table 3). Covalent coupling is considered to occur by nucleophilic attack of amino-groups in the enzyme molecule on the carbon atom of the *trans*-imidocarbonate ring to give either an isourea derivative or an *N*-substituted imidocarbonate or an *N*-substituted carbonate. Recent references to analogous preparations of immunosorbents and affinity chromatography materials from Sepharose cyclic imidocarbonate are listed in Tables 4 and 5, respectively. These

Table 4 *Use of Sepharose cyclic imidocarbonate for the preparation of immunosorbents*

Immunologically active species coupled to CIC-agarose	*Use of product*	*Ref.*
Angiotensin β-amide	Preparation of anti-angiotensin β-amide	2195
Anti-dinitrophenyl	Isolation of dinitrophenyl peptides	2196, 2197, 2174
	Isolation of dinitrophenyl-sulphenyl peptides	2198
Anti-human chorionic somatomammotrophin	Purification of human chorionic somatomammotrophin	2199
Anti-human α-fetoprotein	Purification of α-fetoproteins	2200
Anti-human immunoglobulin α-, γ-, κ-, λ-, and μ-chains	Removal of classes of immunoglobulins from serum	1293
Anti-human immunoglobulin IgG light chain	Purification of immunoglobulin IgA from serum	1271
Anti-human α_2-macroglobulin	Investigation of interaction of α_2-macroglobulin with collagenase	1235
Anti-human myeloma immunoglobulins IgA, IgG, IgM	Removal of classes of immunoglobulins from serum	1293
Anti-human serum albumin	Removal of contaminant human serum albumin	1661
Anti-lactoferrin	Purification of free secretory component	1377
Anti-luteinizing hormone	Purification of ovine luteinizing hormone	1109
Anti-lysozyme	Experimental control	2201

[2195] E. Hurwitz, F. M. Dietrich, and M. Sela, *European J. Biochem.*, 1970, **17**, 273.
[2196] D. Givol and M. Rotman, *Israel J. Med. Sci.*, 1970, **6**, 452.
[2197] D. Givol, Y. Weinstein, M. Gorecki, and M. Wilchek, *Biochem. Biophys. Res. Comm.*, 1970, **38**, 825.
[2198] M. Wilchek and T. Miron, *Biochim. Biophys. Acta*, 1972, **278**, 1.
[2199] B. D. Weintraub, *Biochem. Biophys. Res. Comm.*, 1970, **39**, 83.
[2200] S. Nishi and H. Hirai, *Biochim. Biophys. Acta*, 1972, **278**, 293.
[2201] B. Bonavida, S. Fuchs, and M. Sela, *Biochem. Biophys. Res. Comm.*, 1970, **41**, 1335.

Table 4 (*cont.*)

Immunologically active species coupled to CIC-agarose	*Use of product*	*Ref.*
Anti-mouse immunoglobulin and myeloma immunoglobulins	Search for anti-carbohydrate antibodies in myeloma conditions	2202
Anti-nitrotyrosyl lysozyme	Isolation of nitrotyrosyl-containing peptides	2203
Anti-t-ribonucleic acid	Binding of t-ribonucleic acid	2201
Bovine serum albumin[a]	Isolation of anti-dextran	1198
Dinitrophenyl-ovalbumin	Isolation of anti-dinitrophenyl	2198
Human Bence Jones glycoprotein κ- and λ-chains	Isolation of anti-Bence Jones glycoprotein κ- and λ-chains	1293
Human chorionic gonadotrophin	Separation of specific and non-specific anti-follicle-stimulating hormone	1119
Human immunoglobulin	Specific binding of human immunoglobulin IgG	2204
Human immunoglobulin IgG	Purification of protein-A from *Staphylococcus aureus*	1315
Human myeloma immunoglobulins IgA, IgG, and IgM	Isolation of anti-human myeloma immunoglobulins IgA, IgG, and IgM	1293
Lysozyme and related peptides	Investigation of the antigenicity of hen egg-white lysozyme	1846, 1861
Protein-A from *Staphylococcus aureus*	Purification of human immunoglobulin IgG	1315
Ribonuclease and fragments thereof	Fractionation of anti-ribonucleases	2205
t-Ribonucleic acid	Binding of anti-t-ribonucleic acid	2201
Synthetic antigen	Purification of anti-t-ribonucleic acid	2201

[a] Subsequently reacted with periodate-oxidized dextran.

Table 5 *Use of Sepharose cyclic imidocarbonate for the preparation of affinity chromatography materials*

Ligand or affinant	*Use of product*	*Ref.*
2-Acetamido-2-deoxy-D-glucose	Purification of protein-A of lactose synthetase	2012
Acetylcholinesterase affinants (quaternary ammonium salts)	Purification of acetylcholinesterase	2206—2209

[2202] F. Melchers and M. Sela, *Immunochemistry*, 1970, **7**, 749.
[2203] M. Helman and D. Givol, *Biochem. J.*, 1971, **125**, 971.
[2204] W. G. Palmer, *Biochim. Biophys. Acta*, 1972, **278**, 299.
[2205] G. S. Omenn, D. A. Ontjes, and C. B. Anfinsen, *Nature*, 1970, **225**, 189.
[2206] N. Kalderon, I. Silman, S. Blumberg, and Y. Dudai, *Biochim. Biophys. Acta*, 1970, **207**, 560.
[2207] J. D. Berman and M. Young, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 395.
[2208] Y. Dudai, I. Silman, N. Kalderon, and S. Blumberg, *Biochim. Biophys. Acta*, 1972, **268**, 138.
[2209] Y. Dudai, I. Silman, M. Shinitsky, and S. Blumberg, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2400.

Table 5 (*cont.*)

Ligand or affinant	*Use of product*	*Ref.*
	Purification of acetylcholine receptor	2210
Alkylamines	Purification of glycogen phosphorylase and proteins	2211
Amino-acids and derivatives	Purification of carboxypeptidase	2212
	Purification of chlorismate mutase	2213, 2214
	Purification of plasminogen	2215
	Purification of tyrosine hydroxylase	2216
ε-Aminocaproyl-*N*-(4-chlorobenz)amide	Purification of thrombin	1991
1-{*N*-(6-aminohexyl)}-aminoglycerol 3-phosphate	Isolation of flavin-linked glycerol 3-phosphate dehydrogenase	2217
2-(4-aminophenoxy)-propoxybenzamide	Separation of bovine α- and β-trypsins	2218
Anthranilic acid	Determination of steric requirements for substrate binding by 5-phosphoribosyl pyrophosphate phosphoribosyltransferase	2219
ATPase inhibitor	Affinity chromatography of ATPase	2220
Butyl aminobenzoate	Assay of plasminogen by affinity chromatography	2221
Collagen	Purification of collagenase	810
Concanavalin A	Cell fractionation	734
	Purification of hormonal glycoproteins	731
	Purification of lymphocyte-membrane glycoproteins	732, 733
Deoxyribonucleic acid	Affinity chromatography of deoxyribonucleic acid-binding enzymes	2222
2,4-Dichlorophenoxyacetic acid	Purification of plant proteins	2223
Dihydrofolireductase affinant	Purification of dihydrofolireductase	2224
ε-*N*-Dinitrophenyl-lysine	Affinity chromatography of myeloma immunoglobulin IgA and Fab′ fragment	1302

[2210] J. Schmidt and M. A. Raftery, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 572.
[2211] Z. Er-El, Y. Zaidenzaig, and S. Shaltiel, *Biochem. Biophys. Res. Comm.*, 1972, **49**, 383.
[2212] G. R. Reeck, K. A. Walsh, M. A. Hermodson, and H. Neurath, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 1226.
[2213] B. Sprossler and F. Lingens, *F.E.B.S. Letters*, 1970, **6**, 232.
[2214] M. J. Gething, T. A. A. Dopheide, and B. E. Davidson, *Proc. Austral. Biochem. Soc.*, 1971, **4**, 94.
[2215] D. G. Deutsch and E. T. Mertz, *Science*, 1970, **170**, 1095.
[2216] W. N. Poillon, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 64.
[2217] P. D. Holohan, K. Mahajan, and T. P. Fondy, *Fed. Proc.*, 1970, **29**, 888abs.
[2218] G. W. Jameson and D. T. Elmore, *Biochem. J.*, 1971, **124**, 66P.
[2219] S. L. Marcus and E. Balbinder, *Analyt. Biochem.*, 1972, **48**, 448.
[2220] P. Swanljung and L. Frigeri, *Biochim. Biophys. Acta*, 1972, **283**, 391.
[2221] R. P. Zolton, E. T. Mertz, and H. T. Russell, *Clinical Chem.*, 1972, **18**, 654.
[2222] H. Schaller, C. Nüsslein, F. J. Bonhoeffer, C. Kurz, and J. Nietzschmann, *European J. Biochem.*, 1972, **26**, 474.
[2223] M. A. Venis, *Biochem. J.*, 1972, **127**, 29P.
[2224] B. R. Baker and N. M. J. Vermeulen, *J. Medicin. Chem.*, 1970, **13**, 1143.

Table 5 (*cont.*)

Ligand or affinant	*Use of product*	*Ref.*
Glucagon	Selective binding of glucagon receptors of liver-cell membranes	2225
Glycosides	Isolation of L-fucose-binding proteins	2226
	Purification of soybean agglutinin	729
	Purification of β-xylosidase	1688
Insulin	Isolation of insulin-specific protease	2227
Isoleucyl tRNA synthetase	Purification of isoleucyl tRNA	2228
α-Lactalbumin	Purification of protein-A of lactose synthetase	2009—2012
Neurophysins	Purification of labelled vasopressin	2229
Nicotinamide nucleotides	Affinity chromatography of dehydrogenases dependent upon such nucleotides	2230
Nucleosides	Purification of glycosyltransferases	2231
Nucleotides and derivatives	Affinity chromatography of affinant-related enzymes and dehydrogenases	2232—2234
	Affinity chromatography of protein kinase	2235
	Purification of pyridoxal kinase	2236
	Purification of ribonuclease	2237
Ovomucoids	Purification of α-chymotrypsin and trypsin	1422—1424
Peptides and polypeptides	Affinity chromatography of carboxypeptidase B	2238
	Affinity chromatography of trypsin	2239
	Purification of papain	2240
4-Phenylbutylamine	Affinity chromatography of chymotrypsins A and B	2241
Phytohaemagglutinin from *Lens culinaris*	Isolation of lymphocyte-membrane glycoproteins	736

[2225] F. Krug, B. Desbuquois, and P. Cuatrecasas, *Nature New Biol.*, 1971, **234**, 268.
[2226] S. Blumberg, J. Hildesheim, J. Yariv, and K. J. Wilson, *Biochim. Biophys. Acta*, 1972, **264**, 171.
[2227] W. C. Duckworth, M. H. Heinemann, and A. E. Kitabchi, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3698.
[2228] J. Denburg and M. DeLuca, *Proc. Nat. Acad. Sci. U.S.A.*, 1970, **67**, 1057.
[2229] P. Pradelles, J. L. Morgat, P. Fromageot, M. Camier, D. Bonne, P. Cohen, J. Bockaert, and S. Jard, *F.E.B.S. Letters*, 1972, **26**, 189.
[2230] P. D. G. Dean and C. R. Lowe, *Biochem. J.*, 1972, **127**, 11P.
[2231] J. H. Shaper, R. Barker, and R. L. Hill, *Fed. Proc.*, 1971, **30**, 1265abs.
[2232] K. Mosbach, H. Guilford, R. Ohlsson, and M. Scott, *Biochem. J.*, 1972, **127**, 12P.
[2233] K. Mosbach, H. Guilford, R. Ohlsson, and M. Scott, *Biochem. J.*, 1972, **127**, 625.
[2234] R. Ohlsson, P. Brodelius, and K. Mosbach, *F.E.B.S. Letters*, 1972, **25**, 234.
[2235] M. Wilchek, Y. Salomon, M. Lowe, and Z. Selinger, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 1177.
[2236] J. T. Neary and W. F. Diven, *J. Biol. Chem.*, 1970, **245**, 5585.
[2237] L. Jervis, *Biochem. J.*, 1972, **127**, 29P.
[2238] M. Sokolovsky and N. Zisapel, *Biochim. Biophys. Acta*, 1971, **251**, 203.
[2239] K. Kasei and S. Ishii, *J. Biochem. (Japan)*, 1972, **71**, 363.
[2240] S. Blumberg, I. Schechter, and A. Berger, *European J. Biochem.*, 1970, **15**, 97.
[2241] K. J. Stevenson and A. Landman, *Canad. J. Biochem.*, 1971, **49**, 119.

Table 5 (*cont.*)

Ligand or affinant	*Use of product*	*Ref.*
Polyuridylic acid	Isolation of mRNA	2242
Proteinase inhibitor	Purification of proteinase inhibitor-binding species	2243
Purine derivatives	Fractionation of nucleoside deoxyribosyltransferase	2244
	Purification of guanine deaminase and xanthine oxidase	2245
Pyridoxamine 5′-phosphate	Selection of ribosomes from tissue culture cells	2246
Pyrimidine derivatives	Separation of functional and non-functional xanthine oxidase	2247
Ribonuclease	Affinity chromatography of UDP-labelled peptides	2197
Sulphanilamide	Affinity chromatography of carbonic anhydrase	2248
Trypsin	Affinity chromatography of trypsin	2249
	Isolation of trypsin inhibitor	2250
Trypsinogen	Affinity chromatography of trypsin	2249

derivatives are listed according to the described uses, since, for example, the insoluble form of an enzyme used for affinity chromatography may not have been enzymically active.

The stability of the bridge between the two molecules in the product formed by reaction of adenosine 3′,5′-cyclic monophosphate, to which an aminocaproylcysteamine side-chain had been attached, with Sepharose cyclic imidocarbonate has been examined as a function of pH.[2251] In this particular system, it appeared that the ligand with its side-chain could be liberated from the matrix at pH > 5.

Sepharose cyclic imidocarbonate has been allowed to react with 4-phenylenediamine and 4,4′-methylenedianiline to give matrices to which pepsin could be coupled using 3-dimethylaminopropyl isocyanide according to Scheme 18 (p. 524).[2190] Cross-linked, reduced Sepharose similarly reacted, *via* its cyclic imidocarbonate, with 4-phenylenediamine, cyclohexyl isocyanide being employed as the coupling reagent.

[2242] U. Lindberg and T. Persson, *European J. Biochem.*, 1972, **31**, 246.
[2243] H. Ako, C. A. Ryan, and R. J. Foster, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1639.
[2244] R. Cardinauld and J. Holguin, *Biochem. J.*, 1972, **127**, 30P.
[2245] B. R. Baker and H. U. Siebeneick, *J. Medicin. Chem.*, 1971, **14**, 799.
[2246] J. V. Miller, P. Cuatrecasas, and E. B. Thompson, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 1014.
[2247] V. Massey, D. Edmondson, G. Palmer, L. M. Beacham, and G. B. Elion, *Biochem. J.*, 1972, **127**, 10P.
[2248] S. O. Falkbring, P. O. Göthe, P. O. Nyman, L. Sundberg, and J. Porath, *F.E.B.S. Letters*, 1972, **24**, 229.
[2249] K. K. Stewart and R. F. Doherty, *F.E.B.S. Letters*, 1971, **16**, 226.
[2250] J. Chauvet and R. Acher, *F.E.B.S. Letters*, 1972, **23**, 317.
[2251] G. I. Tesser, H.-U. Fisch, and R. Schwyzer, *F.E.B.S. Letters*, 1972, **23**, 56.

Table 6 *Use of Sepharose cyclic imidocarbonate for the preparation of affinity chromatography materials* via *linkage extension*

Ligand or affinant	*Polysaccharide intermediate*[a]	*Use of product*	*Ref.*
Amethopterin	146	Purification of dihydrofolate reductase	2252
Amino-acids	149A	Affinity chromatography of proteins	2253
Aminoacylated tRNA	141	Purification of aminoacyl-tRNA synthetase	2254
4-Aminobenzamidine	145	Purification of thrombin	2255
2-Aminoethyl 4-nitrophenyl methyl-phosphonate	144	Purification of acetylcholinesterase	2256
4-Aminophenyl 1-β-D-thiogalactopyranoside	144A	Purification of β-galactosidase	2257
Aminopterin	146	Purification of dihydrofolate reductase	2252
Anthranilic acid	140, 142, 144	Determination of steric requirements for substrate binding by 5-phosphoribosyl pyrophosphate phosphoribosyltransferase	2219
Chlorosuccinamic acid	140	Purification of L-asparaginase	2258
Glucagon	142A, 143A	Selective binding of glucagon receptors	2225
Guanosine 2′(3′)-phosphate	145A	Purification of ribonuclease	2237
Insulin	141A–144A, 147A	Affinity chromatography of insulin receptor	2259
Methionine succinimide ester	140	Purification of methionyl-tRNA synthetase	2260
Methotrexate	140	Purification of dihydrofolic reductase	2261
	140	Purification of tetrahydrofolate dehydrogenase	2262
Nicotinamide adenine dinucleotide	145A	Affinity chromatography of cosubstrate-related enzymes	2232, 2233
Norepinephrine	147A	Purification of β-adrenergic receptor protein	2263
Oestrone hemisuccinate	140	Purification of oestradiol-17β-dehydrogenase	2264

Oxalic acid	140	Affinity chromatography of lactate dehydrogenase	2265
Pyridoxal 5′-phosphate	140	Affinity chromatography of aspartate aminotransferase	2266
Trypsin	149A	Affinity chromatography of proteins	2253

[a] See Scheme 19.

[2252] J. M. Whiteley, R. C. Jackson, G. P. Mell, J. H. Drais, and F. M. Huennekens, *Arch. Biochem. Biophys.*, 1972, **150**, 15.
[2253] P. Cuatrecasas and I. Parikh, *Biochemistry*, 1972, **11**, 2291.
[2254] S. Bartkowiak and J. Pawełkiewicz, *Biochim. Biophys. Acta*, 1972, **272**, 137.
[2255] G. Schmer, *Z. physiol. Chem.*, 1972, **353**, 810.
[2256] Y. Ashani and I. B. Wilson, *Biochim. Biophys. Acta*, 1972, **276**, 317.
[2257] E. Steers, P. Cuatrecasas, and H. B. Pollard, *J. Biol. Chem.*, 1971, **246**, 196.
[2258] T. Kristiansen, M. Einarsson, L. Sundberg, and J. Porath, *F.E.B.S. Letters*, 1970, **7**, 294.
[2259] P. Cuatrecasas, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 1277.
[2260] M. Roberto-Gero and J. P. Waller, *European J. Biochem.*, 1972, **31**, 315.
[2261] B. T. Kaufman and J. V. Pierce, *Biochem. Biophys. Res. Comm.*, 1971, **44**, 608.
[2262] J. Gauldie and B. L. Hillcoat, *Biochim. Biophys. Acta*, 1972, **268**, 35.
[2263] R. J. Lefkowitz, E. Haber, and D. O'Hara, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2828.
[2264] J. C. Nicolas, M. Pons, B. Descomps, and A. C. de Paulet, *F.E.B.S. Letters*, 1972, **23**, 175.
[2265] P. O'Carra and S. Barry, *F.E.B.S. Letters*, 1972, **21**, 281.
[2266] E. Ryan and P. F. Fottrell, *F.E.B.S. Letters*, 1972, **23**, 73.

Table 7 *Use of Sepharose cyclic imidocarbonate for the preparation of miscellaneous derivatives*

Species coupled	*Polysaccharide intermediate*[a]	*Use of product*	*Ref.*
Adenosine 3′,5′-cyclic phosphate	—	Investigation of stability of the bond formed on nucleophilic attack of cyclic imidocarbonate	2251
Bovine serum albumin	—	Experimental control	734
Glucagon	142A	Activation of adenyl cyclase	2267
Glycosaminoglycuronans	—	Investigation of the interaction of plasma lipoproteins with glycosaminoglycuronans	888
Human serum albumin	—	Experimental control	1182
Insulin	—	Investigation of dynamics of insulin action	2268
		Stimulation of glycogen synthetase	2269
Luteinizing hormone	142A	Investigation of interaction with adrenal cells	1112
Nicotinamide adenine dinucleotide	145	Investigation of insolubilized co-enzymes	2270
Ribonucleic acid (periodate oxidized)	148	Demonstration of coupling technique	2271

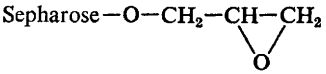
[a] See Scheme 19

[2267] G. B. Johnson, M. Blecher, and N. A. Giorgio, *Biochem. Biophys. Res. Comm.*, 1972, **46**, 1035.
[2268] T. Oka and Y. J. Topper, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 2066.
[2269] L. M. Blatt and K.-H. Kim, *J. Biol. Chem.*, 1971, **246**, 4895.
[2270] P. Larsson and K. Mosbach, *Biotechnol. and Bioeng.*, 1971, **13**, 393.
[2271] D. L. Robberson and N. Davidson, *Biochemistry*, 1972, **11**, 533.

In a number of instances, direct attachment of a ligand to the polysaccharide derivative was unsatisfactory for affinity chromatography, since the approach of the incoming molecule to the ligand was sterically inhibited. In such cases, the problem has been overcome by incorporating a bridge between the matrix and the ligand. The bridging method has also proved useful when functional group(s) on the ligand were incompatible with those on the matrix, since a new type of group can be introduced by means of an appropriate bridging molecule. Sequences recently applied to polysaccharide cyclic imidocarbonates are depicted in Scheme 19 and the final derivatives so obtained from CIC-agarose are listed in Table 6. Miscellaneous products obtained by coupling to CIC-agarose are listed in Table 7.

Macroporous agarose derivatized with 2-amino-4,6-dichloro-*sym*-triazine has been used for the insolubilization of hyaluronidase.[1927] Amino-groups of the enzyme protein are considered to react with chloro-groups of the triazinyl-derivatized polysaccharide.

Sepharose has been converted into an oxiran derivative (139) by reaction with epichlorohydrin.[2272] The epoxide ring was opened with nucleophiles



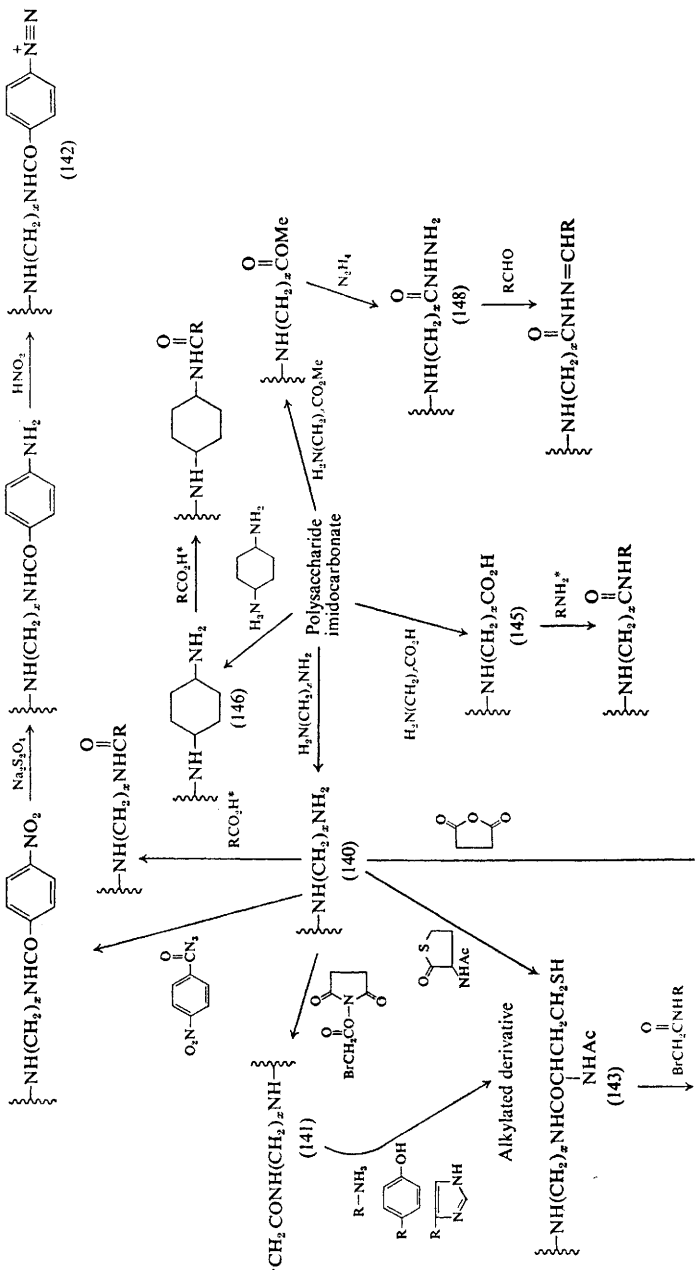
(139)

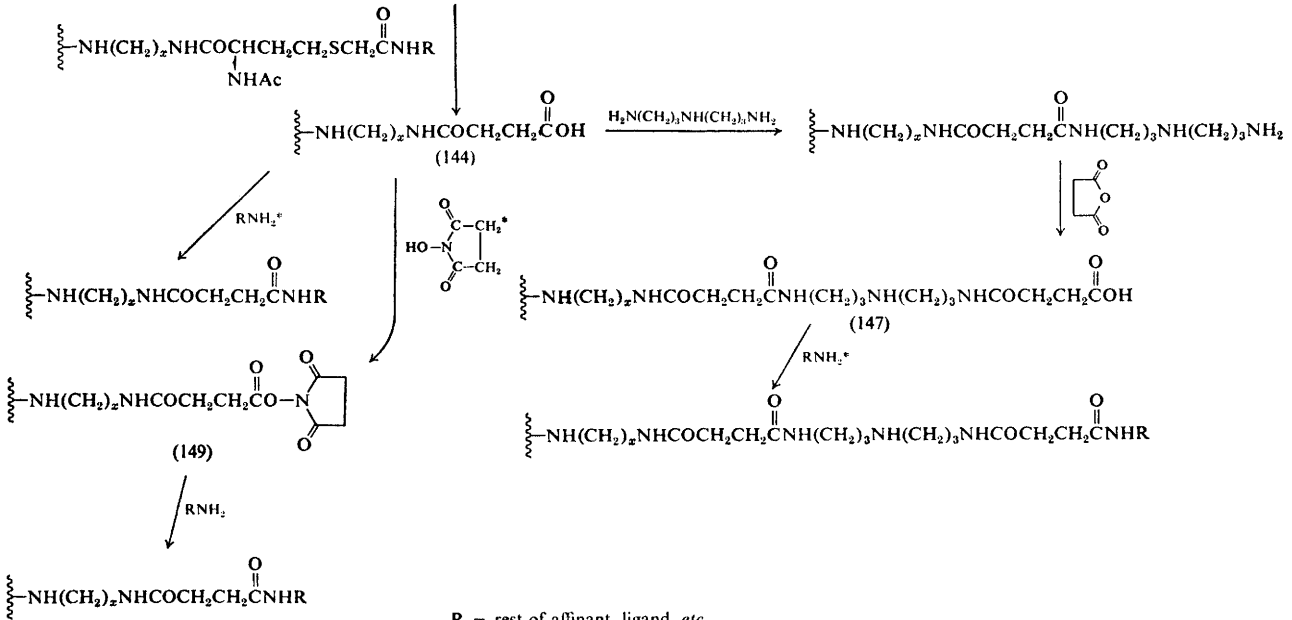
in the presence of alkali and the products obtained from such coupling with arginine and sulphanilic acid have been used as ion-exchangers in the group fractionation of plasma proteins. The oxiran derivative has also been advocated as a matrix for the insolubilization of enzymes and affinity ligands, *etc.*

Alginic Acid.—The photolysis of silver alginate has been investigated.[2273] The primary process involved direct charge transfer from carboxylate anions to silver ions, the products being silver atoms and acyloxyl radicals. The quantum yield of silver was found to depend on the water content and temperature of the silver alginate. During the modification, carbon dioxide and free carboxy-groups were formed simultaneously.

The acetylation of alginic acid from *Cystoseira barbata* has been investigated.[2274] Degradation occurring during acetylation was best evaluated by following the gradual increase in reducing end-groups of the products rather than the decrease in viscosity. Measurement of the optical rotation of the ammonium salt of acetylated alginic acid was found to be of value in determining the extent of acetylation. The relative stiffness of the

[2272] J. Porath and N. Fornstedt, *J. Chromatog.*, 1970, **51**, 479.
[2273] Y. Konishi, H. Hada, and M. Tamura, *J. Chem. Soc. Japan*, 1971, **92**, 829.
[2274] H. M. Salem, A. F. Abdel-Fattah, and M. M. Hussein, *J. Chem. U.A.R.*, 1971, **14**, 297.





In subsequent references to this Scheme, the suffix 'A' denotes the use of $NH_2(CH_2)_2NH(CH_2)_2NH_2$ in place of $NH_2(CH_2)_xNH_2$ for the production of (140)

Scheme 19

molecular chain of acetylated alginate has been estimated from viscosimetric measurements at different ionic strengths.[2275]

Hemiacetals were formed during the periodate oxidation of alginates to polyaldehydo-derivatives.[2276] Hemiacetals occurred between neighbouring hexuronic acid residues and it was not until they were reduced that periodate oxidation could proceed to the Malapradian limit.

Alginic acid has been sulphated without difficulty to a degree of sulphation of 1.8 using a solution of the DMF–sulphur trioxide complex.[2277] Lower degrees of sulphation could be obtained by decreasing the proportion of the complex, while degradation occurred only to a minor extent during the derivatization, as indicated by viscosity measurements. The sulphated alginic acids exhibited reactivity with proteins, the interaction becoming more pronounced as the degree of substitution was increased. Solutions of alginic acid sulphates having degrees of substitution greater than 1.0 were found to gelatinize in the presence of the heavier alkali-metal ions. The molecular weights and sulphate contents of the products arising from treatment of alginic acid with concentrated sulphuric acid for 2 h at 0 °C have been determined.[2163]

Amylopectin.—Reacton Red 2B, a dye containing trichloropyrimidinyl groups, has been allowed to react with amylopectin to give a coloured substrate that was used for the assay of amylase.[1693, 1702] Washing to remove the unreacted dye was found to be inefficient, but a separation of the free dye and the dyed polysaccharide was achieved by gel filtration.[1693] The dyed polymer was precipitable by a solution of benzoic acid, sodium benzoate, and tannic acid in methanol, and this property was utilized in the assay system.

Amylopectin has been phosphorylated using polyphosphoric acid in the presence of a tertiary amine;[2278] phosphorylation imparted interferon-inducing properties to amylopectin.

Methodology for the sulphation of amylopectin has been described,[2158] and the molecular weights and sulphate contents of the products obtained from treatment of amylopectin with concentrated sulphuric acid at 0 °C for 2 h have been determined.[2163]

Amylose.—The pyrolyses of amylose, amylose acetate, and amylose propionate have been studied kinetically by thermogravimetric and differential thermal analyses in an atmosphere of nitrogen.[2279] The thermal stability of amylose was found to increase upon substitution. The e.s.r. spectra of γ-irradiated amylose have been examined at the temperature of liquid nitrogen and above.[2280] Scission of the main chain was considered

[2275] O. Smidsrød and A. Haug, *Biopolymers*, 1971, **10**, 1213.
[2276] T. Painter and B. Larsen, *Acta Chem. Scand.*, 1970, **24**, 813.
[2277] R. G. Schweiger and T. R. Andrew, *Carbohydrate Res.*, 1972, **21**, 275.
[2278] S. Suzuki, M. Suzuki, M. Imaya, and F. Chaki, *Jap. J. Microbiol.*, 1972, **16**, 1.
[2279] D. H. Desai, C. K. Patel, K. C. Patel, and R. D. Patel, *Stärke*, 1972, **24**, 80.
[2280] T. Handa, N. Otsuka, and Y. Ayukawa, *J. Agric. Chem. Soc. Japan*, 1972, **46**, 9.

to be mediated by the transfer of kinetic energy from the electrons and ions formed to a folded part of the crystal surface.

The u.v., o.r.d., and c.d. spectra of amylose acetate and some of its oligomers and of amylose xanthate in trifluoroethanol solution have been recorded.[2281] In the amylose acetate series, both monomer and dimer showed a normal acetyl $n \rightarrow \pi^*$ transition in their c.d. spectrum, whereas the trimer and polymer exhibited an additional band at shorter wavelength, which was presumed to arise from a helical conformation of the amylose chain. This interpretation was substantiated by the observation of a helix–coil transition in the c.d. spectrum of the polymer at elevated temperatures and a reversion to the normal spectrum on partial deacetylation. The o.r.d. and c.d. spectra of amylose xanthate were complex, suggesting the presence of organized structures in solution. The spectra were compared with those of analogous derivatives of other polysaccharides.

Surface pressure–area isotherms of amylose acetate at an air/water interface have been determined on vertical and horizontal film balances.[2282] The polymer exhibited anomalous behaviour on the vertical balance, including higher compressibilities, movement of the vertical plate, and atypical hysteresis of the isotherms. A helical conformation of the polymer at the air/water interface was proposed to explain the low compressibility of the monolayer.

Kinetic evidence has been presented for the formation of inter-residue hemiacetals during the oxidation of amylose to polyaldehydo-derivatives by periodate ion.[2283] Assuming a random oxidation process, it has been calculated that oxidation of amylose with periodate should protect more than 99% of the amylose molecule against attack by an *exo*-enzyme,[1454] but that oxidation is not expected to have any effect in blocking the action of an *endo*-enzyme. These predictions were confirmed by the observations that periodate-oxidized amylose was hydrolysed by pullulanase and α-amylase, but not by β-amylase. Thus, periodate-oxidized amylose is considered to be of general use in distinguishing between *endo*- and *exo*-glucanases.

Methodology has been described for the preparation of perbenzylated amylose[2284] and conditions were also reported for debenzylation. Perbenzylamylose has been used as an intermediate in the preparation of terminal, 4-substituted malto-oligosaccharides.[2285]

The distribution of substituents in carboxymethyl, hydroxyethyl, and sulphopropyl ethers of amylose has been determined by periodate oxidation and tritylation.[2286] In each case, secondary hydroxy-groups appeared to

[2281] S. Mukherjee, A. Sarko, and R. H. Marchessault, *Biopolymers*, 1972, **11**, 303.
[2282] M. Hittmeier, L. S. Sandell, and P. Luner, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 267.
[2283] T. Painter and B. Larsen, *Acta Chem. Scand.*, 1970, **24**, 2724.
[2284] R. E. Wing and J. N. BeMiller, *Methods Carbohydrate Chem.*, 1972, **6**, 368.
[2285] R. E. Wing and J. N. BeMiller, *Methods Carbohydrate Chem.*, 1972, **6**, 378.
[2286] E. Husemann and M. Kafka, *Makromol. Chem.*, 1960, **41**, 208.

react preferentially at the beginning of the etherification reaction. The relative stiffness of the molecular chain of carboxymethylamylose has been estimated from measurements of the viscosity of solutions of the polymer at different ionic strengths.[2275] The conformation of the polyelectrolyte appeared to depend on the stiffness and molecular weight of the chain and on the ionic strength of the solution. The relative stiffness of amylose sulphate has also been deduced from viscosimetric measurements.

Cibachron Blue (a monochlorotriazinyl dye) has been used for covalent dying of amylose and the products were used for the chromogenic estimation of amylase.[1694, 1699]

An investigation of the methanolysis of amylose peresters has shown that esterification stabilizes the glycosidic linkages to methanolysis.[2287] Methanolysis did not occur until an adjacent, acylated D-glucose unit had been extensively deacylated.

Amylose has been phosphorylated using polyphosphoric acid in the presence of a tertiary amine.[2278] Phosphorylation was shown to impart interferon-inducing characteristics to amylose.

From viscosity, sedimentation, and light-scattering measurements carried out in a pyridine–water theta solvent of amylose phenylcarbamate (carbanilate) fractions, it has been shown that the molecular weight can be related to the viscosity number, the radius of gyration, and the sedimentation coefficient.[2288] In the theta solvent mixture, the excluded-volume effect of the polymer disappeared and the molecules behaved as partially free-draining coils. Theories separating short- and long-range force vectors have been applied to solutions of amylose phenylcarbamate.[2289] The unperturbed dimensions obtained in this manner from light-scattering, viscosimetric, and sedimentation measurements have been compared with those obtained directly for solutions of the polymer in the pyridine–water theta mixture. Basic criteria to be satisfied in order to determine the unperturbed coil dimensions of the polymer from measurements made in a good solvent were suggested. The unperturbed, mean-square radius of gyration of amylose phenylcarbamate has been measured for a range of DPs.[2290] The chain exhibited pronounced deviation from a gaussian statistical conformation owing to its stiffness and model calculations were presented to interpret the data. The experimental data were fitted best by a helix of four residues per turn with a pitch height of 10.4 Å. The results were compared with those obtained for the isomeric cellulose phenylcarbamate.

Methodology for the sulphation of amylose has been described.[2158]

Further *X*-ray analysis of the amylose–dimethyl sulphoxide complex has led to a revision of the crystal-lattice parameters.[2291] The reaction between

[2287] G. Entlicher and J. N. BeMiller, *Carbohydrate Res.*, 1971, **16**, 363.
[2288] W. Banks, C. T. Greenwood, and J. Sloss, *Makromol. Chem.*, 1970, **140**, 109.
[2289] W. Banks and C. T. Greenwood, *Makromol. Chem.*, 1971, **144**, 135.
[2290] W. Burchard, *Brit. Polymer J.*, 1971, **3**, 214.
[2291] W. Winter and A. Sarko, *Biopolymers*, 1972, **11**, 849.

the amylose component of starch and iodine to give the blue complex has been shown to involve the polymerization of iodine atoms inside the linear cavity of helically coiled amylose macromolecules.[2292] The kinetics of the reaction of amylose with iodine have been studied for starches from a number of sources and the reaction was found to follow the same rate equation as that established for amylopectin-free amylose fractions.[2292, 2293] Differences among the rate constants for various starches were attributed to different average molecular weights of the amylose fractions and it was concluded that amylopectin does not interfere significantly with complexation. The o.r.d. and c.d. spectra of amylose–iodine complexes have been studied and the complexes have also been investigated by electron microscopy.[1455] The latter technique revealed that the complex crystallizes in the form of fibrils with uniform diameters of 40 Å.[2294] It was shown that an iodine concentration sufficient for complete filling of the amylose helices is necessary for fibril formation. A model in which folded amylose chains are orientated parallel to the fibril axis was proposed for the amylose–iodine complex.

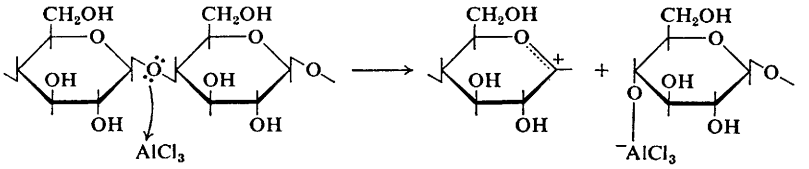
Carrageenans.—The antipeptic activities *in vivo* and *in vitro* of degraded carrageenans have been compared with those of chondroitin sulphate and a sulphated mucosal glycopeptide.[1033, 1034]

Cellulose.—Substitution reactions of cellulose, particularly esterification, etherification, cross-linking, and graft copolymerization, have been described.[2295] A book recently published on cellulose has dealt in detail with the preparation, technological properties, and degradation of cellulose derivatives,[2296] and a second volume briefly covers some cellulose derivatives.[2297] Papers presented at the Seventh Cellulose Conference have been published and they include those describing cellulose derivatives and the reaction of cellulose with ammonia.[2298]

The formation of gels by cellulose derivatives has been discussed from the molecular viewpoint.[2299] The properties and applications of cellulose derivatives exhibiting ion-exchange properties have been discussed.[2300] Methodology for the insolubilization of enzymes by attachment to cellulose has been described.[2170]

[2292] J. C. Thompson and E. Hamori, *J. Phys. Chem.*, 1971, **75**, 272.
[2293] E. Hamori and M. C. Kallay, *Biopolymers*, 1972, **11**, 475.
[2294] H. Bittiger, E. Husemann, and B. Pfannemüller, *Stärke*, 1972, **23**, 113.
[2295] K. Ward and P. A. Seib, in 'The Carbohydrates', Vol. IIA, ed. W. Pigman and D. Horton, Academic Press, New York, 1970, p. 413.
[2296] 'High Polymers', Vol. V, 2nd edn., 'Cellulose and Cellulose Derivatives', Part V, ed. N. M. Bikales and L. Segal, Wiley–Interscience, New York, 1971.
[2297] 'High Polymers', Vol. V, 2nd edn., 'Cellulose and Cellulose Derivatives', Part IV, ed. N. M. Bikales and L. Segal, Wiley–Interscience, New York, 1971.
[2298] 'Proceedings of the Seventh Cellulose Conference', ed. J. C. Jahn, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36.
[2299] D. A. Rees, *Chem. and Ind.*, 1972, 630.
[2300] K. Dorfner, 'Ion Exchangers – Properties and Applications', 3rd edn., 1972, Ann Arbor Science Publishers Inc., Michigan.

The kinetics of the degradation of cellulose by acid have been investigated.[2301] A study of the mechanism involved in the degradation of cellulose by aluminium chloride in non-aqueous solvents has indicated that the Lewis acid attacks the glycosidic oxygen atom (Scheme 20).[2302]



Scheme 20

Differential thermal analysis has been used to determine the degrees of substitution of cellulose derivatives.[2303] Where an endothermic or an exothermic peak characteristic of the modified cellulose could be identified in the differential thermal analysis curve, the peak height and area were found to correlate linearly with the degree of substitution. A theoretical basis for this correlation was developed and the method was applied to the determination of the degree of substitution of samples of benzyl-, benzhydryl-, cyanoethyl-, hydroxyethyl-, sodium carboxymethyl-, and trityl-celluloses and cellulose 2-ethylhexanoate.

The distribution of substituents in a partially acetylated cellulose (DS 2.4), prepared by partial hydrolysis of peracetylated cellulose, has been determined.[2304] Free hydroxy-groups were first protected by reaction with methyl vinyl ether, whereafter *O*-acetyl groups were replaced by *O*-methyl groups using methyl iodide and methylsulphinyl carbanion in such a way that saponification did not occur. Analysis of the methylated monosaccharides subsequently obtained from the polymer indicated that *O*-acetyl groups originally occupied the following positions: 2,3,6-substituted residues (50%), 2,3- (20%), 2,6- (9%), 3,6- (10%), 2- (4%), 3- (4%), 6-substituted residues (2%), and unsubstituted residues (1%).

Cellulose acetate (molecular weight 5600) obtained by acetolysis of the parent polymer has been separated into fifteen fractions by gel-permeation chromatography.[2305] The intrinsic viscosities of solutions of the fractions were determined, and the number- and weight-average molecular weights calculated. The axial ratios of a prolate ellipsoid, calculated from the viscosities of fractions with the lowest degrees of polymerization, when

[2301] V. I. Ivanov, V. A. Afanasiev, and R. I. Sarybaeva, *Doklady Akad. Nauk S.S.S.R.*, 1971, **196**, 380.

[2302] V. I. Ivanov, V. A. Afanasiev, and R. I. Sarybaeva, *Doklady Akad. Nauk S.S.S.R.*, 1970, **192**, 1043.

[2303] P. K. Chatterjee and R. F. Schwenker, *Tappi*, 1972, **55**, 111.

[2304] H. Björndal, B. Lindberg, and K. G. Rosell, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 523.

[2305] H. A. Swenson, J. A. Carlson, and H. M. Kaustinen, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 293.

compared with a molecular model and a fully extended contour length were concordant with the persistence length calculated for high molecular weight molecules from viscosity theory.

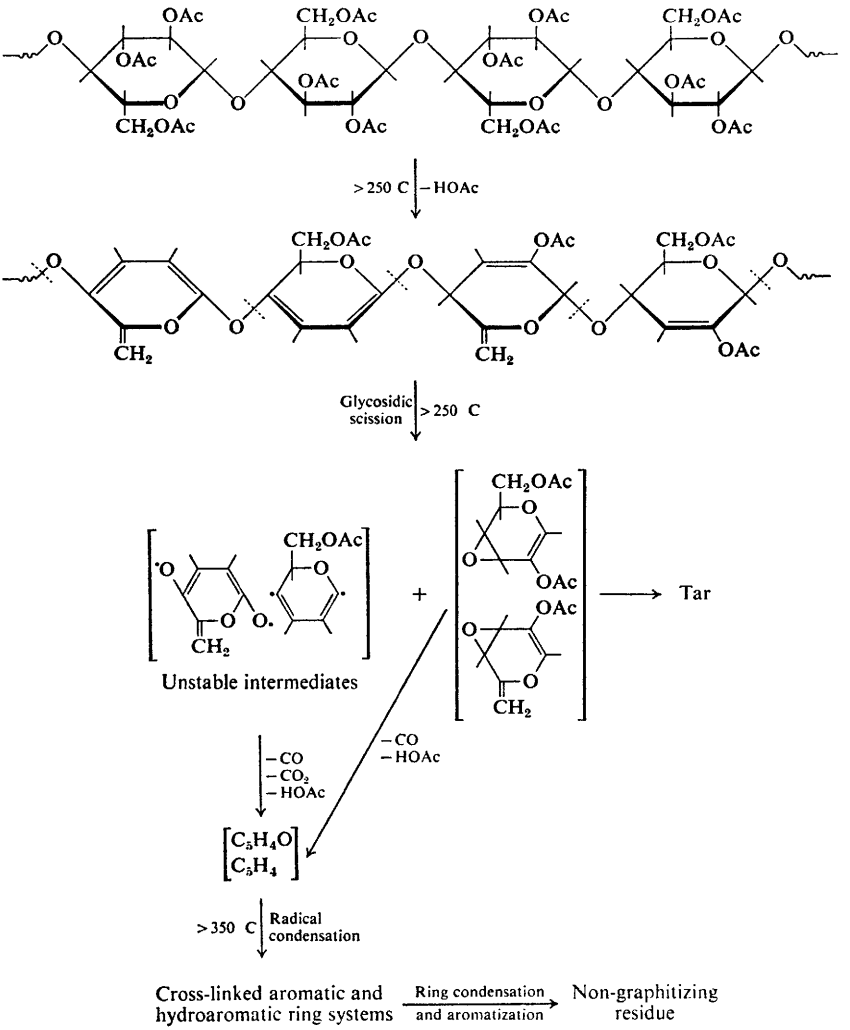
The crystallization of partially substituted cellulose acetates has been studied.[2306] The adsorption hysteresis of a cellulose acetate–water vapour system and the effects of relative humidity and temperature upon the adsorption have been examined.[2307] Surface pressure–area isotherms for cellulose acetate have been determined on vertical and horizontal film balances.[2282] The polymer showed anomalous behaviour on the vertical balance, including higher compressibilities, movement of the vertical plate, and atypical hysteresis of the isotherms. This behaviour of the cellulose acetate film was attributed to heterogeneous gelation of the monolayer at low pressures. The properties of the film could be accounted for by a mechanism involving lateral tilting of pyranose units at the interface and subsequent stabilization of the monolayer by van der Waals' interactions.

Gases evolved on thermal degradation of cellulose acetate *in vacuo* were found to resemble those reported for that of cellulose.[2308] Early evolution of large quantities of the oxides of carbon was indicative of extensive breakdown of the pyranose rings, whereas the liberation of acetic acid as the major product was attributed to thermal deacetylation. The tar-like material remaining appeared to be a mixture of unsaturated mono- and di-acetylated monosaccharides and oligomers. Changes in the structure and composition of the carbonaceous residue were also investigated [2309] and a mechanism was proposed for the overall degradation (Scheme 21).[2310]

Measurements have been made of the degree of crystallinity of heat-treated cellulose acetate.[2311] *X*-Ray diffraction traces of cellulose acetate films heated in the range 220—290 °C have been resolved by a computational method that fitted gaussian or Cauchy functions to the peak profiles.[2312] A polynomial background factor was fitted to the data at the same time so that the total area enclosed by the resolved peaks is a parameter for estimation of the crystallinity, whereas the area under the background is a parameter for estimating the non-crystalline or disordered components. The crystallites obtained from fibres annealed at 290 °C were found to have considerably greater widths than those obtained from fibres at either 250 or 220 °C.

Graft copolymers of cellulose acetate and nylon-66 have been obtained by successive, alternate, controlled additions of adipoyl chloride and 1,6-diaminohexane to a potassium alkoxide derivative of cellulose diacetate (DS 0.67) in THF (Scheme 22).[2313] The graft copolymers were

[2306] P. V. Kozlov and E. V. Gert, *Doklady Akad. Nauk S.S.S.R.*, 1972, **202**, 840.
[2307] R. H. S. Bhatia, *Indian J. Chem.*, 1971, **9**, 1158.
[2308] A. Scotney, *European Polymer J.*, 1972, **8**, 163.
[2309] A. Scotney, *European Polymer. J.*, 1972, **8**, 175.
[2310] A. Scotney, *European Polymer J.*, 1972, **8**, 185.
[2311] A. M. Hindeleh and D. J. Johnson, *Polymer*, 1970, **11**, 666.
[2312] A. M. Hindeleh and D. J. Johnson, *Polymer*, 1972, **13**, 27.
[2313] A. Bar-Ilan and A. Zilkha, *Israel J. Chem.*, 1971, **9**, 149.

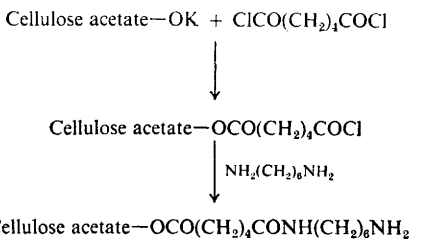


Scheme 21

separated from the homopolymer by fractionation. Increase of the monomer concentration gave rise to increased contents of nylon-66 in the product. An investigation of the grafting of styrene to pre-irradiated cellulose acetate revealed that, whereas pure styrene gave a poor grafting yield, the addition of alcohols increased the grafting yield dramatically.[2314]

Aldehydocellulose (produced by periodate oxidation of the polysaccharide) has been used for the insolubilization of proteins, the aldehydo-

[2314] T. Yasukawa, Y. Sasaki, and K. Murakami, *Makromol. Chem.*, 1972, **153**, 323.



Scheme 22

groups reacting with free amino-groups in the proteins.[1196] The carbinolamine structure of the bridge formed between the protein and cellulose was stabilized by reduction to alkylamino-groups with sodium borohydride and this reduction step also served to remove unreacted aldehyde groups. The coupled products have been used as immunoadsorbents.

The reactions of ammonia with cellulose and wood have been studied with particular reference to the mechanical, physical, and chemical characteristics of the products.[2315–2318]

Soybean trypsin inhibitor linked covalently to an aminoalkyl ether of cellulose and to carboxymethylcellulose has been used as an affinity chromatography matrix for the purification of kallikrein.[2319] The aminoethyl ether of cellulose has been further derivatized by reaction with the carboxy-groups of aminopterin and amethopterin, and the products have been used in the purification of dihydrofolate reductases by affinity chromatography.[2252] Aminoethylcellulose has also been used for the isolation of polynucleotide fragments derived from the terminals of ribonucleic acids.[2320] Aldehydo-derivatives obtained as a result of periodate oxidation of the terminal fragments were bound by the polysaccharide derivative, whereas unoxidized polynucleotide fragments could be eluted. The terminal fragments were released subsequently by a β-elimination reaction involving alkylammonium salts.

4-Aminobenzylcellulose in its diazotized form has been used to insolubilize guanylribonuclease with retention of activity.[2187] Anti-human serum albumin has also been coupled to 4-diazobenzylcellulose and the immunoadsorbent produced was used in an assay for albumin fragments.[1173] Antibodies to human chorionic gonadotrophin and to rabbit immunoglobulin have been covalently coupled to diazotized 3-aminobenzyloxy-

[2315] M. Bariska and R. Popper, *J. Polymer. Sci., Part C, Polymer Symposia*, 1971, No. 36, 199.
[2316] C. Schuerch and R. W. Davidson, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 231.
[2317] R. C. Peterson and R. W. Strauss, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 241.
[2318] M. Mahdalík, J. Rajčan, M. Mlčoušek, and O. Lábsky, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 251.
[2319] H. Fritz, G. Wunderer, and B. Dittmann, *Z. physiol. Chem.*, 1972, **353**, 893.
[2320] J. C. Lee, H. L. Weith, and P. T. Gilham, *Biochemistry*, 1970, **9**, 113.

methylcellulose and cellulose cyclic imidocarbonate for use in immunoassays.[1120] α-Lactalbumin coupled to a similar diazo-derivative of cellulose has been applied to the affinity chromatography of the A-protein of lactose synthetase.[2012]

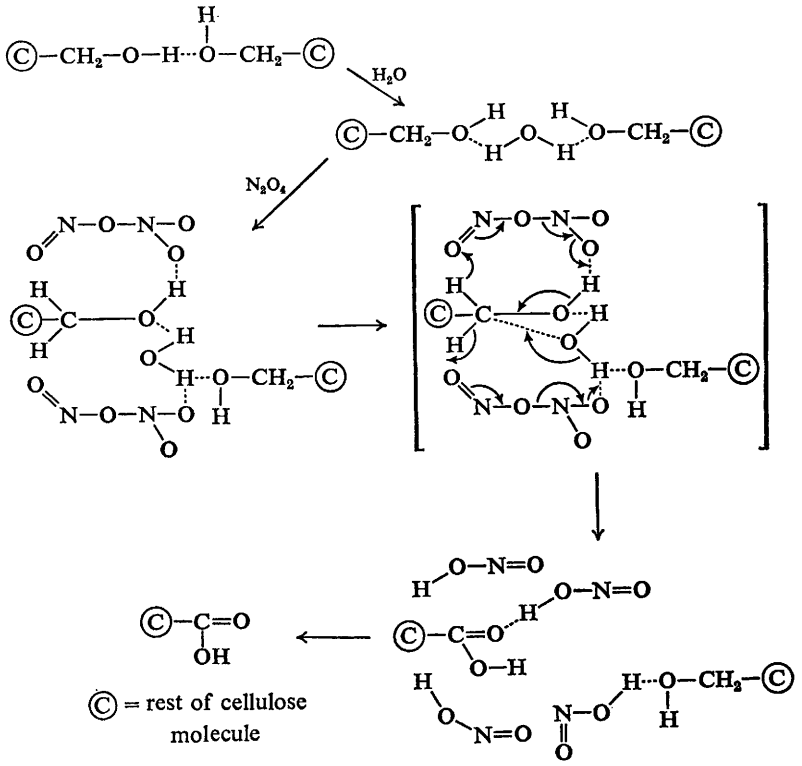
Cellulose interacted with boron alkoxides to produce compounds that acted as Lewis acids.[2321] The degree of interaction was found to decrease as the molecular weight of the polysaccharide increased, but the use of such bases as pyridine or ethylenediamine generally increased the interaction. In the case of a sample of cellulose having a molecular weight of 7×10^5, interaction with boron alkoxides only occurred when ethylenediamine was present. Partial methylation of cellulose also increased the reactivity of the remaining hydroxy-groups to the boron alkoxides, although the molecular weight of cellulose was decreased by methylation.

Bromoacetylcellulose has been produced by treating cellulose with a mixture of bromoacetyl chloride and bromoacetic acid; chloroacetylcellulose was formed in identical fashion using chloroacetyl chloride and chloroacetic acid.[2322] Iodoacetylcellulose was prepared by an exchange reaction on the bromo- or chloro-derivatives with sodium iodide. The three products were used for the preparation of active, insoluble aminoacylase, the iodo-derivative proving to be the best carrier. Insolubilized pronase has been formed in an analogous way using bromoacetylcellulose.[2323] Two immunologically active lysozyme peptides have been coupled to bromoacetylcellulose and the products were used for the isolation of homologous antibodies.[1851]

Sodium cellulose carboxylate has been prepared by oxidation of cellulose with gaseous nitrogen tetroxide, followed by treatment with water and buffer at pH 7.[2324] The efficiency of the buffer system was found to be critical if gel formation and loss of fibrous character were to be avoided. Sodium cellulose carboxylate absorbed much more water than cellulose, but high adsorptivity was only achieved by paying careful attention to the pH of the buffer system; low pHs led to impaired absorptivity, whereas high pHs led to gel formation. The rate of salt formation by the carboxylate appeared to be a diffusion-controlled process that is highly sensitive to localized depletion of buffer solution. A mechanism (Scheme 23) for the oxidation of cellulose with nitrogen tetroxide has been proposed.[2325]

General and qualitative characteristics of sodium carboxymethyl-, hydroxyethyl-, and methyl-celluloses have been described.[2326] Gravimetric and titrimetric methods for assaying these water-soluble cellulose ethers

[2321] M. S. Bains and J. C. Arthur, jun., *Carbohydrate Res.*, 1971, **19**, 365.
[2322] T. Sato, T. Mori, T. Tosa, and I. Chibata, *Arch. Biochem. Biophys.*, 1972, **147**, 788.
[2323] S. Shaltiel, R. Mizrahi, Y. Stupp, and M. Sela, *European J. Biochem.*, 1970, **14**, 509.
[2324] M. P. Godsay, V. N. Gupta, R. L. Lafond, and D. M. MacDonald, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 529.
[2325] B. G. Yasnitsky, E. B. Dolberg, and V. A. Oridoroga, *Ukrain. khim. Zhur.*, 1972, **38**, 76.
[2326] L. Grosse and W. Klaus, *Z. analyt. Chem.*, 1972, **259**, 195.



Scheme 23

and their degrees of substitution were also reported. Procedures for measuring the viscosity of solutions of the polymers, the degrees of polymerization, and the content of gel particles were discussed. Typical examples were presented for dealing with mixtures containing only a small percentage of cellulose ether.

Donnan equilibria between carboxymethylcellulose and sodium ions have been measured by equilibrium dialysis.[921] The degree of fixation of ions by the polymer was determined and compared with values obtained for other polyionic polysaccharide derivatives and glycosaminoglycuronans. The relative stiffness of the molecular chain of carboxymethylcellulose has been estimated from viscosity measurements made at different ionic strengths.[2275] The effects of molecular weight, degree of neutralization, and concentration and nature of the cation on the ultrasonic absorption by aqueous solutions of salts of carboxymethylcellulose have been studied.[2327] The processes involved in extensive absorption were shown to be due to

[2327] G. Atkinson, E. Baumgartner, and R. Fernández-Prini, *J. Amer. Chem. Soc.*, 1971, **93**, 6436.

interaction of the counterion with the repeating units of the polyionic chain rather than the chain as a whole. Alkaline-earth metal counterions produced large increases in the absorption. In general, the absorption was found to differ qualitatively from that due to isolated interactions between single carboxylate ions and the same counterions. Several possible mechanisms of absorption by the polymer were discussed.

The degradation of carboxymethyl-, carboxymethyl-hydroxyethyl-, hydroxyethyl-, and methyl-celluloses by bacterial cellulase has been investigated.[1882] Both carboxymethyl- and hydroxyethyl-celluloses appeared to be attacked by the enzyme in a similar fashion and the two types of substituent were equally effective in limiting hydrolysis. Analysis of the products of hydrolysis indicated that the distribution of substituents in the cellulose derivatives was not uniform and that accessibility of the hydroxy-groups in cellulose was important in determining the pattern of derivatization. Results obtained with various preparations of carboxymethylcellulose showed that carboxymethylation is influenced by the original fibre structure of the cellulose. Sodium carboxymethylcellulose was converted into carboxymethyl-hydroxyethylcellulose by treatment with dihydropyran, followed by partial reduction of the derivatized form. The product was found to contain equal proportions of carboxymethyl and hydroxyethyl groups.

The distribution of substituents in sodium carboxymethylcellulose has been studied in relation to its stabilizing effect upon milk proteins.[1137] Although the distribution of substituents per D-glucose residue did not vary markedly among a number of samples, the distribution along the cellulose chain varied and was more heterogeneous in samples which could stabilize α_s-casein best. Carboxymethylcellulose has been investigated as an artificial lubricant for joints.[2328]

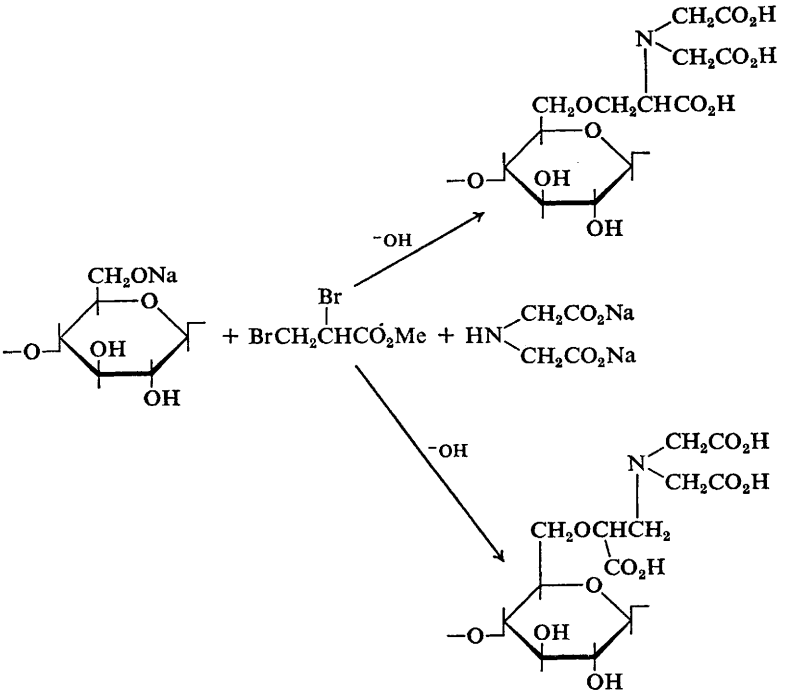
In carbodi-imide-mediated reactions, carboxymethylcellulose has been allowed to react with ε-dinitrophenyl-lysine, 4-aminophenylazophenylarsonic acid, and bovine serum albumin.[1197] In the same study, carboxymethylcellulose was acetylated with acetic anhydride, and cellulose was ethylated with ethyl chloride at high temperatures and pressures. The products of both reactions were coupled to 4-aminophenylazophenylarsonic acid with the aid of DCC.

Carboxymethylcellulose has been converted into the reactive acid azide *via* its methyl ester and acid hydrazide; the azide reacted with proteases (*e.g.* bromelain and ribonuclease) to give active, carrier-bound forms of the enzymes.[2329] Coupling of alkaline phosphatase to the azide, however, caused complete inactivation.[2330] Ribonuclease[2331] and guanid-

[2328] E. L. Radin, I. L. Paul, and P. A. Weisser, *Arthritis Rheum.*, 1971, **14**, 126.
[2329] W. Brümmer, N. Hennrich, M. Klockow, H. Lang, and H. D. Orth, *European J. Biochem.*, 1972, **25**, 129.
[2330] R. A. Zingaro and M. Uziel, *Biochim. Biophys. Acta*, 1970, **213**, 371.
[2331] A. A. M. Gribnau, J. G. G. Schoenmakers, M. van Kraaikamp, and H. Bloemendal, *Biochem. Biophys. Res. Comm.*, 1970, **38**, 1064.

ated trypsin–kallikrein inhibitor[2332] have been coupled similarly to carboxymethylcellulose azide and the products were used for affinity chromatography of ribonuclease inhibitor and kallikreins, respectively. α-Amylase has been immobilized by reaction with a triazinyl derivative of carboxymethylcellulose, which was prepared by treatment of the polysaccharide derivative with 2-amino-4,6-dichloro-*sym*-triazine.[1758]

Carboxymethylcellulose has also been modified by reaction with methyl 1,2-dibromopropionate and substitution of the residual bromo-groups with di(carboxymethyl)amine to give products containing complex ethers bearing three carboxy-groups (Scheme 24).[2333] The carboxy-groups



Scheme 24

contributed extensively to the ability of the polysaccharide derivative to chelate with metal ions and this property has been utilized for metal-ion separations.

Cellulose cyclic imidocarbonate, prepared by treating cellulose with cyanogen bromide, has been used for covalent insolubilization of anti-immunoglobulin IgG and the product was applied to an enzyme-linked,

[2332] H. Fritz and B. Fòrg-Brey, *Z. physiol. Chem.*, 1972, **353**, 901.
[2333] I. Kojdl, *Z. analyt. Chem.*, 1972, **259**, 118.

immunosorbent assay of immunoglobulins.[1314] Adenosine 3′,5′-cyclic phosphate bearing an attached aminocaproylcysteamine side-chain reacted with cellulose cyclic imidocarbonate and the stability of the bridge formed between the two molecules has been examined as a function of pH.[2251] For the particular system examined, it appeared that the ligand and its side-chain could be liberated from the matrix at pH >5.

Benzoylated DEAE-cellulose has been shown to bind nucleotides, particularly those modified to contain substituted phenol groups.[2334] The products have been used in the synthesis of deoxyribo-oligonucleotides bearing 5′-phosphate end-groups. Treatment of DEAE-cellulose with 2-amino-4,6-dichloro-*sym*-triazine afforded a triazinyl derivative, which has been used for the covalent attachment of glucoamylase [1777] and amidase [2335] with retention of activity.

Dyed derivatives of cellulose have been produced by reaction with the Ramazol-type dyes β-chlorovinyl aryl sulphone and ββ-dichloroethyl aryl sulphone.[2336] Some assessment was made of the location of the dye ether groups in the cellulose derivatives.

Cellulose reacted rapidly with dicarboxylic acids on stirring in a mixture of a dipolar, aprotic solvent and triethylamine.[2337] On pouring the resultant viscous solutions into aqueous acid, the corresponding dicarboxylic acid half-esters of cellulose were precipitated. Products were obtained using phthalic, glutaric, succinic, cyclohexane-1,2-*cis*-dicarboxylic, and Δ^4-*cis*-tetrahydrophthalic acids.

The diffusion of organic diluents in ethylcellulose has been studied by sorption and microinterferometric techniques.[2338] The general features of diffusion of compounds of low molecular weight in the polysaccharide derivative were found to be similar to those for other polymers. The use of quaternary ammonium ethylcellulose in the fractionation of tyrosine-rich proteins has been reported.[2339]

Cotton cellulose has been shown to react readily with solutions of thionyl chloride in DMF.[2340] Subsequent hydrolysis yielded cellulose formate as the major product, presumably formed by way of reaction of cellulose with the iminium salt $Me_2\overset{+}{N}=CHCl\ Cl^-$. The minor products of the reaction were identified as a sulphurous acid monoester of cellulose and a chlorodeoxycellulose. The effects of various pretreatments of the cellulose and of reaction conditions on the type and rate of reaction were determined. The stability of the formyl and sulphurous monoester groups

[2334] S. A. Narang, O. S. Bhanot, J. Goodchild, and R. Wightman, *Chem. Comm.*, 1970, 91.
[2335] D. Warburton, K. Balasingham, P. Dunnill, and M. D. Lilly, *Biochim. Biophys. Acta*, 1972, **284**, 278.
[2336] F. Wolf and H. Rabe, *Z. Chem.*, 1972, **12**, 104.
[2337] K. Garves, *Tappi*, 1972, **55**, 263.
[2338] M. I. Artsis, A. E. Chalykh, N. A. Khalturinskii, Y. V. Moiseev, and G. E. Zaikov, *European Polymer J.*, 1972, **8**, 613.
[2339] J. M. Gillespie, *J. Chromatog.*, 1972, **72**, 319.
[2340] T. L. Vigo and D. J. Daigle, *Carbohydrate Res.*, 1972, **21**, 369.

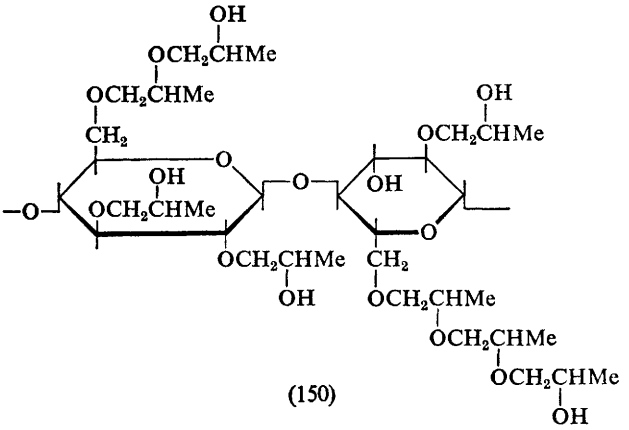
to hot water and to dilute ammonium hydroxide, as well as the tensile properties of yarns treated with thionyl chloride and DMF, were investigated.

The interactions of nucleotides, polynucleotides, and nucleic acids with *N*-(3-dihydroxyborylphenyl)carbamylmethylcellulose and *N*-{*N'*-(3-dihydroxyborylphenyl)succinamyl}aminoethylcellulose (see Vol. 4, pp. 258—259) have been studied.[2341] Nucleotides and polynucleotides containing a free 2',3'-diol group were capable of forming specific complexes with the cellulose derivatives. In column chromatography with these derivatives, the retention volume of a particular mono- or poly-nucleotide was found to depend on the pH of the eluant, the ionic strength and nature of the cations in the eluant, the number of negative charges in the vicinity of the diol group, and the nature of the nucleotide bases in the vicinity of the diol group. A method was also devised whereby polynucleotides not possessing a 2',3'-diol group could be bound to the substituted celluloses.

The effect of increasing the degree of substitution of water-soluble hydroxyethyl- and hydroxypropyl-celluloses was to decrease their affinities for water.[2342] Ionizable substances were found to have a considerable influence on the temperature–solubility behaviour of hydroxyalkylcelluloses. When the ions were present as inorganic salts in solution, they caused hydration of the polymer to be reduced with some loss of solubility. In the case of hydroxypropylcellulose, ions derived from substituent groups present in small amounts were shown to improve the solubility in hot water and to impart unusual viscosity–temperature characteristics to the polymer. The mechanical properties of films of hydroxyethylcellulose and the thermoplasticity of hydroxypropylcellulose were found to be functions of the extent of substitution rather than the degree of hydrophilicity. A highly cross-linked gel has been obtained by cross-linking hydroxyethylcellulose with epichlorohydrin.[2343] The gel was found to swell readily in a wide range of polar organic solvents as well as in water. Good separations were obtained with respect to molecular weight when the gels were used for the filtration of oligosaccharides and poly(ethylene oxides) of low molecular weight. The influences of temperature and solvent upon the separations were discussed.

Two independent n.m.r. spectroscopic procedures have been described for determining the number of moles of alkylene oxide combined per D-glucose unit (molar substitution, MS) in propene oxide derivatives of cellulose.[2344] One of these procedures can also be used for determining the average length of the substituent chains and, therefore, the average number of hydroxy-groups substituted per D-glucose unit (degree of substitution, DS). A possible structure for hydroxypropylcellulose is (150), which illustrates a product of MS 4.0 and DS 2.5. A solution fractionation

[2341] M. Rosenberg, J. L. Wiebers, and P. T. Gilham, *Biochemistry*, 1972, **11**, 3623.
[2342] E. D. Klug, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 491.
[2343] W. Brown and K. Chitumbo, *Chem. Scripta*, 1972, **2**, 88.
[2344] F. F. L. Ho, R. R. Kohler, and G. A. Ward, *Analyt. Chem.*, 1972, **44**, 178.



(150)

technique has been developed for water-soluble hydroxypropylcellulose utilizing anhydrous ethanol (solvent) and n-heptane (non-solvent) mixtures of varying composition.[2345] The fractionation data indicated that substitution of the polysaccharide chain was uniform, although there was a tendency for shorter chains to be more highly substituted. The physicochemical, rheological, biological, and thermoplastic characteristics of hydroxypropylcellulose have been investigated and attention has been drawn to the potential industrial importance and application of this polysaccharide derivative.[2346]

Metal chelates of cellulose have been formed by treatment of the polysaccharide with Ti^{3+}, Ti^{4+}, Sn^{2+}, Sn^{4+}, Zr^{4+}, V^{3+}, Fe^{3+}, and Fe^{2+} ions in the presence of hydrochloric acid.[1555, 1780, 1781] The cellulose chelates were able to accept functional groups of proteinaceous molecules as additional ligands and so provided a new way of insolubilizing enzymes.

The effects of sucrose and citric acid on the sol–gel transformation of methylcellulose in water have been investigated.[2347] The phase equilibrium of a methylcellulose–water system has been determined by differential thermal analysis.[2348] Methylated cellulose dried in the presence of RNA has been used for the column purification of ribosomal RNA genes.[2349]

A procedure described for preparing cellulose nitrate has been used in extracting undegraded cellulose in high yield from pea (*Pisum sativum*) tissues.[2350] Osmotic-pressure measurements and gel-permeation chromatography have been carried out on solutions of nitrated cotton celluloses in THF.[2351] Peak elution volumes for the cellulose nitrates were converted

[2345] M. G. Wirick and M. H. Waldman, *J. Appl. Polymer Sci.*, 1970, **14**, 579.
[2346] E. D. Klug, *Food Technol.*, 1970, **24**, 51.
[2347] N. Iso and D. Yamamoto, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 1867.
[2348] A. Kagemoto, Y. Baba, and R. Fujishiro, *Makromol. Chem.*, 1972, **154**, 105.
[2349] I. Smith, H. Smith, and S. Pifko, *Analyt. Biochem.*, 1972, **48**, 27.
[2350] F. S. Spencer and G. A. Maclachlan, *Plant Physiol.*, 1972, **49**, 58.
[2351] R. L. Kennedy, *Tappi*, 1972, **55**, 1262.

into the corresponding extended chain length using a calibration curve (from polystyrene standards) and an effective density factor ($\bar{Q}$) was obtained by dividing the osmometrically obtained molecular weight by the extended chain length. The value of $\bar{Q}$ per unit length appeared to be independent of the molecular size and the degree of polymerization distribution of the cellulose nitrate. Molecular weight values for the fractions based on $\bar{Q}$ were compared with values obtained by other methods. The analysis by g.l.c. of cellulose nitrate-based propellants has been described [2352] and collodion membranes have been used for the insolubilization of alkaline phosphatase by adsorption.[2353]

Methodology has been described for the covalent binding of nucleotides, polynucleotides, and nucleic acids to cellulose with the aid of a carbodiimide.[2354] Oligothymidylic acid derivatives of cellulose formed in this way have been used for the affinity chromatography of DNA polymerase [2355] and for the purification of mRNA.[2356] Deoxyribo-oligonucleotides have been similarly linked to cellulose papers.[2357] On conversion into forms suitable for column chromatography, the products were capable of retaining deoxyribo-oligonucleotides of complementary sequences.

Methodology has also been described for the attachment of DNA to cellulose without the aid of a reagent.[2358] The process involved a drying step, the conditions for which appear to be critical. DNA bound to cellulose in this way has been used to study the binding of protein to the nucleic acid.[2359] Cellulose has been shown to bind selectively polyadenylic acid and RNA containing polyadenylic acid in solutions of high and low salt concentrations at pHs <5.6.[2360] The polysaccharide did not bind heteropolymeric RNA, but exhibited some affinity for DNA, particularly in its single-stranded form. The binding properties were applied to the isolation of certain types of RNAs.

Standard methodologies reported for the determination of carbonyl groups in oxidized cellulose involved measurements of oxime formation and the ammonia released on hydrolysis of the oxime, or their measurement by a combination of oxidation with chlorous acid and reduction with borohydride.[2361] Free carboxy-groups in oxycelluloses may be differentiated from lactone groups on the basis of their different rates of reaction with potassium iodide–potassium iodate solution.[2362] An attempt has been made to identify the various lactone groups present by comparison of the reaction

[2352] B. J. Alley and H. W. H. Dykes, *J. Chromatog.*, 1972, **71**, 23.
[2353] R. Goldman, O. Kedem, and E. Katchalski, *Biochemistry*, 1971, **10**, 165.
[2354] P. T. Gilham, *Methods Enzymol.*, 1971, **21**, 191.
[2355] B. I. Gerwin and J. B. Milstein, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 2599.
[2356] H. Aviv and P. Leder, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 1408.
[2357] C. R. Astell and M. Smith, *Biochemistry*, 1972, **11**, 4114.
[2358] B. Alberts and G. Herrick, *Methods Enzymol.*, 1971, **21**, 198.
[2359] J. Salas and H. Green, *Nature New Biol.*, 1971, **229**, 165.
[2360] P. A. Kitos, G. Saxon, and H. Amos, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 1426.
[2361] M. Pašteka, *Methods Carbohydrate Chem.*, 1972, **6**, 81.
[2362] W. B. Achwal and G. Shanker, *J. Appl. Polymer Sci.*, 1972, **16**, 1873.

rates of different oxycelluloses with those of model compounds. Blocking of the free carboxy-groups did not affect the determination of the lactone groups in this way. Extensive treatment of oxycelluloses with sodium borohydride was incapable of reducing all the lactones present, whereas treatment with chlorous acid increased the values obtained for the free carboxy-groups and lactones.

The unperturbed mean-square radii of gyration of the isomeric chains of cellulose and amylose phenylcarbamates (carbanilates) have been measured over a range of degrees of polymerization.[2290] Both chains exhibited pronounced deviations from the gaussian statistical conformation because of a marked stiffness of the chains. Model calculations were presented to interpret the data obtained and, in the case of cellulose phenylcarbamate, could be reasonably interpreted by a model chain with a symmetric potential of the hindered rotation.

Methodology has been described for the phosphorylation of cellulose with an amine salt of tetra(polyphosphoric acid).[2363] Cellulose phosphate and glycerylcellulose phosphate have been used for the affinity chromatography of phosphatases.[2364]

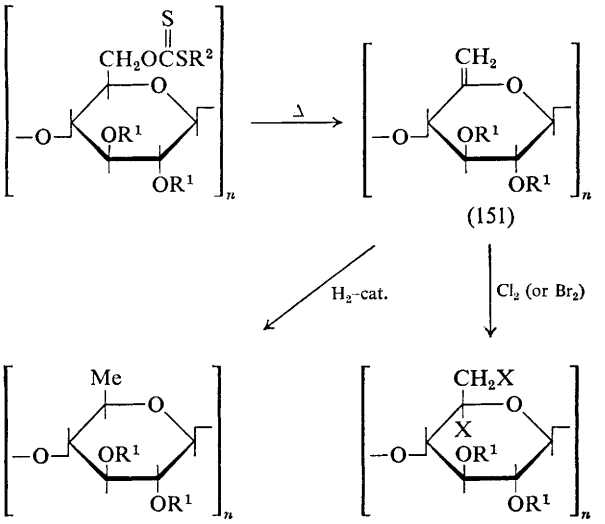
Use of a complex of sulphur trioxide and DMF for the sulphation of cellulose has been shown to overcome the problems of excessive degradation and incomplete substitution experienced with other methods.[2365] Products obtained by the new method had degrees of polymerization greater than 2.0, while the high viscosities of the solutions indicated that little degradation had occurred. The cellulose sulphate reacted with proteins and formed thermoreversible gels in aqueous solutions in the presence of potassium, rubidium, or caesium ions. Overall, the properties of the cellulose sulphate resembled those of carrageenan. The molecular weights and sulphate contents of the products resulting from treatment of cellulose with concentrated sulphuric acid for 2 h at 0 °C have been determined.[2163] Considerable depolymerization was found to occur during the sulphation reaction.

The periodate oxidation of 6-*O*-tritylcellulose has been investigated and compared with those of methyl α-D-glucopyranoside and methyl 6-*O*-trityl-α-D-glucopyranoside.[2366]

Unsaturated cellulose (5,6-cellulosene) (151; R^1 = H) and its acetyl derivatives (151; R^1 = Ac) have been prepared by thermolysis of cellulose allyl- and benzyl-xanthates.[2367] The compounds obtained were identified by hydrogenation and other addition reactions (Scheme 25).

The ability of the cellulose xanthate–hydrogen peroxide system to initiate graft copolymerization with styrene has been demonstrated.[2368]

[2363] G. A. Towle and R. L. Whistler, *Methods Carbohydrate Chem.*, 1972, **6**, 408.
[2364] P. D. G. Dean, S. R. Willetts, and J. E. Blanch, *Analyt. Biochem.*, 1971, **41**, 344.
[2365] R. G. Schweiger, *Carbohydrate Res.*, 1972, **21**, 219.
[2366] R. G. Krylova, S. N. Ryadovskaya, L. I. Kostelian, and A. I. Usov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1972, 2068.
[2367] G. Descotes, A. Faure, and J.-C. Martin, *Bull. Soc. chim. France*, 1971, 4590.
[2368] B. V. Kokta and J. L. Valade, *Tappi*, 1972, **55**, 366.



R^1 = H or Ac; R^2 = allyl or benzyl; X = Cl or Br

Scheme 25

It was shown that addition of either acrylonitrile or acrylamide to the system resulted in increases in the degree of conversion, the grafting efficiency, and the polymer loading. Explanations for this synergistic effect involving the electron-acceptor characteristics of the monomers, the higher reactivities of the resulting cellulose–polymer radicals, and swelling and gel effects were presented.

Beads of cross-linked cellulose have been prepared by reduction of viscose, followed by treatment with epichlorohydrin and further reduction.[2369] Solvent selection permitted beads of 20—70 μm diameter to be formed. The products were found to adsorb cellodextrins, whereas xylodextrins and mannodextrins of comparable size were only weakly adsorbed. Thus, it appears that special binding forces operate when the adsorbent and adsorbate structures correspond, and these effects were exploited in the separation of cello-, xylo-, and manno-dextrins.

The physical, chemical, and morphological effects of free-radical-initiated, oxidative reactions of cellulose have been summarized and the formation of free radicals in cellulose has been shown to increase the reactivity of cellulose towards copolymerization with vinyl monomers.[2370] The chemical properties of cellulose–polyvinyl copolymers were reported.

[2369] K. Chittumbo and W. Brown, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 279.

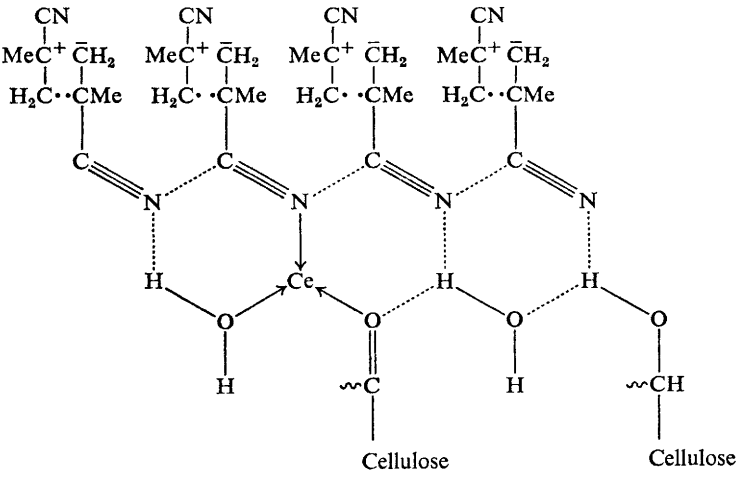
[2370] J. C. Arthur and O. Hinojosa, *J. Polymer Sci., Part C, Polymer Symposia*, 1971, No. 36, 53.

The nature of the propagating radical in the copolymerization of methacrylic acid with γ-irradiated cellulose has been investigated by e.s.r. spectroscopy.[2371] Grafts of different levels of poly(methyl methacrylate) and poly(butyl methacrylate) on to cellulose have been obtained using a ferrous ion–hydrogen peroxide initiated system.[2372] I.r. spectroscopy of the copolymers and of blends of the homopolymers with cellulose indicated that the absorbance of the carbonyl peak at 1730 cm^{-1} is lower for the grafts than for the blends. Dynamic differential thermal analyses permitted distinctions to be made between the different types of polymer, the levels of polymer bound to cellulose, and the types of bonding of the polymer to cellulose. Whereas both i.r. spectroscopy and differential thermal analysis may be used for general characterization of cellulose copolymers, the latter method appeared to be more precise. Thermogravimetric analysis and differential scanning calorimetry have been used to examine the effects of molecular structure on the thermal behaviour of poly(alkyl acrylates) and poly(methyl methacrylate) grafted on to cellulose with the aid of the ferrous ion–hydrogen peroxide system.[2373] The physical properties of the polymers were again found to depend on the nature of the graft and the polymer loading.

The graft copolymerization of acrylamide to cellulose has been carried out using a ceric salt as initiator.[2374] The total conversion and grafting ratio increased with increasing concentration of acrylamide, but decreased with an increase in the concentration of ceric ion. Evidence was obtained to indicate that the reaction occurred inside the cellulose grains and the results of *X*-ray diffraction measurements suggested that graft copolymerization did not occur in the crystalline region of cellulose particles. The flocculation of aqueous suspensions of microcrystalline cellulose powder grafted with polyacrylamide has been studied.[2375] A correlation between the initial flocculation rate and the length and number of grafted polymer chains was established by turbidimetric measurements; the relationships were discussed in terms of a polymer-bridging mechanism.

An investigation of the polymerization of methacrylonitrile (using ceric ammonium nitrate and ammonium persulphate as initiators) in the presence and in the absence of cellulose has provided support for the role of monomer–cellulose interactions in the graft copolymerization and has permitted the structure of the monomer–cellulose–water complex to be predicted.[2376] It was suggested that, with ceric ion, cellulose–monomer–water and ceric ion–monomer–water complexes are present. The complex containing ceric ion is anchored to the cellulose matrix by the interaction of

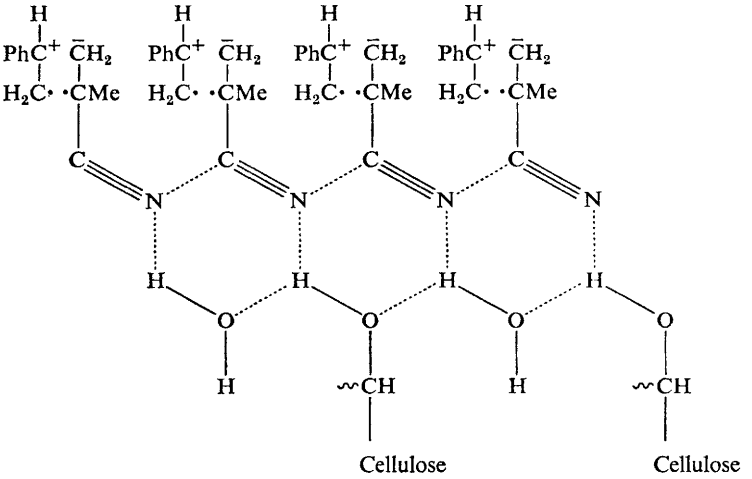
[2371] O. Hinojosa and J. C. Arthur, *J. Polymer Sci., Part B, Polymer Letters*, 1972, **10**, 161.
[2372] B. V. Kokta, P. Le Poutre, and J. L. Valade, *Tappi*, 1972, **55**, 370.
[2373] B. V. Kokta and J. L. Valade, *Tappi*, 1972, **55**, 375.
[2374] H. Narita and S. Machida, *J. Soc. Textile and Cellulose Industry, Japan* (*Sen-I Gakkaishi*), 1971, **27**, 162.
[2375] S. Machida, H. Narita, and T. Katsura, *Angew. Makromol. Chem.*, 1971, **20**, 47.
[2376] N. G. Gaylord and L. C. Anand, *J. Polymer Sci., Part B, Polymer Letters*, 1972, **10**, 285.



(152)

ceric ion with carbonyl groups either present initially or generated by the redox reaction [see (152)].

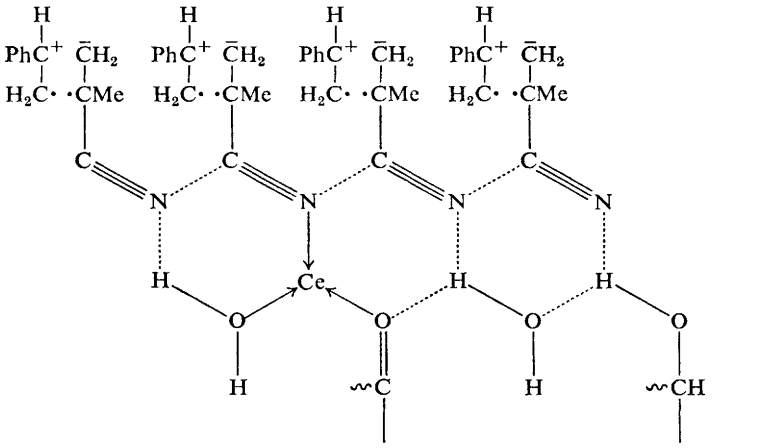
Alternating graft copolymers of cellulose have been obtained by grafting alternating styrene–acrylonitrile copolymers on to cellulose in the presence of zinc chloride.[2377] In the absence of the metal halide, the graft copolymerization involved the complex (153), but analogous structures can



(153)

[2377] N. G. Gaylord and L. C. Anand, *J. Polymer Sci., Part B, Polymer Letters*, 1971, **9**, 617.

be considered for other pairs of donor and acceptor molecules.[2378] When ceric ions are involved in the complexes, they are aligned on the cellulose matrix as shown in (154). The similar high molecular weights of grafted and ungrafted alternating copolymers are consistent with the proposal that both polymers arise from complexes aligned on the cellulose matrix.



(154)

The graft copolymerization of methyl vinyl ketone in such solvents as water, methanol, and DMF and their mixtures to pre-irradiated fibrous cellulose has been investigated.[2379] Addition of methanol and high concentrations of DMF to aqueous solutions of methyl vinyl ketone inhibited copolymerization, whereas low concentrations of DMF increased the rate of the reaction. The copolymers exhibited a strong i.r. absorption band characteristic of the carbonyl group of the grafted ketone. The technological properties of the fibrous copolymers were investigated. Nitrogenous derivatives of fibrous cellulose–poly(methyl vinyl ketone) copolymers have been prepared by reaction of the carbonyl groups with hydroxylamine, semicarbazide, and potassium cyanide;[2380] quantitative derivatization was found to occur with hydroxylamine.

Cellulose has been shown to adsorb cellulase strongly at pH 4—5 and this property has been applied to the continuous degradation of cellulose to D-glucose.[1885]

Chitin.—The preparation of oligosaccharides from chitin has been described.[430]

[2378] N. G. Gaylord and L. C. Anand, *J. Polymer Sci., Part B, Polymer Letters*, 1972, **10**, 305.
[2379] A. H. Reine, J. C. Arthur, and M. George, *J. Appl. Polymer Sci.*, 1972, **16**, 1893.
[2380] N. A. Portnoy, A. H. Reine, and J. C. Arthur, *Carbohydrate Res.*, 1972, **24**, 198.

Glycol–chitin, agar–chitin, and agar–chitosan have been used as substrates for investigations of chitinase activities.[1505] The molecular weights and sulphate contents of the products formed on treatment of chitin and chitosan with concentrated sulphuric acid for 2 h at 0 °C have been determined.[2163] Extensive sulphation and depolymerization occurred when chitin was dissolved in the acid and the effect of temperature on depolymerization was also studied.

Treatment of chitin with concentrated sodium hydroxide solutions at elevated temperature did not cause de-*N*-acetylation, but affected the intrinsic viscosity of the polysaccharide.[2381] Degradation was less pronounced when argon, rather than air or nitrogen, was used as the surrounding atmosphere. A method was also described for the direct isolation of chitosan from crab shells without the preliminary isolation of chitin. The physical and tensile properties, *X*-ray patterns, and degree of molecular weight heterogeneity in films cast from chitosan were investigated.

Chitosan is considered to form chelates with a number of metal ions and its chelating ability compared favourably with that of the chelating resin Chelex.[2382] Chitosan has been proposed, therefore, as a chromatographic support suitable for pollution detection, survey, and abatement, water purification, and the recovery of trace metal ions for analytical purposes. Chitosan was used for the collection of transition metals from salt solutions and sea water by column chromatography.

The depolymerization and *N*-sulphation of chitosan by concentrated sulphuric acid have been investigated.[2383] Whereas neither the reaction time nor temperature appeared to affect the degree of sulphation, the temperature of the solvent ether used to separate the sulphated products was found to have a marked effect. The stability of chitosan to concentrated sulphuric acid was reduced by the presence of the remaining *N*-acetyl groups. Use of aqueous acid also caused depolymerization, but the degree of sulphation was less than that achieved using concentrated acid. The anticoagulant activities of *N*- and *O*-sulphated and of oxidized chitosans have been investigated.[2384]

Chitosan has been shown to form a complex with dextran sulphate and this property has been applied to the detection of chitosan in gels.[2161]

The peracetylated 2-acetamido-2-deoxy-{2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)}$_{3-6}$-α-D-glucoses have been prepared by acetolysis of chitin.[1809] The tetrasaccharide was isolated by chromatography on silicic acid and was converted into the chloro-compound by treatment with acetyl chloride and hydrogen chloride. Reaction of the product with sodium 3,4-dinitrophenolate in DMF, followed by deacetylation, yielded

[2381] L. A. Nudga, E. A. Plisko, and S. N. Danilov, *Zhur. obshchei Khim.*, 1971, **41**, 2555.
[2382] R. A. A. Muzzarelli, G. Raith, and O. Tubertini, *J. Chromatog.*, 1970, **47**, 414.
[2383] K. Nagasawa and N. Tanoura, *Chem. and Pharm. Bull. (Japan)*, 1972, **20**, 157.
[2384] R. L. Whistler and M. Kosik, *Arch. Biochem. Biophys.*, 1971, **142**, 106.

3,4-dinitrophenyl 2-acetamido-2-deoxy-{2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)}$_3$-β-D-glucopyranoside, which proved to be a good chromophoric substrate for lysozyme.

Cycloamyloses.—Crystal structure data for modified cycloamyloses have been reviewed.[1783]

Carboxymethyl ethers of cyclohexa- and cyclohepta-amyloses have been prepared by treatment of the cyclodextrins with chloroacetic acid.[2385] Sulphopropyl ethers were formed by the reaction of the cycloamyloses with propanesulphone. The molecular weights of these derivatives were determined in a number of ways. Water-soluble, cross-linked resins have been prepared from cyclohexa- and cyclohepta-amyloses by reaction with epichlorohydrin. Use of these derivatives facilitated an examination of the formation of inclusion compounds by cycloamyloses with apolar substances in solution. In particular, the interaction of the derivatives with 3-chlorobenzoic acid was studied. Further studies on the inclusion of this acid by the cross-linked cyclohepta-amylose have been reported.[2386]

Preparations of cyclohexa-amylose pyridine-2,5-dicarboxylate [2387] and a spin-labelled monoester of cyclohepta-amylose [2388] have been described.

The structures of cyclohexa-amylose complexes with a number of guest organic molecules have been studied by *X*-ray diffraction analysis and the type of crystal structure was found to vary according to the nature of the included molecule.[2389] In an investigation of the interaction of cyclohepta-amylose with pharmaceuticals, complexes were formed with several barbituric acid derivatives.[2390] Derivatives containing cyclic residues showed higher degrees of interaction, which were generally found to have a stabilizing effect upon the barbituric acids.

Dextran.—The properties and applications of cross-linked, dextran-based ion-exchangers have been discussed.[2300]

The u.v., o.r.d., and c.d. spectra of dextran acetates and xanthates in trifluoroethanol have been recorded.[2281] The c.d. spectra of the acetates did not exhibit any conformational effects, but were sensitive to β-(1 → 6)- and branched linkages. Dextran xanthate showed only a simple o.r.d. spectrum and no observable c.d. spectrum.

An aldehydodextran, produced by periodate oxidation of the native polysaccharide, has been allowed to react with the free amino-groups on the surface of sheep red-blood cells and the product was used to assess the active synthesis of immunoglobulin IgM by tumour cells.[1367] Periodate-oxidized dextran also reacted with bovine serum albumin derivatives of Sepharose cyclic imidocarbonate to form a product that was used in the isolation of an anti-dextran by immunoadsorption.[1198]

[2385] J. N. J. J. Lammers, J. L. Koole, and J. Hurkmans, *Stärke*, 1971, **23**, 167.
[2386] N. Wiedenhof and R. G. Trieling, *Stärke*, 1971, **23**, 129.
[2387] R. Breslow and L. E. Overman, *J. Amer. Chem. Soc.*, 1970, **92**, 1075.
[2388] R. M. Paton and E. T. Kaiser, *J. Amer. Chem. Soc.*, 1970, **92**, 4723.
[2389] K. Takeo and T. Kuge, *Agric. and Biol. Chem. (Japan)*, 1970, **34**, 568.
[2390] K. Koizumi and K. Fujimura, *J. Pharm. Soc. Japan*, 1972, **92**, 32.

Aminoacyl derivatives of dextran have been obtained by condensation of dextran with N-protected amino-acids.[2391] Treatment of dextran with imidazolide derivatives of amino-acids resulted in the introduction of 2—4 amino-acid residues per 100 D-glucose units.[2392]

The relative stiffness of the molecular chain in the polyelectrolytes carboxymethyl-, DEAE-, quaternary aminoethyl-dextrans, and dextran sulphate has been estimated from viscosimetric measurements made at different ionic strengths.[2393] The formation of an insoluble complex between soluble DEAE-dextran and acidic polysaccharides has been applied to the detection of the latter in gels;[2161] this technique was useful for electrophoretograms and Ouchterlony-type diffusions. DEAE-dextran can be precipitated using dextran sulphate. Subcutaneous injections of vaccine adjuvant DEAE-dextran have been shown to induce local sarcomas in mice.[2394]

Dextrans of the linear and branched types have been converted into complex ether derivatives by reaction with 1,2-epoxy-3-(4-nitrophenoxy)-propane.[2395] The resulting 2-hydroxy-3-(4-nitrophenyoxy)propyl groups were reduced with titanous chloride and the product was found to contain 3—10 amino-groups per dextran chain. The diazotized form of the reduced derivative was coupled with 2-naphthol, insulin and its derivatives, L-tyrosine, poly(L-tyrosine), and human serum albumin to give conjugates that were tested for their ability to induce hypersensitivity reactions in guinea-pigs.

Dextran cyclic imidocarbonates have been prepared by treating dextran with either cyanogen bromide or organic cyanates.[2396] The imidocarbonates reacted with glycine, insulin, and ampicillin to give soluble conjugates; the antibiotic derivative exhibited a low level of bacteriostatic activity. Water-soluble insulin derivatives of dextran activated with cyanogen bromide have been tested for biological activity *in vivo* and *in vitro*, and they have aided studies of the mode of action of insulin.[2397, 2398]

The structure of the chromophoric group of Blue Dextran 2000, a water-soluble dye derivative of dextran (molecular weight 2×10^6) that is commercially available, has now been disclosed.[2399] This derivative is useful for determining the void volumes of gel-filtration columns and has also been applied to the purification of proteins and glycoproteins on

[2391] A. E. Vasilyiev, A. A. Khachaturjan, and G. Y. Rosenberg, *Khim. prirod. Soedinenii*, 1971, **7**, 698.
[2392] G. N. Koltsova, A. A. Khachaturjan, T. N. Doronina, A. E. Vasilyiev, and G. Y. Rosenberg, *Khim. prirod. Soedinenii*, 1972, **8**, 266.
[2393] O. Smidsrød, *Acta Chem. Scand.*, 1971, **25**, 2770.
[2394] J. M. Rice and R. M. Madison, *Nature New Biol.*, 1972, **236**, 28.
[2395] S. A. Barker, C. N. D. Cruickshank, H. M. Disney, and P. J. Somers, *Immunology*, 1972, **23**, 689.
[2396] L. Kågedal and S. Åkerström, *Acta Chem. Scand.*, 1971, **25**, 1855.
[2397] K. J. Armstrong, M. W. Noall, and J. E. Stouffer, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 354.
[2398] F. Suzuki, Y. Daikuhara, M. Ono, and Y. Takeda, *Endocrinology*, 1972, **90**, 1220.
[2399] Blue Dextran 2000, Pharmacia, Uppsala, 1972.

account of its protein-binding characteristics. The ability of dextranase to liberate a dye complex from Dextran Blue 2000 has been applied to the estimation of unknown amounts of the enzyme.[1513, 1899] The suitability of Blue Dextran 2000 as a void-volume marker has been compared with that of dye derivatives of bacterial lipopolysaccharides.[2400]

An aqueous two-phase system of hydroxypropyldextran and dextran has been used as a model in adsorption studies of Sephadex gels.[2401] The results of these studies supported the theory that the hydroxypropyl ether groups in Sephadex gels are the source of adsorption occurring during gel chromatography.

The oxidation of dextran by Fenton's reagent (hydrogen peroxide and ferrous sulphate) has been studied by redox titrimetry.[2402]

Dextran has been phosphorylated using polyphosphoric acid in the presence of a tertiary amine [2278] and derivatization was shown to impart interferon-inducing properties to dextran.

Homogeneous sulphation of dextran with piperidine-*N*-sulphonic acid in DMSO has been described and the effects of varying a number of parameters on the sulphation of high and low molecular weight dextrans were investigated.[2403] Conditions for sulphation without accompanying depolymerization were established, and the anticoagulant and lipaemia-clearing activities of dextran sulphates produced in this way were investigated. The molecular weights and sulphate contents of the products formed on treatment of dextran with concentrated sulphuric acid for 2 h at 0 °C have been determined.[2163] From viscometric measurements made at different ionic concentrations, an estimate was made of the relative stiffness of the molecular chain of dextran sulphate.[2275] Donnan equilibria between solutions of dextran sulphate and sodium ions have been measured by equilibrium dialysis and estimates were made of the degree of fixation of sodium ions by the polyanion.[921] The results were related to those similarly obtained for glycosaminoglycuronans. Heparinoid activities of dextran sulphate have been compared with those of chondroitin poly-sulphates,[885] and dextran sulphate has been shown to interact with low-density lipoproteins in plasma.[2404] Analysis of the sedimentation patterns of dextran sulphate–lipoprotein mixtures revealed that there is a maximum of six binding sites on the dextran sulphate molecule.[2405]

Triazinyl derivatives of dextran and DEAE-dextran have been prepared by treating the polysaccharides with 2-amino-4,6-dichloro-*sym*-triazine,[1758] and these derivatives were used to produce covalent derivatives of α-amylase that were water-soluble, but of restricted mobility.

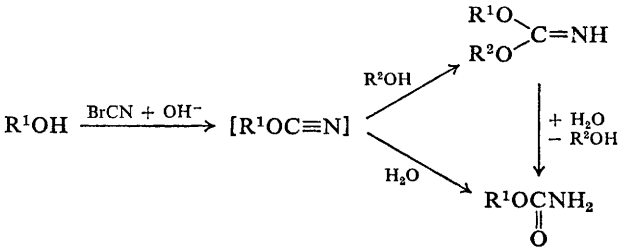
[2400] G. L. Hawk and J. A. Cameron, *J. Chromatog.*, 1972, **72**, 395.
[2401] K. Lampert and H. Determann, *J. Chromatog.*, 1971, **63**, 420.
[2402] D. L. Ingles, *Austral. J. Chem.*, 1972, **25**, 105.
[2403] K. Nagasawa, H. Harada, S. Hayashi, and T. Misawa, *Carbohydrate Res.*, 1972, **21**, 420.
[2404] T. Nishida and U. Cogan, *J. Biol. Chem.*, 1970, **245**, 4689.
[2405] M. Janado and T. Nishida, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 301.

Sephadex (a commercially available, macroporous, cross-linked dextran) after conversion into an aldehydo-derivative by periodate oxidation reacted with the free amino-groups of protein molecules to insolubilize the macromolecules.[1196] The carbinolamine-coupling groups so formed were reduced with sodium borohydride to the more stable alkylamine linkage and this reduction also served to remove unreacted aldehydo-groups. Products of the coupling have been used as immunoadsorbents.

Sephadex modified to contain 3-amino-4-methoxyphenylsulphonylethyl groups has been allowed to react in diazotized form with immunoglobulins and the product was used as an immunoadsorbent in assaying antibodies.[1267]

Carboxymethyl-Sephadex has been derivatized with Pronase to yield a water-insoluble derivative;[2323] the reaction was accomplished using a carbodi-imide. Carboxymethyl-Sephadex converted into its azido-derivative reacted with ε-aminocaproyl-D-arginine and -D-phenylalanine to form substrate analogue-coupled matrices that were used in the affinity chromatography of carboxypeptidase.[2406]

Treatment of Sephadex with cyanogen bromide has been investigated and, in addition to cyclic imidocarbonates, carbamate groups were considered to be formed (Scheme 26).[2407] Whereas cyclic imidocarbonate



R^1 and R^2 are parts of the Sephadex molecule

Scheme 26

groups are involved in the coupling reaction with amino-groups, a low, but significant, halogen content of the activated Sephadex suggested the presence of a small proportion of bromotriazinyl groups. These groups could be formed by trimerization of cyanogen bromide, followed by reaction with the polysaccharide, and would take part in the coupling reaction. The preparation of amino-acid and peptide derivatives of the cyclic imidocarbonate were described. Other cyanogen halides were equally suitable for this type of activation of Sephadex and the products were used for insolubilization of chymotrypsin and trypsin.[2186] Adenosine 3′,5′-cyclic phosphate to which an aminocaproylcysteamine side-chain had been attached reacted with Sephadex cyclic imidocarbonate and the stability

[2406] H. Akanuma, A. Kasuga, T. Akanuma, and M. Yamasaki, *Biochem. Biophys. Res. Comm.*, 1971, **45**, 27.

[2407] R. Axén and P. Vretblad, *Acta Chem. Scand.*, 1971, **25**, 2711.

of the bridge between the two molecules was examined as a function of pH.[2251] For the particular system examined, it appeared that the ligand and its side-chain could be liberated from the matrix at pH >5.

Further reports on the use of Sephadex cyclic imidocarbonate for the preparation of active, water-insoluble enzymes have described its reaction with guanyloribonuclease[2187] and trypsin.[2193] However, alkaline phosphatase became inactivated on attachment to the modified polysaccharide.[2330] Fluorescence spectral analysis of chymotrypsin, trypsin, and β-alanyl-L-tryptophan,[2185] and trypsin[2194] coupled with Sephadex cyclic imidocarbonate has been used to investigate changes occurring in the conformations of the enzymes on insolubilization.

Chymotrypsin and trypsin attached to Sephadex cyclic imidocarbonate have been investigated as affinity chromatography matrices for the selective isolation of ovomucoid,[1418] and an analogous derivative of sulphanilamide has been used for the affinity chromatography of carbonic anhydrase.[2248] Nicotinamide adenine dinucleotide coupled to cyclic imidocarbonate derivatives of Sephadexes of a number of types has been used in a micromethod for determining the apparent molecular weights of dehydrogenases; the technique is based on their exclusion from the affinity-gel filtration matrices.[2408]

Ion-exchangers containing the grouping $=\overset{+}{N}H(CH_2)_nCO_2^-$ have been prepared by the reaction of glycine, β-alanine, and ε-aminocaproic acid with Sephadex cyclic imidocarbonate.[2409] The dipolar, ionic adsorbents proved to have advantages over conventional ion-exchange matrices, since their charge properties were less intense and could be adjusted by changing the distance between the charges. Reversible adsorption of proteins was shown to occur and the molecular-sieving properties seemed to predominate in the derivative having $n = 1$, whereas the weak cation-exchanger effect was dominant in derivatives having $n = 2$ and 5.

DEAE-Sephadex in its hydroxide-counterion form has been used to produce an active derivative of aminoacylase by adsorption.[2410]

A dye derivative of Sephadex has been prepared by reaction of the cross-linked polysaccharide with Cibachron Blue F_3G-A;[2411] the product was used for the affinity chromatography of phosphofructokinase.

A nucleic acid derivative of Sephadex was formed by reaction of the polysaccharide with DNA in the presence of a water-soluble carbodiimide.[2412] Sonication was necessary to effect covalent binding, which was considered to involve phosphodiester linkages. The product was used in studies with deoxyribonuclease and in those of templates for ribonucleic acid polymerase.

[2408] C. R. Lowe and P. D. G. Dean, *F.E.B.S. Letters*, 1971, **18**, 31.
[2409] J. Porath and L. Fryklund, *Nature*, 1970, **226**, 1169.
[2410] T. Tosa, T. Mori, and I. Chibata, *J. Ferment. Technol.*, 1971, **49**, 522.
[2411] H.-J. Böhme, G. Kopperschläger, J. Schulz, and E. Hofmann, *J. Chromatog.*, 1972, **69**, 209.
[2412] D. Rickwood, *Biochim. Biophys. Acta*, 1972, **269**, 47.

Sephadexes have been trimethylsilylated (with trimethylchlorosilane–pyridine) to extend their range of application, especially with regard to the polarity of the developing solvent.[2413] The trimethylsilylated gels could be swollen sufficiently in non-polar solvents and their permeability limits on gel chromatography increased with decreasing degrees of cross-linking in the original Sephadex. The modified gels were hydrolytically stable and could be used for the separation of solute mixtures in either straight-phase or reversed-phase chromatography according to the polarity of the developing solvent.

Sephadex has been used for the purification (by adsorption) of an anti-agglutinin from *Helix aspersa*;[2414] the material was subsequently desorbed by elution with 2-acetamido-2-deoxy-D-glucose.

Glycogen.—Glycogen has been modified by enzymic extension of its branched chains.[2294] The iodine complexes of these molecules appeared star-shaped in the electron-microscope picture.

Glycosaminoglycuronans.—The molecular weights and sulphate contents of the products arising from treatment of chondroitin 6-sulphate with concentrated sulphuric acid for 2 h at 0 °C have been determined.[2163] It has been shown that when chondroitin 6-sulphate is dissolved in concentrated sulphuric acid, sulphation and depolymerization take place simultaneously.[2415] The degree of depolymerization depended on the reaction temperature, which also slightly affected the degree of sulphation. However, the degree of sulphation was found to be controlled largely by the temperature at the time when ether was added to the reaction mixture for the separation of the products. By controlling the reaction conditions of the sulphation, products with various combinations of molecular weight and sulphate content were obtained. Although the products appeared to be polydisperse when subjected to gel filtration, neither degradation of uronic acid residues nor deacetylation occurred during sulphation. Periodate oxidation and the i.r. spectra of sulphated chondroitin 6-sulphate indicated that the hydroxy-groups in the disaccharide repeating unit had been randomly sulphated. Similar results were obtained for chondroitin 4-sulphate.

Over forty samples of sulphated chondroitin 6-sulphate, differing in both the degree of sulphation and the number-average molecular weight (5.6—15.6% S, $\bar{M}_n$ 600—73 800), have been examined for their heparinoid activities in relation to their chemical properties.[885] The anticoagulant activity was low, but the lipaemia-clearing activity was comparable to that of heparin. The clearing activity was found to be proportional to the degree of sulphation of the sample and the range of values of $\bar{M}_n$ for optimal activity was found to be $2.5—4.0 \times 10^4$.

[2413] H. Tanaka and K. Konishi, *J. Chromatog.*, 1972, **64**, 61.
[2414] I. Ishiyama, G. Uhlenbruck, and G. Hermann, *Blut*, 1972, **24**, 178.
[2415] K. Nagasawa and Y. Inoue, *Chem. and Pharm. Bull. (Japan)*, 1971, **19**, 2617.

Desulphation of chondroitin sulphate and heparin reduced their ability to inhibit the action of RNA polymerase.[974] Dermatan (desulphated dermatan sulphate) has been produced by alkaline hydrolysis of dermatan sulphate, which had been pre-treated with methanol and hydrogen chloride.[1658] Modification of hyaluronic acid by ferrous ions in the presence of oxygen gave rise to an increase in the number of acidic groups and the formation of residues yielding β-formylpyruvic acid on periodate oxidation.[880] Degradation of the polysaccharide with ferrous ions rendered it incompletely hydrolysable by the combined actions of hyaluronidase, β-acetamidodeoxyglucosidase, and β-glucuronidase.

Hydroxy-acids reacted with water-soluble carbodi-imides to form lactones, which could be reduced with sodium borohydride to the corresponding alcohols. Glycosaminoglycuronans have been shown to react in an analogous way and, although the exact natures of the products have not been established, they appeared to be either lactones or some other form of intramolecular esters.[862] Borohydride reduction of the products converted the hexuronic acid residues into the corresponding neutral sugars. This reduction facilitated acidic hydrolysis of glycosyluronic acid bonds by replacing them with more acid-labile glycosyl bonds. When hyaluronic acid, chondroitin sulphate, and heparin were subjected to the reaction sequence, more than 90% of the original glycosyluronic acid bonds were subsequently cleaved with acid. The remaining, acid-resistant 2-amino-2-deoxy-D-hexosyl bonds could then be cleaved with nitrous acid to complete an essentially quantitative, non-destructive depolymerization of these polymers. Applications of the combined reactions to component analysis of glycosaminoglycuronans were discussed.

The interaction of glycosaminoglycuronans with Alcian Blue to form coloured complexes has been quantitated;[864] conditions for maximum interaction were established and employed in the quantitative estimation of glycosaminoglycuronans. The complexes could be dissociated with sodium sulphosuccinate dibutyl ester. Similar complexes of glycosaminoglycuronans with Toluidine Blue have been applied to the quantitative determination of the polysaccharides in cellulose acetate membranes.[865] Complexation of glycosaminoglycuronans with hexamine cobaltichloride has been shown to affect the transport of calcium ions in rat liver mitochondria.[2416]

Chondroitin 6-sulphate, dermatan sulphate, heparin, and heparan sulphate have been insolubilized by reaction with Sepharose cyclic imidocarbonate.[888] The reaction was considered to involve the amino-groups of short peptide chains attached to the polysaccharides. The insolubilized glycosaminoglycuronans and a cross-linked hyaluronic acid were used in a study of the interactions of lipoproteins with glycosaminoglycuronans.

[2416] B. A. Tashmukhamedov, A. I. Gagelgans, K. Matatkulov, and E. M. Makhmudova, *F.E.B.S. Letters*, 1972, **28**, 239.

Guaran.—Pyranosidic pentose and hexose residues having the aglycone in an equatorial position in the most stable chair form were readily oxidized by chromium trioxide, whereas those in which the aglycone occupies an axial position were more resistant to oxidation. Acetylated guaran, which consists of chains of β-(1 → 4)-linked D-mannose residues having 60% substituted by an α-D-galactopyranose residue in the C-6 position, when subjected to the oxidation yielded a product containing relatively less D-mannose than the parent polysaccharide.[2417] This result accorded with the selective oxidation of β-linked residues.

Inulin.—Methanolysis of inulin for the preparation of methyl β-D-fructofuranoside has been described.[2418]

Lichenin.—A carboxymethyl derivative of lichenin (a soluble β-glucan related to cellulose) has been produced by reaction of the polysaccharide with chloroacetic acid.[1882] Reaction of the polysaccharide with ethylene oxide or dimethyl sulphate gave hydroxyethylated and partially methylated derivatives, respectively. Degradation of these derivatives with cellulase was investigated.

Mannan.—The fibre structure of a fully acetylated mannan from *Tubera salep* has been investigated.[2419] *X*-Ray analysis diagrams, together with conformational data based on minimum energy considerations, implied that a three-fold axis exists along the chain.

Yeast mannan has been phosphorylated using polyphosphoric acid in the presence of a tertiary amine to give a product exhibiting interferon-inducing activity.[2278]

Nigeran.—The u.v., o.r.d., and c.d. spectra of nigeran acetate in trifluoroethanol have been recorded.[2281] The c.d. spectrum did not exhibit any conformational effects.

Pachyman.—Methodology for the carboxymethylation of pachyman has been described.[2420] Treatment with monochloroacetic acid was carried out in propan-2-ol and variation of the time of heating allowed degrees of substitution up to 0.7 to be achieved. The preparation of carboxymethylpachyman has also been described by others and the product was used as a substrate for oligo-1,3-glucosidase.[1946]

Pectic Acid.—The esterification of pectic acid using a water–alcohol–hydrochloric acid mixture has been studied.[2421] With saturated and unsaturated alcohols, ethylene glycol, and glycerol, esterification was found to proceed readily at first, but did not reach completion. The degree of esterification decreased with an increase in the ratio of the number of

[2417] J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 1972, **26**, 661.
[2418] J. Szejtli, R. D. Henriques, and M. Castiñeira, *Acta Chim. Acad. Sci. Hung.*, 1972, **72**, 459.
[2419] H. Bittiger and R. H. Marchessault, *Carbohydrate Res.*, 1971, **18**, 469.
[2420] B. A. Stone, *Methods Carbohydrate Chem.*, 1972, **6**, 384.
[2421] M. Manabe, *J. Agric. Chem. Soc. Japan*, 1971, **45**, 417.

carbon atoms to hydroxy-groups in the alcohol. The viscosity of the esters decreased with increasing ester content.

Pectic acid and pectic acids of low methoxy-group contents were esterified readily with aqueous methanol containing small amounts of hydrogen chloride.[2422] According to the proportions of the reagents and reaction conditions, up to 86% of the hydroxy-groups could be esterified and pectic acids containing a range of contents of methoxy-groups were prepared.

The oxidation of pectin with Fenton's reagent (ferrous ions and hydrogen peroxide) has been studied using redox titrimetry to monitor the reaction.[2402] Pectic acids oxidized by sodium hypoiodite and then reduced with sodium borohydride were found to be resistant to the action of a polygalacturonate lyase from a *Pseudomonas* sp.[1967]

Pectic acid has been sulphated with ease to a degree of substitution of 1.8 using a DMF–sulphur trioxide complex.[2277] Lower degrees of substitution were achieved by decreasing the proportion of the complex and, in all cases, viscosimetric measurements of the products indicated that little degradation had occurred. Pectic acid sulphates formed complexes with proteins and the reactivity was more pronounced with high degrees of sulphation.

Reaction of pectic acid with water-soluble carbodi-imides yielded products which appeared to be lactones or some form of intramolecular esters.[862] Reduction with sodium borohydride yielded a product in which the hexuronic acid residues had been converted into neutral sugar residues.

Pectic acid has been cross-linked by reaction with epichlorohydrin.[1951, 1952] A cross-linked, water-insoluble pectic acid was shown to adsorb selectively *endo*-polygalacturonase and this property was used for purification of the enzyme by affinity chromatography.[1951] The enzyme was competitively inhibited by the cross-linked substrate and formation of the enzyme–inhibitor complex stabilized the enzyme to heat denaturation.[1952]

A pectate gel medium has been used for the isolation of pectinolytic bacteria.[2423]

Pullulan.—That pullulan is randomly oxidized by periodate ion has been proved by Smith degradation and gel filtration of the reaction products.[1454] It has been calculated that random oxidation of pullulan to an extent of 5% causes less than 1% of the polysaccharide to remain unprotected from attack by *exo*-enzymes. On this basis, periodate-oxidized pullulan has been used to distinguish between *exo*- and *endo*-glucanases, since 5% oxidation did not appear to inhibit the action of the latter type of enzyme.

Starch.—Corn and potato starches were fully acetylated with little degradation using acetic anhydride in an alkaline medium.[2424]

[2422] M. Manabe, *J. Agric. Chem. Soc. Japan*, 1971, **45**, 195.
[2423] S. N. Thorne, *J. Appl. Bacteriol.*, 1972, **35**, 357.
[2424] A. M. Mark and C. L. Mehltretter, *Stärke*, 1972, **24**, 73.

Factors affecting the hydrolysis of periodate-oxidized starch to D-erythrose and glyoxal by sulphurous acid have been investigated systematically.[2425] The folate antagonists aminopterin and amethopterin have been covalently attached by way of their carboxy-groups to aminoethylstarch with the aid of a carbodi-imide.[2252] The products were used in the purification of dihydrofolate reductases by affinity chromatography. Starch anthranilates (DS 0.10 and 0.01) have been cyanoethylated to give products (DS 0.1 and 1.0, respectively) that were used in their diazotized forms for the insolubilization of papain.[2426]

Starch has been dyed with Cibachron Blue F_3G-A and the product was cross-linked with butane-1,4-diol glycide ether to give a material suitable for inclusion in agar gels.[1701] In this form, the dyed starch was used as a substrate in determinations of α-amylase.

Starch has been etherified to give benzylstarch and esterified to give starch benzoate, laurate, octanoate, palmitate, and stearate.[2427] The effect of substitution on the reactivity of starch with isocyanates was investigated and maximum reactivity was achieved with a degree of substitution of 0.7. The starch derivatives were used in plastic formulations.

Carbamoylethyl ethers of wheat flour have been prepared by alkali-catalysed addition of acrylamide[2428] and the properties of the products were investigated. Hypochlorite-treated, carbamoylethylated corn starch has been used in paper-making and was found to impart greater tensile strength to the product.[2429]

Maize starch has been carboxymethylated with monochloroacetic acid using a reaction medium of aqueous alkali and acetone.[2430] Degrees of substitution up to 0.81 were achieved at 20—40 °C, whereas omission of acetone resulted in much lower degrees of substitution. Viscosimetric measurements on carboxymethylstarch in sodium hydroxide solutions revealed a correlation between the viscosity and the degree of substitution only when the series of ethers had been synthesized and isolated by the same procedure.

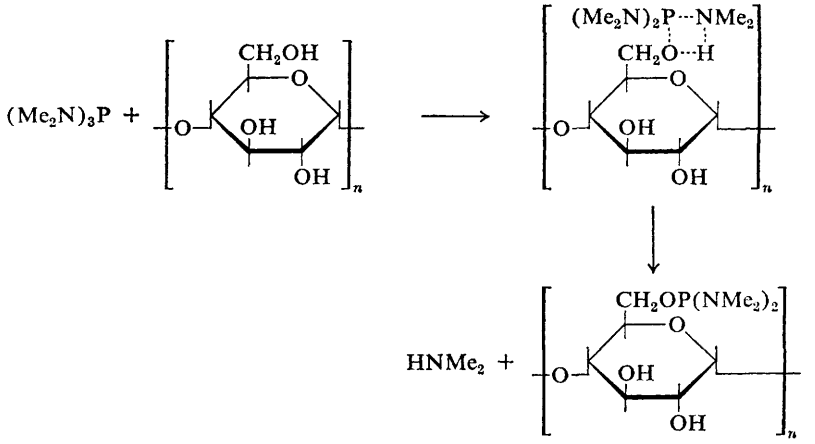
From a study of the digestibility of hydroxypropylstarches by relatively large amounts of pancreatin, it was found that digestibility decreased exponentially with increasing degrees of substitution.[1713] This relationship was explained by means of a statistical model based on the assumption that the hydroxypropyl groups are distributed randomly over the starch molecule.[1714] In such cases, the reducing power of the pancreatic digests was proportional to the function (1 − degree of substitution) and, from available data, the proportionality constant was assigned a value 5.3.

[2425] C. A. Wilham, T. A. McGuire, and C. L. Mehltretter, *Stärke*, 1971, **23**, 201.
[2426] C. L. Mehltretter and F. B. Weakley, *Biotechnol. and Bioeng.*, 1972, **14**, 281.
[2427] F. H. Otey, R. P. Westhoff, and C. L. Mehltretter, *Stärke*, 1972, **24**, 107.
[2428] H. E. Smith, H. C. Katz, S. H. Gordon, and C. R. Russell, *Cereal Chem.*, 1972, **49**, 336.
[2429] H. E. Smith, S. H. Gordon, C. R. Russell, and C. E. Rist, *Tappi*, 1970, **53**, 1704.
[2430] M. Čeh, *Stärke*, 1972, **24**, 124.

This result suggested that the pancreatic enzymes act only upon chains containing five or more α-(1 → 4)-linked D-glucopyranose units.

The oxidation of starch by Fenton's reagent (ferrous ions and hydrogen peroxide) has been investigated and the products were analysed by redox titrimetry.[2402] The mild oxidation of starch with aqueous bromine has been further studied with respect to the physicochemical properties of the products.[2431] Oxystarches prepared at pH 7 had a slightly lower intrinsic viscosity in solution than those prepared at pH 8 and it was concluded that the oxystarches do not undergo degradation at pH 8. Furthermore, oxystarches were found to be stable in 1M potassium hydroxide for considerable periods. It was considered that the extensive depolymerization of starch observed during oxidation may be due to β-alkoxy-elimination reactions engendered either by carbonyl groups at C-2 or C-3, or by aldehydic or carboxy-functions at C-6. Alternatively, depolymerization could be caused by brominolysis of glycosidic bonds.

Phosphorylation of starch has been accomplished using an amine salt of tetra(polyphosphoric acid).[2363] The reaction of starch with HMPT has been studied for a variety of conditions and the highest product yields and phosphorus contents (0.7%) were obtained by refluxing the mixture in organic solvents.[2432] The reaction was considered to occur according to Scheme 27. The modified starch granules so obtained were found to swell



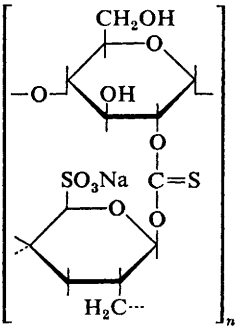
Scheme 27

in cold water and to be insoluble in organic solvents; they were strongly anionic and their gelatinization temperatures decreased with increasing degrees of substitution.

Methodology has been described for the sulphation of starch.[2158]

[2431] J. Bel-Ayche and I. Ziderman, *Carbohydrate Res.*, 1972, **24**, 159.
[2432] F. R. T. Rosenthal and V. M. R. H. de Araújo, *Stärke*, 1971, **23**, 404.

Starch xanthates (DS 0.08—0.58) reacted with high and low molecular weight polyethylenimines to form starch poly(ethyleniminothiourethanes).[2433] Aqueous solutions of the thiourethanes on mixing with styrene–butadiene and acrylonitrile–butadiene latices gave precipitable compounds that were used in the reinforcement of rubber. Sodium starch xanthate has been shown to react with a polyacrolein bisulphite adduct.[2434] The reaction was considered to occur by elimination of sodium hydrogen sulphite between a xanthate group and a hydroxy-group of the polyacrolein adduct to give a thionocarbonate bridge [see (155)].



(155)

Starch has been cross-linked using a bisepoxide and the product was then dyed with Cibachron Blue F_3G-A.[1700] Incorporation of this material into agar gel afforded a material suitable for the determination of α-amylase by a gel-diffusion technique.

The cross-linking of starch with epichlorohydrin under homogeneous and heterogeneous conditions has been studied with a view to measuring the extent of the side-reaction whereby epichlorohydrin becomes substituted on to starch as a monoether.[2435] The extent of this reaction, compared with the formation of diether cross-linkages, was found to depend on the reaction conditions and up to 25% of the applied epichlorohydrin could be bound in the product as a glycerol monoether. The degree of swelling of the cross-linked starch was shown to be linearly related to the molar ratio of water and starch in the reaction mixture. It was also shown that the cross-linking of starch proceeded efficiently when epichlorohydrin was applied in the vapour phase. The reaction between iodine and starch cross-linked with phosphorus oxychloride has been investigated.[2436] The cross-linked starch had the same capacity for iodine as native starch, although the rate of reaction was much slower.

[2433] J. A. Douglas, G. G. Maher, C. R. Russell, and C. E. Rist, *J. Appl. Polymer Chem.*, 1972, **16**, 1937.
[2434] G. G. Maher, J. A. Douglas, C. R. Russell, and C. E. Rist, *J. Polymer Sci., Part A*-1, *Polymer Chem.*, 1970, **8**, 1637.
[2435] L. Kuniak and R. H. Marchessault, *Stärke*, 1972, **24**, 110.
[2436] R. Collison and J. O. Ogundiwin, *Stärke*, 1972, **24**, 258.

Monosaccharides that inhibit the activity of haemagglutinins have been cross-linked to starch with the aid of epichlorohydrin to provide specific affinity adsorbents.[724] L-Fucose and 2-acetamido-2-deoxy-{2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 4)}$_2$-D-glucose coupled to starch in this way were used for the purification of a number of phytohaemagglutinins by affinity chromatography.

The content of carboxy-groups and the distribution of modified D-glucose residues in alcohol-soluble and alcohol-insoluble fractions arising following γ-irradiation of starch have been studied.[2437] The number of modified D-glucose residues was found to increase in proportion to the size of the radiation dose, but was independent of the water content of the sample. However, the development of carboxy-groups provided evidence of the important protecting role that water plays in this type of modification. When the proportion of alcohol-soluble fraction was expressed as a function of the proportion of water-soluble, alcohol-insoluble fraction, a linear relationship is obtained that is independent of the radiation dose, the degree of hydration, and the origin of the starch.

Graft copolymers of starch with acrylamide and acrylic acid have been induced by γ-irradiation.[2438] A graft terpolymer of starch, acrylamide, and acrylic acid was prepared by base-catalysed hydrolysis of a starch–acrylonitrile copolymer. The mixed polymers were evaluated as thickeners of textile print-paste and were most suitable when used in conjunction with sodium alginate. The graft copolymerization of methyl methacrylate on to starch and triethylaminostarch has been reported.[2439] The grafting of polyacrylics with starch by the Scott–xanthation process has also been described.[2440] On the basis of the solubility of the products and their electron-microscope pictures, a model for the grafted starch granule was proposed which consists of an ungrafted amylose core surrounded by a thick shell of amylopectin graft copolymer.

The reaction between starch and iodine to give a blue complex has been shown to involve polymerization of iodine atoms inside the linear cavity of helically coiled amylose macromolecules.[2292] The kinetics of the reaction with iodine have been studied for starches from a number of sources and the reaction was shown to follow the same rate equation as that established in a previous study using amylopectin-free fractions.[2292, 2293] The rate differences for various starches were attributed to different average molecular weights of the amylose fractions and it was concluded that amylopectin does not interfere significantly with complexation.

The action of bacterial α-amylase on the gelatinization characteristics of waxy-rice flour has been investigated.[2441] Electron-microscope studies of starch granules modified by bacterial α-amylase have been reported.[2442]

[2437] T. Tollier and A. Guilbot, *Stärke*, 1972, **24**, 285.
[2438] D. A. Jones and L. F. Elmquist, *Stärke*, 1972, **24**, 23.
[2439] T. Nishiuchi and K. Ozaki, *Nippon Kagaku Kaishi*, 1972, 1728.
[2440] E. Gruber, S. Alloush, K. John, and J. Schurz, *Stärke*, 1972, **24**, 251.
[2441] M. Tawfik and R. M. Attia, *Cereal Chem.*, 1972, **49**, 343.
[2442] D. Gallant, C. Mercier, and A. Guilbot, *Cereal Chem.*, 1972, **49**, 354.

Cholinesterase occluded in a starch gel has been employed as an immobilized form of the enzyme.[2443]

Xylan.—Hemiacetal structures were shown to be formed during the modification of xylan by oxidation with periodate ions.[2444] These inter-residue hemiacetal structures were responsible for an early halt in the oxidation process and it was not until they were removed that oxidation proceeded to the expected limit.

Reaction of a mixture of xylan and D-xylose with phosphorus pentoxide–DMSO yielded a modified polysaccharide containing phosphorus.[2142]

Miscellaneous.—Desulphation of the sulphated polysaccharide obtained from sea urchin (*Anthocidaris crassispina* and *Hemicentrotus pulcherrimus*) embryos reduced its ability to inhibit the action of RNA polymerase.[974]

An extracellular polysaccharide from an *Aerobacter* sp. has been modified by complete reduction of its constituent D-glucuronic acid to D-glucose residues.[704]

Treatment of the capsular polysaccharides from two strains of *Aerobacter aerogenes* with carbodi-imides gave rise to products that appeared to be lactones or some form of intramolecular ester.[862] Reduction of the polymeric products with sodium borohydride converted the uronic acid residues into the corresponding neutral sugars; reduction facilitated the acidic hydrolysis of glycuronosyl bonds by replacing them with acid-labile glycosyl bonds.

A polysaccharide from *Cryptococcus neoformans* has been coupled to sheep erythrocytes by the addition of a solution of chromic chloride to a suspension of the cells in a solution containing the polysaccharide.[2445]

Non-acylated derivatives of naturally acetylated, formylated, and pyruvylated oligosaccharides containing uronic acid were prepared from *Escherichia coli*, *Klebsiella* (*Aerobacter*) *aerogenes*, and *Salmonella typhimurium* by partial acidic hydrolysis.[2446]

Pyranosidic pentose and hexose residues in which the aglycone occupies an equatorial position in the most stable chair form were readily oxidized by chromium trioxide, whereas corresponding residues having the aglycone in an axial position were resistant to oxidation. This property has been used in determining the anomeric configuration of monosaccharide units in a lipid-free lipopolysaccharide from *S. typhimurium*.[2417] Abequopyranose, D-mannopyranose, D-galactopyranose, and D-glucopyranose units of the lipopolysaccharide were not modified by chromium trioxide (indicative of the α-configuration), whereas L-rhamnopyranose units were oxidized (indicative of the β-configuration). These conclusions were confirmed by other structural investigations. The lipopolysaccharide of *S. typhimurium* has been converted into a dye derivative by reaction with Procion Scarlet

[2443] G. G. Guilbault and J. Das, *Analyt. Biochem.*, 1970, **33**, 341.
[2444] T. Painter and B. Larsen, *Acta Chem. Scand.*, 1970, **24**, 2366.
[2445] T. R. Kozel and J. Cazin, *Infection and Immunity*, 1972, **5**, 35.
[2446] I. W. Sutherland, *J. Chromatog.*, 1971, **59**, 476.

MGS.[2400] The properties of the product were compared with those of Blue Dextran 2000, particularly with regard to its use as a void-volume marker for permeation chromatography.

Modification of Glycoproteins and Uses of Modified Glycoproteins

The modification of glycoproteins by periodate oxidation has been used in their detection.[636] The excess of periodate reacted with metabisulphite, which also formed addition products with the aldehydo-groups generated by oxidation. The formation of addition products enhanced the acidity of the glycoprotein and so increased its reactivity with the staining agent Alcian Blue.

Sialoglycoproteins have been modified with a factor isolated from animal tissues that caused the sialyl residues to react as free sialic acid in the Warren assay.[1380] In particular, mucins of the Cowper's gland of boar and ovine submaxillary mucins were modified in this way and it appeared that the factor renders the C-4—C-5 bond of bound neuraminic acid susceptible to oxidation by periodate.

[^{3}H]Acetyl concanavalin A has been prepared by acetylation of the phytohaemagglutinin with [^{3}H]acetic anhydride.[711] Crystals of concanavalin A containing 2-iodophenyl β-D-glucopyranoside have been used to locate the saccharide-binding site of the haemagglutinin.[701] Mercury-labelled concanavalin A has been used as a marker in the electron-microscopic localization of mannan in yeast cell walls.[2447]

A cellulosic glycoprotein from the haptophycean alga *Pleurochrysis scherffelii* has been nitrated and viscometric measurements have been carried out on the product.[675] Modification of cell-wall material from group A Streptococci by partial removal of carbohydrate, substitution of free amino-groups, and saponification of *O*-acyl groups rendered the material susceptible to lysozyme and lysosomal enzymes from human phagocytes.[2448]

The carbohydrate residues of human blood-group substances A and B have been removed by Smith-degradation sequences.[750] However, the oxidized and reduced fragment of 2-acetamido-2-deoxy-D-galactose linked glycosidically to hydroxy-amino-acids in the peptide chain was resistant to hydrolysis under the mild conditions normally employed in the Smith degradation, but was liberated by more vigorous hydrolysis.

Oxidation at C-6 of the D-galactosyl residues of the side-chains of guinea-pig collagen has been achieved using galactose oxidase, but the properties of the original collagen could be restored by reduction with sodium borohydride.[799] Collagen and other glycoproteins have been modified by partial removal of the carbohydrate residues with the aid of glycosidases as follows: collagen minus D-glucose; fetuin minus *N*-acetylneuraminic acid, minus *N*-acetylneuraminic acid and D-galactose, and

[2447] M. Horisberger, H. Bauer, and D. A. Bush, *F.E.B.S. Letters*, 1971, **18**, 311.
[2448] A. D. Glick, J. M. Ranhand, and R. M. Cole, *Infection and Immunity*, 1972, **6**, 403.

minus *N*-acetylneuraminic acid, D-galactose, and 2-acetamido-2-deoxy-D-glucose; bovine submaxillary mucin minus *N*-acetylneuraminic acid, and minus *N*-acetylneuraminic acid, L-fucose, and D-galactose.[680] The modified glycoproteins were used as model acceptors for studies with glycosyltransferases.

Bovine nasal cartilage proteoglycan yielded the protein core without cleavage of peptides when subjected to Smith degradation.[1001] The products of the degradation have been used as substrates for glycosyltransferases.

Ovine submaxillary glycoprotein has been modified by treatment with neuraminidase.[745] Treatment with neuraminidase and α-acetamidodeoxygalactosidase completely removed the carbohydrate residues.[1026] Sialoglycoprotein from porcine duodenum has been sulphated as a suspension in pyridine with either oleum or chlorosulphonic acid to give a product containing 14.1% sulphur.[2449] Various metal salts of the polysulphate could be obtained by exchange reactions with the sodium salt. A glycopeptide derived from the same source has also been sulphated to give a product exhibiting antipeptic activity.[1033] A number of studies were carried out on this product and its inhibitory action was ascribed to its ionic character.[1034, 2450, 2451]

A methylated derivative of human pituitary follicle-stimulating hormone has been prepared and isolated.[1092] Derivatization of the hormone by maleylation, citraconylation, t-butyloxycarbonylation, and acetylation have also been described.[1095] Ovine-luteinizing hormone and its α- and β-subunits have been allowed to react with tetranitromethane [2452] and the *S*-carboxymethyl derivatives of the two subunits have been prepared.[1110, 1111] Terminal *N*-acetylneuraminic acid and D-galactose residues of human chorionic gonadotrophin have been removed enzymically.[731, 1113, 1114] The asialo-glycoprotein was held more firmly by insolubilized concanavalin A than the intact molecule, but the asialoagalacto-species could not be desorbed.[731]

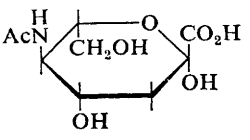
Electrophoretic and ion-exchange chromatographic studies of enzymically desialylized human thyroglobulin indicated that the modified glycoprotein possesses a different conformation from the intact molecule.[1128] The asialo molecule was found to aggregate at pH 5.6. An arginyl derivative of bovine thyroglobulin has been produced in a reaction catalysed by arginyl-transfer RNA-protein transferase.[2453]

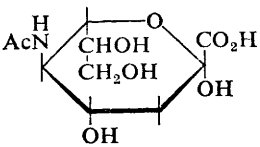
Changes occurring in casein during roasting have been investigated.[1142] Methylated, ethylated, succinylated, and acetylated derivatives of α_s-casein

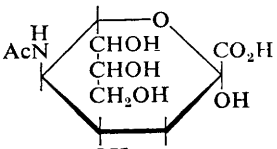
[2449] G. Bertellini, A. Butti, B. Piantanida, G. Prino, A. Riva, A. Rossi, and S. Rossi, *Arzneim.-Forsch.*, 1971, **21**, 247.
[2450] G. Prino, S. Paglialunga, G. Nardi, and A. Lietti, *European J. Pharmacol.*, 1971, **15**, 119.
[2451] G. Prino, S. Paglialunga, A. Lietti, and R. Niada, *European J. Pharmacol.*, 1972, **17**, 279.
[2452] M. R. Sairam, H. Papkoff, and C. H. Li, *Biochim. Biophys. Acta*, 1972, **278**, 421.
[2453] R. L. Soffer, *J. Biol. Chem.*, 1971, **246**, 1481.

have been prepared.[1144] The secondary structures of these derivatives were investigated by i.r. and o.r.d. spectroscopy. Dansyl derivatives of α_s-, β-, and κ-caseins of bovine origin have also been prepared and, in the latter case, hydrolysis of the derivative yielded a dansyl derivative of 2-amino-2-deoxy-D-galactose.[1145] κ-Casein has been carbamylated and subsequently esterified to give the glycine methyl ester of the carboxy-groups.[2454]

Asialo human α_1-acid glycoprotein has been prepared enzymically from the parent glycoprotein.[665, 1157] The asialoagalacto-derivatives of human α_1-acid glycoprotein and transferrin and the asialo-derivative of bovine fetuin have similarly been prepared.[665] Oxidation of human α_1-acid glycoprotein, ovine submaxillary mucin, and human erythrocyte glycoprotein with very low concentrations of periodate was found to oxidize selectively the *N*-acetylneuraminic acid units.[1166] Reduction of the oxidized glycoproteins with sodium borohydride yielded glycoproteins containing seven- (156) and eight-carbon analogues (157) of *N*-acetylneuraminic acid, as well as the parent nine-carbon unit (158), in proportions depending upon


(156)


(157)


(158)

the extent of the oxidation. Oxidation of other monosaccharides could not be detected. The action of neuraminidase on the modified glycoproteins was investigated.

Human serum albumin formed a ternary co-ordination complex with copper(II) and L-histidine, and the physicochemical properties of the complex were reported.[1181] Human serum albumin has been dinitrophenylsulphenylated,[2198] and tosyl[734] and dinitrophenyl[2198, 734] derivatives of bovine serum albumin have been described. Conjugates of bovine serum albumin with the 4-diazophenyl β-glycosides of *O*-α-D-glucopyranosyl-(1 → 6)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose (panose) and other carbohydrates have been prepared and used as artificial antigens.[2150] The interaction of bovine serum albumin with tetracycline[1191] and a sialoglycopeptide from human erythrocytes[1192] has been studied. The binding of Methyl Orange to the albumin has been investigated by Raman spectroscopy[1193] and the fluorescence characteristics of a bovine serum albumin–retinol complex have been studied.[2455] Bovine serum albumin has been used in the micro-encapsulation of carbonic anhydrase.[1194]

Human fibrinogen has been *S*-carboxymethylated[1217] and sulphitolysed.[1210] A study of the mode of complex formation between rabbit

[2454] R. F. L. Clarke and S. Nakai, *Biochim. Biophys. Acta*, 1972, **257**, 70.
[2455] S. Futterman and J. Heller, *J. Biol. Chem.*, 1972, **247**, 5168.

α_2-macroglobulin and either trypsin or chymotrypsin revealed that the complexes involve two moles of enzyme per mole of macroglobulin, irrespective of the conditions used.[1236] Complexes of bovine prothrombin with calcium ions and phospholipids have been studied.[1238] Hydroxy-groups of the carbohydrate moieties of glycoproteins isolated from the serum of an Antarctic fish (*Trematomus borchgrevinki*) have been modified by acetonation, acetylation, and oxidation with galactose oxidase.[1227] The ability of the modified glycoproteins to depress the freezing point of water was examined.

Human erythrocyte sialoglycoproteins have been labelled by sequential periodate oxidation and reduction with sodium borotritide.[1255] The label was found to reside in oligosaccharide chains of the glycoproteins and predominantly in residues which were formerly bound as sialic acid; a ten molar excess of periodate relative to sialic acid was employed.

Carbohydrate-depleted forms of immunoglobulins have been prepared by the use of glycosidases.[1301] The formation of a reduced, alkylated derivative of a mouse myeloma immunoglobulin IgA has been reported.[1302] Human immunoglobulin IgG has been modified by acetylation, acetamidation, and reaction with 2,4-dinitrofluorobenzene and 2-hydroxy-5-nitrobenzyl bromide.[1326] The citraconylated and carbamylated derivatives of human immunoglobulin IgG have been examined by c.d. spectroscopy.[1324] The preparation of alkylated heavy-chains of rabbit immunoglobulin IgG [1342] and of citraconylated derivatives of the μ- and κ-chains of human Waldenström immunoglobulin IgM [2456] have been reported.

Sialic acid and 2-amino-2-deoxyhexose residues of bovine submaxillary mucin have been removed enzymically with the aid of specific glycosidases and the products were used as substrates for studies of glycosyltransferases.[1379]

Methods for insolubilization of biologically active glycoproteins have been reviewed.[638] Preparations of many insolubilized and coupled glycoproteins have been described and these derivatives, together with their applications, are summarized in Table 8.

Modifications of Enzymes and Uses of Modified Enzymes

The maleyl derivative of β-galactosidase has been prepared [1593] and the reaction of the enzyme with *N*-bromoacetyl β-D-galactopyranosylamine served to label the active site.[1592] A reduced, alkylated derivative of β-glucosidase has been formed.[1632]

An *S*-carboxymethylated derivative of α-amylase has been used to study the functions of thiol groups in the enzyme molecule.[1739]

Changes occurring in lysozyme during roasting have been studied.[1142] γ-Irradiation of lysozyme in the dry state and *in vacuo* produced a species of molecular weight larger than that of the native enzyme.[1828] Physicochemical measurements on this species, together with the results of

[2456] A. F. S. A. Habeeb, R. E. Schrohenloher, and J. C. Bennett, *Biochim. Biophys. Acta*, 1972, **263**, 339.

Table 8 *Modification of glycoproteins by covalent coupling to insoluble matrices and other macromolecules*

Glycoprotein	*Matrix or macromolecule coupled*	*Use of product*	*Ref.*
Anti-bovine serum glycoprotein	Disulphide cross-linked	Purification of bovine serum glycoprotein by immunoadsorption	639
Anti-dinitrophenyl	Sepharose cyclic imidocarbonate	Isolation of 2,4-dinitrophenylated peptides by affinity chromatography	2174, 2196—2198
Anti-hemocyanin	Polystyrene tubes	Radioimmunoassay of anti-hemocyanin	2457
Anti-human chorionic gonadotrophin	Cellulose cyclic imidocarbonate	Radioimmunoassay of human chorionic gonadotrophin	1120
	3-Diazobenzyloxymethylcellulose	Radioimmunoassay of human chorionic gonadotrophin	1120
Anti-human chorionic somatomammotrophin	Sepharose cyclic imidocarbonate	Purification of human chorionic somatomammotrophin	2199
Anti-human α-fetoprotein	Sepharose cyclic imidocarbonate	Purification of human α-fetoprotein by immunoadsorption	2200
Anti-human immunoglobulin	Disulphide cross-linked	Purification of immunoglobulins by immunoadsorption	639
Anti-human immunoglobulin α-, γ-, κ-, λ-, and μ-chains	Sepharose cyclic imidocarbonate	Removal of classes of immunoglobulins from serum	1293
Anti-human immunoglobulin IgG light chain	Sepharose cyclic imidocarbonate	Purification of human immunoglobulin IgA by immunoadsorption	1271
Anti-human α_2-macroglobulin	Sepharose cyclic imidocarbonate	Immunoadsorbent chromatography of human α_2-macroglobulin and its complex with collagen	1235
Anti-human myeloma immunoglobulins IgA, IgG, and IgM	Sepharose cyclic imidocarbonate	Removal of classes of immunoglobulins from serum	1293
Anti-human serum albumin	4-Diazobenzylcellulose	Immunoassay of albumin fragments in human serum	1173
	Disulphide cross-linked	Purification of human serum albumin by immunoadsorption	639
Anti-lactoferrin	Sepharose cyclic imidocarbonate	Isolation of free secretory component by immunoadsorption	1377

Anti-lysozyme	Sepharose cyclic imidocarbonate	Experimental control	2201
	Disulphide cross-linked	Purification of lysozyme by immuno-adsorption	639
Anti-mouse serum	Sepharose cyclic imidocarbonate	Experimental control	2202
Anti-mouse serum myeloma glycoproteins	Sepharose cyclic imidocarbonate	Search for anti-carbohydrate antibodies in myeloma conditions	2202
Anti-nitrotyrosyl-lysozyme	Sepharose cyclic imidocarbonate	Isolation of nitrotyrosinyl peptides by immunoadsorption	2203
Anti-ovalbumin	Disulphide cross-linked	Purification of ovalbumin by immuno-adsorption	639
Anti-ovine luteinizing hormone	Sepharose cyclic imidocarbonate	Isolation of ovine luteinizing hormone by immunoadsorption	1109
Anti-rabbit immunoglobulin	Cellulose cyclic imidocarbonate	Solid-phase radioimmunoassay of human chorionic gonadotrophin	1120
	3-Diazobenzyloxymethylcellulose	Solid-phase radioimmunoassay of human chorionic gonadotrophin	1120
Anti-rabbit immunoglobulin IgG	Cellulose cyclic imidocarbonate	Immunoassay of immunoglobulin IgG	1314
Anti-ribonuclease	Ethylene–maleic anhydride copolymer	Isolation of polyribosomes by affinity chromatography	2458, 2459
	Ethyl chloroformate cross-linked	Isolation of polyribosomes by affinity chromatography	2458, 2459
Anti-tRNA	Sepharose cyclic imidocarbonate	Binding of tRNA	2201
Anti-sheep β-lipoprotein	Disulphide cross-linked	Purification of sheep β-lipoprotein by immunoadsorption	639
Anti-transferrin	Glutaraldehyde cross-linked	Isolation of transferrin by immuno-adsorption	1250
Bovine immunoglobulin	Acrylamide copolymers	Immunoassay of anti-sera	1195
Bovine serum albumin	Acrylamide copolymers	Immunoassay of anti-sera	1195
	Aldehydocellulose	Insoluble antigen	1196
	Aldehydo-Sephadex	Insoluble antigen	1196
	Aldehydo-Sepharose	Insoluble antigen	1196
	Carboxymethylcellulose[a]	Studies of antigen–antibody interactions	1197

[2457] A. O. Kotoulas and L. A. Moroz, *J. Immunol.*, 1971, **106**, 1630.
[2458] W. Y. Lee, L. Gyenes, and A. H. Sehon, *Immunochemistry*, 1971, **8**, 751.
[2459] W. Y. Lee and A. H. Sehon, *Immunochemistry*, 1972, **9**, 210.

Table 8 (*cont.*)

Glycoprotein	*Matrix or macromolecule coupled*	*Use of product*	*Ref.*
	Glutaraldehyde-treated Bio-Gel	Isolation of anti-bovine serum albumin by immunoadsorption	2460
	Glutaraldehyde cross-linked and glutaraldehyde cross-linked with ovomucoid, peroxidase, or trypsin inhibitor	Affinity chromatography of specifically interacting macromolecules	691
	Nylon[a]	Cell fractionation	734
	Sepharose cyclic imidocarbonate[b]	Isolation of anti-dextran antibody	1198
	Sepharose cyclic imidocarbonate	Experimental control	734
Concanavalin A	Acrylic polymer (Enzacryl AA)	Investigation of interactions of concanavalin A with lymphocytes	735
	Sepharose cyclic imidocarbonate	Cell fractionation	734
		Purification of glycoprotein hormones and their derivatives by affinity chromatography	731
		Purification of lymphocyte plasma-membrane glycoprotein by affinity chromatography	732, 733, 1051
	Glutaraldehyde cross-linked	Purification of mammalian glycoproteins by affinity chromatography	691, 737, 1058
		Purification of enzymes by affinity chromatography	691
	Glutaraldehyde cross-linked with coliphage K 29	Affinity density perturbation	2461
Collagen	Sepharose cyclic imidocarbonate	Purification of collagenase by affinity chromatography	810
Collagen (membrane form)	Urease	Electrochemical studies	2462
Dinitrophenylated bovine serum albumin	Nylon[a]	Cell fractionation	734

Dinitrophenylated ovalbumin	Sepharose cyclic imidocarbonate	Isolation of anti-dinitrophenyl antibodies	2198
Hemocyanin	Polystyrene	Detection of antibody to hemocyanin	2457
Human chorionic gonadotrophin	Glutaraldehyde cross-linked with peroxidase	Enzyme-immunoassay procedure	1120
Human Bence Jones glycoprotein κ- and λ-chains	Sepharose cyclic imidocarbonate	Purification of antibodies by immunoadsorption	1293
Human immunoglobulin	Sepharose cyclic imidocarbonate	Specific binding of human immunoglobulin IgG	2204
Human immunoglobulin IgG	Sepharose cyclic imidocarbonate	Isolation of bacterial protein-A by affinity chromatography	1315
	Glutaraldehyde cross-linked	Immunoassay of serum immunoglobulins	1283
Human myeloma immunoglobulins IgA, IgG, and IgM	Sepharose cyclic imidocarbonate	Isolation of anti-immunoglobulins by immunoadsorption	1293
Human myeloma immunoglobulins IgG	Chromic chloride cross-linked to erythrocytes	Investigation of monocyte binding	2463
Human serum albumin	Sepharose cyclic imidocarbonate	Experimental control	2147
Immunoglobulins	*N*-Acetylhomocysteine thiolactone cross-linked	Immunoadsorbents	2464
Immunoglobulin IgG	Nylon[a]	Experimental control	734
	Glutaraldehyde cross-linked to peroxidase	Enzyme-immunoassay procedure	1313
β-Lactoglobulin	Acrylamide copolymers	Immunoassay for anti-β-lactoglobulin	1195
Luteinizing hormone	Diazo-derivative of agarose[c]	Investigation of interaction of luteinizing hormone with cell surfaces	1112
Mouse immunoglobulin IgG	Glutaraldehyde-treated Bio-Gel	Isolation of anti-immunoglobulin IgG by immunoadsorption	2460
Ovalbumin	Nylon[a]	Experimental control	734
Ovomucoids	Sepharose cyclic imidocarbonate	Purification of proteases by affinity chromatography	1422–1424

[2460] T. Ternynck and S. Avrameas, *F.E.B.S. Letters*, 1972, **23**, 24.
[2461] D. F. H. Wallach, B. Kranz, E. Ferber, and H. Fischer, *F.E.B.S. Letters*, 1972, **21**, 29.
[2462] I. Karube and S. Suzuki, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 51.
[2463] N. Abramson and P. H. Schur, *Blood*, 1972, **40**, 500.
[2464] N. E. Crook, J. W. Chidlow, J. Stephen, and H. Smith, *Immunochemistry*, 1972, **9**, 749.

Table 8 (*cont.*)

Glycoprotein	*Matrix or macromolecule coupled*	*Use of product*	*Ref.*
	Glutaraldehyde cross-linked with bovine serum albumin and/or rabbit immunoglobulin IgG	Isolation of enzymes and phytohaemagglutinins by affinity chromatography	691
Phytohaemagglutinin (*Lens culinaris*)	Sepharose cyclic imidocarbonate	Isolation of lymphocyte plasma-membrane glycoproteins by affinity chromatography	736
Prothrombin	Poly-(4-diazo-DL-phenylalanine-L-leucine)	Studies of thrombin formation	1241
Rabbit immunoglobulin	3-Diazo-4-methoxyphenyl-sulphonethyl-Sephadex	Immunoadsorbent microdetections of phage antibodies	1267
Rabbit immunoglobulin IgG	Glutaraldehyde-treated Bio-Gel	Isolation of anti-immunoglobulin IgG by immunoadsorption	2460
	Glutaraldehyde cross-linked with alkaline phosphatase	Enzyme-immunoassay procedures	1314, 1339
	Glutaraldehyde cross-linked with bovine serum albumin and/or ovomucoid	Isolation of proteases by affinity chromatography	691
Sheep immunoglobulin IgG	Plastic tubes	Radioimmunoassay of immunoglobulin IgG	1339
Thyroglobulin	Sepharose cyclic imidocarbonate	Isolation of haemagglutinins by affinity chromatography	724
Tosylated bovine serum albumin	Nylon[a]	Cell fractionation	734

[a] Coupling with carbodi-imide; [b] subsequently allowed to react with periodate-oxidized dextran; [c] see 142A, Scheme 19.

γ-irradiation of reduced, alkylated lysozyme, suggested that heavily damaged lysozyme molecules with exposed disulphide bonds can undergo free-radical polymerization initiated by $-CH_2\dot{S}$ radicals.

The immunological properties of acetylated lysozymes have been assessed [1848, 1849] and the effects of acetylation, guanidation, maleylation, and succinylation on the enzymic and immunological properties of the enzyme have been examined.[1850] Alkylated and mercury derivatives,[1430] and reduced, alkylated derivatives [1804, 1805] of lysozymes have been prepared. Aminoethylated and carboxymethylated, reduced lysozymes have been formed.[2465] Modification of lysozyme to the reduced, carboxymethylated derivative [1854] was found to induce cross-reactions between the enzymes from hen egg-white and humans.[1791]

Lysozyme has been conjugated with carboxymethylmorphine–isobutyl chloroformate mixed anhydride [2466] and the c.d. spectrum of the product indicated that an average of four hapten molecules were bound per enzyme molecule. The arginyl residues of lysozyme have been modified by treatment with cyclohexanedione.[1826] The u.v., c.d., and o.r.d. spectra of lysozyme derivatized with di-isopropyl phosphorofluoridate have been compared with those of the native enzyme.[1821] Lysozyme derivatized by treatment with 1-dimethylaminonaphthalene-5-sulphonyl chloride has been used in an investigation of complex formation between lysozyme and thyroglobulin.[1129]

The effects of 2,4-dinitrophenylation on the physicochemical and enzymic properties of lysozyme have been examined in detail for derivatization carried out at a series of pHs.[1823–1825] The β-ethyl ester,[1843] nitrotyrosyl and reduced, alkylated, nitrotyrosyl,[2203] and oxidized [1820] derivatives of lysozyme have been prepared. Monosulphenylation of lysozyme with 2-nitrophenylsulphenyl chloride resulted in loss of enzymic activity, but the product exhibited cross-reactivity with an anti-lysozyme antibody.[2467] A dye derivative of lysozyme has been produced by its reaction with 2-(4-toluidinyl)naphthalene-6-sulphonate.[1822] The extrinsic fluorescence properties of this derivative were compared with those of the analogous derivative of α-lactalbumin. The enzyme was also esterified with triethyloxonium fluoroborate.[1818]

Carboxymethylation of thrombin has been shown to eliminate its clotting and esterase activities.[1992] Nitration of thrombin with tetranitromethane had different effects upon the proteinase and esterase activities of the enzyme.[1993]

Alkaline phosphatases have been modified by removal of their neuraminic acid units.[1310] Whereas this modification impaired the heat stability of the enzyme from human liver, it did not impair that of a hepatoma variant nor

[2465] M. Sokulovsky and H. Neumann, *Israel J. Chem.*, 1971, **9**, 233.
[2466] K. E. Rubenstein, R. S. Schneider, and E. F. Ullman, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 846.
[2467] Y. Schechter, Y. Burnstein, and A. Patchornik, *Biochemistry*, 1972, **11**, 653.

did it cause inactivation. α-Lactalbumin has been carboxymethylated and the properties of this derivative have been compared with those of lysozyme.[2006] α-Lactalbumin has also been derivatized with tetranitromethane,[2007] 2-hydroxy-5-nitrobenzyl bromide, and dimethyl-(2-hydroxy-5-nitrobenzyl)sulphonium bromide.[2468] The characteristics of the products were investigated.

The modification of enzymes to produce water-insoluble, but active, derivatives has been reviewed [2164, 2165, 2168] and standard methodology for insolubilization of a number of enzymes has been reported.[2169, 2170] Applications of carrier-bound enzymes have been reviewed [638, 2166, 2167] and the effect of the micro-environment on the mode of action of immobilized enzymes has been discussed in detail.[1449] The kinetic behaviour of enzymes immobilized in artificial membranes has been studied [1448] and kinetic laws for solid-supported enzymes have been derived.[2469] Preparations of many insolubilized enzymes and enzymes derivatized by coupling to other macromolecules have been reported; derivatives described recently, together with their applications, are summarized in Table 9.

Further details of insolubilized carbohydrases worthy of mention are as follows. β-Fructofuranosidase coupled to a bentonite–cyanuric chloride complex exhibited a pH optimum of 6.3 compared with a value of 5.2 for the free enzyme.[1553] The same enzyme coupled to glass was shown to have a half-life of forty days.[1554] Chelation of cellulose, nylon, and glass with transition-metal ions has been found to be a simple method of activating surfaces for use in the insolubilization of β-fructofuranosidase and other carbohydrases;[1555, 1780, 1781] the enzyme molecules presumably act as further ligands in the chelates.

β-Glucosidase insolubilized by attachment to poly(allyl cyclic carbonate) has been stabilized for storage in a dry state by freeze drying in the presence of a polyol,[1642] which is considered to supply a hydrophilic network in the solid state.

α-Amylase derivatized by reaction with soluble polysaccharide derivatives, obtained by treatment of dextran, DEAE-dextran, and carboxymethylcellulose with 2-amino-4,6-dichloro-*sym*-triazine, retained up to 67% of the specific activity of the free enzyme.[1758] The pH optima of the derivatized enzyme showed shifts of 0.5 units according to the charge on the support. Attachment of the enzyme to carboxymethylcellulose and DEAE-dextran increased the stability of the enzyme at neutral pH and attachment to the former matrix also enhanced the heat stability of the enzyme. This stability was demonstrated by the continuous hydrolysis of starch in an ultrafilter reactor.

Cellulase was adsorbed by cellulose at pH 4.0—5.0 – conditions that are optimum for enzymic reaction.[1885] The adsorbed enzyme was sufficient to digest cellulose without need of replenishment. The enzyme released

[2468] T. E. Barman, *Biochim. Biophys. Acta*, 1972, **257**, 297.
[2469] P. V. Sundaram, A. Tweedale, and K. J. Laidler, *Canad. J. Chem.*, 1970, **48**, 1498.

Table 9 *Modification of enzymes by covalent coupling to insoluble matrices and other macromolecules*

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled*	*Use(s) of product*	*Ref.*
Alkaline phosphatase	3.1.3.1	Carboxymethylcellulose azide	Inactive insoluble enzyme	2330
		Maleic anhydride copolymers	Active insoluble enzyme	2330
		Sephadex cyclic imidocarbonate	Inactive insoluble enzyme	2330
		Glutaraldehyde cross-linked with immunoglobulin IgG	Enzyme-linked immunoassay	1339
D-Amino-acid oxidase	1.4.3.3	Amino-derivative of agarose[a, b]	Active insoluble enzyme	2181
Aminoacylase	3.5.1.14	Halogenoacetylcelluloses	Active insoluble enzyme	2322
Aminopeptidases	3.4.1.2/3	Enzacryls	Active insoluble enzyme	2183
		Sepharose cyclic imidocarbonate	Active insoluble enzyme	2182, 2183
α-Amylase	3.2.1.1	Amino-*sym*-triazinyl derivatives of dextran, carboxymethylcellulose, and diethylaminoethyldextran	Active soluble enzyme	1758
		Poly(allyl cyclic carbonate)	Active insoluble enzyme	1642
		Ti^{3+} chelates of various materials	Active insoluble enzymes	1555
L-Asparaginase	3.5.1.1	Glutaraldehyde cross-linked with partially hydrolysed nylon	Active insoluble enzyme	2470
Bacterial proteinase	—	Sepharose cyclic imidocarbonate	Active insoluble enzyme	2191
Bromelain	3.4.4.24	Carboxymethylcellulose azide	Active insoluble enzyme	2329
Carbonic anhydrase	4.2.1.1	Bovine serum albumin	Active microencapsulated enzyme	1194
Catalase	1.11.1.6	Glutaraldehyde cross-linked	Active insoluble enzyme	2471
Cellulase	3.2.1.4	Cellulose	Active insoluble adsorbed enzyme	1885
Chymotrypsins	3.4.4.5/6	Ethylene–maleic anhydride copolymer	Active insoluble enzyme	2472

[2470] J. P. Allison, L. Davidson, A. Gutierrez-Hartman, and G. B. Kitto, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 66.
[2471] L. K. Ferrier, T. Richardson, and N. F. Olson, *Enzymologia*, 1972, **42**, 273.
[2472] L. Goldstein, *Analyt. Biochem.*, 1972, **50**, 40.

Table 9 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled*	*Use(s) of product*	*Ref.*
		Modified polysaccharides[c]	Active insoluble enzyme	2176
		Polymethacrylate derivative (Poliodal)	Active insoluble enzyme	2473
		Sephadex cyclic imidocarbonate	Active insoluble enzyme	2186
			Affinity chromatography control	1418
			Conformation–fluorescence studies	2185
		Sepharose cyclic imidocarbonate	Active insoluble enzyme	2184—2186
			Conformation–fluorescence studies	
		Glutaraldehyde cross-linked	Affinity chromatography of enzyme inhibitors	691
Cholinesterase	3.1.1.8	Starch gel	Active occluded insoluble enzyme	2443
Dehydrogenases	—	*NN*-Dimethylpropane-1,3-diamine-treated nylon tubes	Automated analyses	2474
β-Fructofuranosidase	3.2.1.26	Diazosilane derivative of glass	Active insoluble enzyme	1554
		Ti^{3+} chelates of various materials	Active insolubilized enzymes	1555
		Bentonite–cyanuric chloride complex	Active adsorbed insoluble enzyme	1553
β-Galactosidase	3.2.1.23	Acrylic polymers polymerized in the presence of the enzyme	Active entrapped insoluble enzyme	1610, 1611
Glucoamylase	3.2.1.3	Amino-*sym*-triazinyldiethylaminoethylcellulose	Active insoluble enzyme	1777
		Transition-metal chelates of various materials	Active insoluble enzymes	1555, 1780, 1781
		Sepharose cyclic imidocarbonate	Active insoluble mixed enzymes	1778

		Charcoal, clay, diatomaceous earth	Active adsorbed insoluble enzymes	1779
Glucose isomerase	—	Diazosilane derivative of glass	Active insoluble enzyme	1996
Glucose oxidase	1.1.3.4	Sepharose cyclic imidocarbonate	Active insoluble mixed enzymes	1778
		Polyacrylamide–platinum matrix	Electrochemical measurements	1986
		Glutaraldehyde cross-linked to nylon	Automated analyses	1987
β-Glucosidase	3.2.1.21	Poly(allyl cyclic carbonate)	Active insoluble enzyme	1642
Guanyloribonuclease	2.7.7.26	4-Diazobenzylcellulose	Active insoluble enzyme	2187
		Sephadex and Sepharose cyclic imidocarbonates	Active insoluble enzymes	2187
Hyaluronidase	3.2.1.35	Chloro-*sym*-triazinyl derivative of agarose	Active insoluble enzyme	1927
α-Lactalbumin	—	Acrylamide copolymers	Affinity chromatography of A-protein	1195
		Diazo-derivative of cellulose	Affinity chromatography of A-protein	2012
		Sepharose cyclic imidocarbonate	Affinity chromatography of A-protein	2009—2012
Lipoxygenase	1.13.1.13	Agarose cyclic imidocarbonate	Active insoluble enzyme	2188
Lysozyme peptides	—	Sepharose cyclic imidocarbonate	Affinity chromatography of anti-lysozyme	1861
		Amino-derivative of Sepharose[a, d]	Affinity chromatography of anti-lysozyme	1861
Pancreatopeptidase E	3.4.4.7	Sepharose cyclic imidocarbonate	Active insoluble enzyme	2189
Papain	3.4.4.10	Cyanoethylated starch anthranilates	Active insoluble enzymes	2426
Penicillin amidase	3.5.1.11	Chloro-*sym*-triazinyl derivative of diethylaminoethylcellulose	Active insoluble enzyme	2335

[2473] E. Brown and A. Racois, *Bull. Soc. chim. France*, 1971, 4357.
[2474] W. E. Hornby, D. J. Inman, and A. McDonald, *F.E.B.S. Letters*, 1972, **23**, 114.

Table 9 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled*	*Use(s) of product*	*Ref.*
Pepsin	3.4.4.1	Sepharose derivative[f]	Active insoluble enzyme	2190
Peroxidase	1.11.1.7	Glutaraldehyde-treated Bio-Gel	Isolation of anti-peroxidase by immunoadsorption	2460
		Glutaraldehyde cross-linked with bovine serum albumin	Isolation of phyto-haemagglutinins by affinity chromatography	691
		Glutaraldehyde cross-linked with human chorionic gonadotrophin	Enzyme-linked immunoassay	1120
		Glutaraldehyde cross-linked with immunoglobulin IgG	Enzyme-linked immunoassay	1313
Proteinases	—	Carboxymethylcellulose azide	Active insoluble enzymes	2329
Pullulanase	—	Acrylic copolymer[a]	Active insoluble enzyme	1973
Renin	3.4.4.15	Sepharose cyclic imidocarbonate	Active insoluble enzyme	2417
Ribonuclease	2.7.7.16/17	Carboxymethylcellulose azide	Active insoluble enzyme	2329
			Purification of ribonuclease inhibitor by affinity chromatography	2331
		Sepharose cyclic imidocarbonate	Fractionation of anti-nucleases by immuno-adsorption	2205
			Isolation of labelled peptides by affinity chromatography	2197
RNA nucleotidyl-transferase	—	Sepharose cyclic imidocarbonate	Active insoluble enzyme	2192
Trypsin	3.4.4.4	Bio-Gel azide	Purification of protease inhibitors by affinity chromatography	2475
		Cellulose derivative	Isolation of protease inhibitors by affinity chromatography	2476
		Poly(allyl cyclic carbonate)	Active insoluble enzyme	1642

		Polymethacrylate derivative (Poliodal)	Active insoluble enzyme	2477
		Polystyrene	Active insoluble enzyme	2478
		Sephadex cyclic imidocarbonate	Active insoluble enzyme	2186
			Conformational studies	2185, 2193, 2194
			Isolation of ovomucoid by affinity chromatography	1418
		Sepharose cyclic imidocarbonate	Active insoluble enzyme	2186
			Conformational studies	2185, 2193, 2194
			Affinity chromatography of trypsin inhibitors	2249, 2250
		Succinimide derivative of glass	Affinity chromatography of proteins	2253
		Succinimide derivative of Sepharose[e]	Affinity chromatography of proteins	2253
		Ti^{3+} chelates of various materials	Active insolubilized enzymes	1555
		Glutaraldehyde cross-linked	Purification of trypsin inhibitors by affinity chromatography	691
Urease	3.5.1.5	Collagen	Active enzyme membrane	2462
		Polymethacrylate derivative (Poliodal)	Active insoluble enzyme	2477
		Poly(vinyl alcohol)	Active insoluble enzyme	1448, 2479
		Ti^{4+} chelates of various materials	Active insoluble enzymes	1555

[a] Coupling with carbodi-imide; [b] see (140A), p. 536; [c] see (135)—(137), p. 524; [d] see (140), p. 536; [e] see (149A), p. 537; [f] see text, p. 531.

[2475] H. Fritz, M. Gebhardt, R. Meister, and H. Schult, *Z. physiol. Chem.*, 1970, **351**, 1119.
[2476] H. Fritz, M. Gebhardt, R. Meister, K. Illchmann, and K. Hochstrasser, *Z. physiol. Chem.*, 1970, **351**, 571.
[2477] E. Brown and A. Racois, *Bull. Soc. chim. France*, 1971, 4351.
[2478] J. B. Taylor and H. E. Swaisgood, *Biochim. Biophys. Acta*, 1972, **284**, 268.
[2479] W. J. Blaedle and R. C. Boguslaski, *Biochem. Biophys. Res. Comm.*, 1972, **47**, 248.

as the cellulose was digested became re-adsorbed on to other cellulose molecules and the released carbohydrates were separated from this complex in a column. In this way, adsorbed cellulase has been used to produce syrups containing 5—15% of D-glucose continuously from either stirred reactors or cellulose columns.

The catalytic activity of glucoamylase attached to DEAE-cellulose treated with 2-amino-4,6-dichloro-*sym*-triazine has been studied in packed-bed and continuous-feed, stirred, tank reactors.[1777] At low flow rates, mass-transfer limitations led to lower conversions in the bed reactor than in the tank reactor. Differences in the kinetic parameters and pH profiles of the immobilized and free enzymes were apparent and the immobilized enzyme also showed greater stability at 50 °C. The dependence of the reaction rate on temperature was found to be the same for both types of enzyme. Glucoamylase insolubilized by adsorption on to clay, charcoal, and diatomaceous earth was partially released during its catalytic action.[1779]

The pH–activity profile of glucoamylase immobilized with glucose oxidase on Sepharose cyclic imidocarbonate has been compared with that of analogous systems comprising the free enzymes in solution.[1778] Whereas immobilization did not alter the pH optimum of either enzyme, the pH optimum of the sequential two-enzyme reaction could be displaced towards alkalinity by 0.75 pH units compared with the system in solution. The displacement was found to be a function of the ratio of the two enzyme activities after binding.

Hyaluronidase bound to 2-amino-4,6-dichloro-*sym*-triazine was active against glycosaminoglycuronans of high molecular weight and is potentially useful for the preparation of the protein cores of glycosaminoglycuronans from intact molecules.[1927] Insolubilization of the enzyme did not affect its pH optimum.

Modification of Gangliosides and Glycolipids and Uses of Modified Gangliosides and Glycolipids

D-Galactosyl-2-acetamido-2-deoxy-D-galactosyl-di-(*N*-acetylneuraminosyl)-D-galactosyl-D-glucosylceramide has been modified by removal of the terminal D-galactose residue with β-galactosidase.[2093] 2-Acetamido-2-deoxy-D-galactosyl-(*N*-acetylneuraminosyl)-D-galactosyl-D-glucosylceramide has been modified by addition of a D-galactosyl residue to the terminal unit with the aid of a mammalian glycosyltransferase. In an analogous way, a D-galactosyl residue has been added to the non-reducing terminal of 2-acetamido-2-deoxy-D-glucosyl-D-galactosyl-D-glucosylceramide.[2077]

The terminal D-galactosyl residues of D-galactosyl-D-galactosyl-D-glucosylceramide [1581] and D-galactosylsphingosine [1575] have been labelled by oxidation with galactose oxidase followed by reduction with sodium borotritide. The labelled molecules were used in experiments with galactosidases. Terminal 2-acetamido-2-deoxy-D-galactosyl residues of glycosphingolipids have been labelled in the same way.[2480] In instances where

[2480] Y. Suzuki and K. Suzuki, *J. Lipid Res.*, 1972, **13**, 687.

high specific activity was not achieved, pre-treatment with sodium borohydride was necessary to reduce the amount of non-specific labelling that occurred. The labelled glycosphingolipids were useful substrates for a study of specific sphingolipid acetamidodeoxygalactosidases.

Two pentabenzoates were obtained on benzoylation of natural *N*-cerebronyl-D-*erythro*-sphingosyl β-D-galactopyranoside (phrenosine).[2481] Two isomeric monomethylated D-galactoses were obtained by methylation of the free hydroxy-groups of phrenosine pentabenzoate followed by debenzoylation and hydrolysis. It was shown that the C-2 and C-4 hydroxy-groups of phrenosine have low reactivities. Heating of bovine brain cerebrosides with barium hydroxide in *p*-dioxan gave psychosine.[2482] Treatment of psychosine with 4-nitrophenyl palmitate gave D-*erythro*-1-*O*-β-D-galactopyranosyl-*N*-palmitoylsphingosine, which on treatment with benzoyl chloride in pyridine gave D-*erythro*-2′,3,3′,6′-tetra-*O*-benzoyl-1-*O*-β-D-galactopyranosyl-*N*-palmitoylsphingosine. D-*erythro*-3,6′-Di-*O*-benzoyl-1-*O*-β-D-galactopyranosyl-*N*-palmitoylsphingosine was prepared similarly.

Starting from naturally occurring mono- and di-galactosyldiglycerides, 1′- and 2′-*O*-acyl-3′-*O*-β-D-galactopyranosyl-*sn*-glycerol and 1′-*O*-acyl-3′-*O*-(6-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-*sn*-glycerol have been prepared.[2483]

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[2482] A. Y. Veinberg, S. Y. Mel'nik, G. I. Roslovtseva, and G. I. Samokhvalov, *Zhur. obshchei Khim.*, 1970, **40**, 2742.

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