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Volume 11

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Carbohydrate Chemistry

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A Review of the Literature Published during 1977

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Preface

This Report, the eleventh in the series, covers the literature available to us between mid-January 1977 and mid-January 1978. The subject matter has been rearranged slightly in this Report, since interest in certain areas of carbohydrate chemistry has waned over the years, and in other areas the material can be dealt with equally well in other chapters or in remodelled chapters, so avoiding unnecessary repetition. In Part I, hydrazones and osazones, *etc.*, are now covered in Chapter 9, which has been enlarged to include all nitrogen-containing sugar derivatives apart from amino-sugars and nucleosides; all thio-sugars, including derivatives with a sulphur atom in the sugar ring, are dealt with in Chapter 10; alditols and cyclitols have been combined in Chapter 17; and references to the polarimetry of sugars are discussed in Chapter 21, which now includes references to all physical techniques except n.m.r. spectroscopy. There is no longer a separate chapter on the oxidation and reduction of sugars, since the resulting derivatives are used, more often than not, as intermediates in the synthesis of amino-sugars and branched-chain sugars, *etc.*, so that they fall naturally into the chapters dealing with these sugars.

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

Dr. B. J. Catley, Dr. J. M. Williams, and I retire as Reporters after this Report. I feel that, after eight Reports, it is time for me to move on, so that fresh minds can be brought in and new energies harnessed for the welfare of the Report. This, then, is the last opportunity for me to express publicly my gratitude to Reporters, past and present, for their contributions; to Dr. L. C. N. Tucker for reading and commenting on innumerable manuscripts; to Mrs. Alice Duncan, Miss Moira Endersby, and Miss Lynda Esplin for the excellence of their typing; to Philip Gardam and his staff at the Chemical Society for their help in the production of the Reports; and to those who have made a number of helpful suggestions. We have tried each year to provide in a digestible form comprehensive, accurate, and up-to-date coverage of the literature in carbohydrate chemistry, highlighting what in our opinions are important developments. If the Reports over the past eight years have come somewhere close to achieving this goal, our labours have been worth while.

September 1978 J.S.B.

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Abbreviations

The following abbreviations have been used:

ADP	adenosine 5′-diphosphate
AMP	adenosine 5′-monophosphate
ATP	adenosine 5′-triphosphate
c.d.	circular dichroism
CDP	cytidine 5′-diphosphate
CMP	cytidine 5′-monophosphate
DBU	1,5-diazabicyclo[5.4.0]undec-5-ene
DCC	dicyclohexylcarbodi-imide
DEAE	diethylaminoethyl
DMF	*NN*-dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dpm	dipivaloylmethanato
e.s.r.	electron spin resonance
fod	2,2-dimethyl-6,6,7,7,8,8,8-heptafluoro-3,5-octanedionato
g.l.c.	gas–liquid chromatography
HMPT	hexamethylphosphoutriamide
i.r.	infrared
NAD^+	nicotinamide adenosine dinucleotide
NBS	*N*-bromosuccinimide
n.m.r.	nuclear magnetic resonance
o.r.d.	optical rotatory dispersion
py	pyridine
RNA	ribonucleic acid
THF	tetrahydrofuran
Thp	tetrahydropyranyl
TMS	trimethylsilyl
UDP	uridine 5′-diphosphate

Part I

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

By
J. S. Brimacombe
R. J. Ferrier
J. M. Williams
N. R. Williams

1
Introduction

The general terms of reference remain those set out in the Introduction to Volume 1 (p. 3).

Several novel approaches to the synthesis of *O*-glycosides are reported in Chapter 3, which also contains details of the latest studies by Angyal's group on the Fischer glycosidation of sugars in the presence of metal cations. The use of imidates for selective activation of the anomeric centre of monosaccharides appears to be very promising for the stereocontrolled synthesis of 1,2-*cis*-related disaccharides, *etc.* (Chapter 3). The use of a deuteriated substrate has revealed that the enzyme-catalysed reaction of glycerol (and, most likely, of water) with D-galactal is a *trans* addition (Chapter 3).

A new procedure for the tritylation of secondary hydroxy-groups, which is based on the use of triphenylmethylium perchlorate or tetrafluoroborate in the presence of a sterically hindered base, should be widely exploitable in carbohydrate chemistry (Chapter 4). So should a new approach to the regioselective acylation of polyhydroxy-compounds through selective enhancement of the nucleophilicity of hydroxy-groups by trialkylstannylation using a limited amount of bis(tributylstannyl) oxide (Chapter 6). Moreover, the reaction of alkyl tributylstannyl sulphides with glycosyl halides or acetates in the presence of tin(IV) chloride has provided a mild and efficient route to alkyl 1-thioglyco-pyranosides and -furanosides (Chapter 10). Several new procedures for the specific deoxygenation of sugar derivatives are mentioned in Chapter 11. In the absence of large neighbouring groups, deuteriated Raney nickel in refluxing deuterium oxide causes deuterium-exchange with carbon-bonded hydrogen atoms geminal to the hydroxy-groups in mono- and di-saccharide derivatives, but isomerization, although much slower than deuteriation, is sometimes observed at a centre undergoing deuterium-exchange (Chapter 14). The simple, but elegant, way in which this exchange reaction can be utilized to facilitate the analysis of the ^{13}C n.m.r. spectra of carbohydrates is illustrated in Chapter 20.

Stoddart's group has pursued its interest in the incorporation of carbohydrate residues into the 18-crown-6 framework (Chapter 17). The ability of these chiral cryptands to complex with primary alkylammonium cations indicates their potential to provide the binding requirements of an enzyme analogue. No year would be complete without a crop of newly discovered antibiotics: the structures of new antibiotics are given in Chapter 18, which also indicates the considerable efforts that are being devoted to the synthesis of antibiotics of various types and their analogues. The troublesome problem of assigning the anomeric configuration to nucleosides has been overcome by a method based on ^{1}H n.m.r. spectro-

scopy of nucleoside 3′,5′-phosphates, since the value of $J_{1',2'}$ in these conformationally rigid molecules is, without exception, $\leqslant 1$ Hz for 1′,2′-*trans*-isomers and $\geqslant 3.5$ Hz for 1′,2′-*cis*-isomers (Chapter 19). A more sophisticated, but equally successful, approach based on the spin–lattice relaxation rates of the protons has been used to distinguish between the α- and β-anomers of *C*-nucleosides (Chapter 19). Both methods are applicable when only one anomer is available. The likelihood that the anomeric proton of the non-reducing residue of disaccharides receives relaxation contributions from the protons on both sugar rings was noted in last year's Report (Vol. 10, p. 183). The use of specifically deuteriated analogues has enabled Hall and his colleagues to identify and measure the relaxation contribution that the anomeric proton on a sugar ring receives from the protons of an aglycone (Chapter 20). Conformational studies of cell-surface oligosaccharides and aminoglycoside antibiotics by this approach should be of great interest.

Carbohydrates have again been used as chiral precursors for the total synthesis of other natural products, including thromboxane B_2, (−)-isoavenaciolide, and insect pheromones – but pride of place must be given to a total synthesis of (+)-biotin from D-glucose in which a biomimetic transformation was used in forming the tetrahydrothiophen ring (Chapter 23).

Various aspects of sucrochemistry,[1] general carbohydrate synthesis,[2] and naturally occurring carbohydrates of unusual structure (*e.g.* antibiotics),[2a] and the use of Lewis acids in carbohydrate chemistry[3] have been reviewed. Several books[4] of interest have appeared during the past year.

The May[5] and August[6] issues of *Carbohydrate Research* were dedicated to the memory of Professor J. K. N. Jones (1912—1977) and Professor Sir Edmund Hirst (1898—1975), respectively.

[1] Amer. Chem. Soc. Symposium Series, vol. 41, 'Sucrochemistry', ed. J. L. Hickson, American Chemical Society, Washington, D.C., 1977; W. M. Nicol, *Chem. and Ind.*, 1977, 427; R. S. Shallenberger and G. G. Birch, 'Sugar Chemistry', The Avi Publishing Co., 1975.

[2] W. A. Szarek and D. M. Vyas, in 'Carbohydrates', ed. G. O. Aspinall (M.T.P. International Review of Science, Series 2), Butterworths, London, 1976, vol. 7.

[2a] T. Goto, Y. Kishi, Y. Okumura, N. Suzuki, T. Kondo, M. Isobe, H. Tanino, S. Nakatsuka, and F. Nakatsubo, *Natural Product Chem.*, 1975, **2**, 87.

[3] R. I. Sarybaeva, V. A. Afanas'ev, G. E. Zaikov, and L. S. Shchelokhova, *Uspekhi Khim.*, 1977, **46**, 1395.

[4] J. Lehmann, 'Chemistry of Carbohydrates; Monosaccharides, and their Derivatives' (Thieme Pocket Textbooks of Organic Chemistry), Thieme, Stuttgart, 1976, vol. 10; *Methods in Carbohydrate Chem.*, 1976, vol. 7; *Adv. Carbohydrate Chem. Biochem.*, 1977, vol. 34; H. Paulsen and F. W. Lichtenthaler, 'Memoirs German Research Society. Memoirs on the Chemistry of Carbohydrates: Development, Present Status, and Perceivable Lines of Research Development', Boldt-Boppard, 1975.

[5] *Carbohydrate Res.*, 1977, vol. 55.

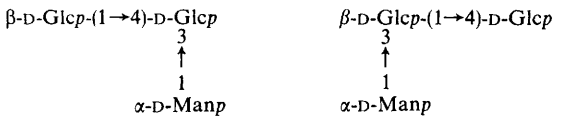
[6] *Carbohydrate Res.*, 1977, vol. 57.

2
Free Sugars

Reviews have appeared on the sugars present in honey[7] and on various aspects of the chemistry of sucrose.[8,9]

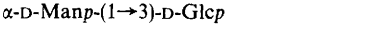
Isolation and Synthesis

Glucose, galactose, and mannose have been identified as the principal monosaccharides in sedimentary rocks aged 200 million years.[10] The oligosaccharides (1)—(3) were among those isolated following deacetylation of the products



(1)

(2)



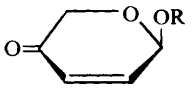
(3)

obtained on partial acetolysis of the extracellular polysaccharide from *Xanthomonas campestris*,[11] while partial acid hydrolysis of the extracellular polysaccharide from the red alga *Porphyridium cruentum* yielded the hexuronic acid-containing disaccharides 3-*O*-(α-D-glucopyranosyluronic acid)-L-galactose, 3-*O*-(2-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose, and 3-*O*-(2-*O*-methyl-α-D-glucopyranosyluronic acid)-D-glucose.[12] 3-*O*-α-D-Glucopyranosyl-L-sorbose has been identified as a metabolite of L-sorbose in such plants as alfalfa (*Medicago sativa*), tomato (*Lycopersicon esculentum*), and kidney bean (*Phaseolus vulgaris*).[13] [U-^{14}C]-Labelled maltosaccharides, [U-^{14}C]maltose, and D-[U-^{14}C]glucose have been obtained by selective enzymic hydrolysis of a soluble [U-^{14}C]-labelled glycogen synthesized from $^{14}CO_2$ by the blue-green bacterium *Anacystis nidulans*.[14]

Stereoselective syntheses of 2,3-*O*-isopropylidene-DL-ribofuranose and methyl

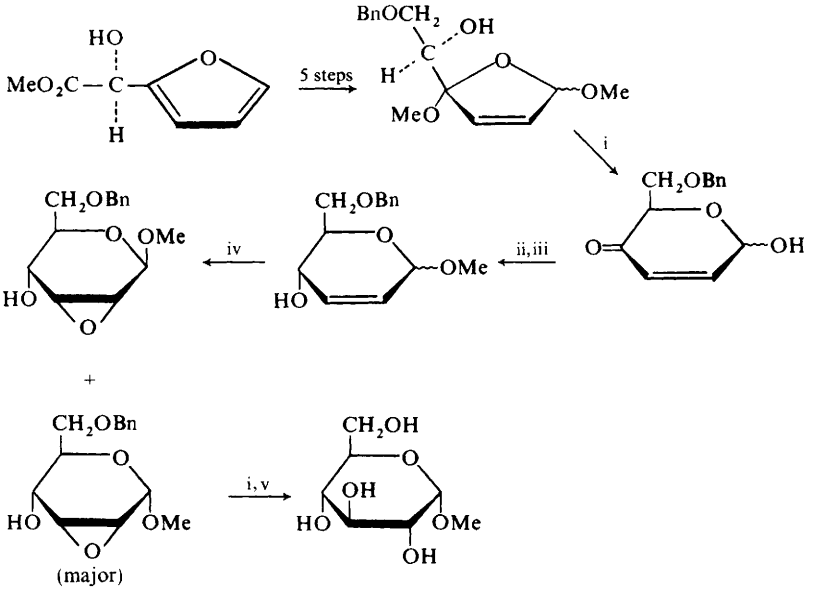
[7] L. W. Doner, *J. Sci. Food Agric.*, 1977, **28**, 443 (*Chem. Abs.*, 1977, **87**, 132 346*y*).
[8] L. Hough, *Amer. Chem. Soc. Symposium Ser.*, 1977, 41, Sucrochemistry Symposium, 1976, 9.
[9] R. Khan, *Amer. Chem. Soc. Symposium Ser.*, 1977, 41, Sucrochemistry Symposium, 1976, 40.
[10] R. Ishiwatari, *Proc. Japan Acad., Ser. B*, 1977, **53**, 47 (*Chem. Abs.*, 1977, **87**, 154 392*n*).
[11] C. J. Lawson and K. C. Symes, *Carbohydrate Res.*, 1977, **58**, 433.
[12] J. Heaney-Kieras and D. J. Chapman, *Carbohydrate Res.*, 1976, **52**, 169.
[13] E. A. McComb and V. V. Rendig, *Carbohydrate Res.*, 1977, **54**, 145.
[14] M. Lehmann and G. Wöber, *Carbohydrate Res.*, 1977, **56**, 357.

β-DL-ribopyranoside from furfuryl alcohol have been described.[15] *cis*-Hydroxylation of the enones (4) and (5) with silver chlorate–osmium tetroxide gave methyl



(4) R = Me
(5) R = Bz

β-DL-*erythro*-pentopyranosid-4-ulose and 1-*O*-benzoyl-β-DL-*erythro*-pentopyranos-4-ulose, respectively, which were converted into DL-ribose derivatives by reduction of their isopropylidene derivatives. In further work on the total synthesis of monosaccharides, Achmatowicz's group has synthesized methyl α-L- and α-D-glucopyranosides from methyl (*R*)- and (*S*)-(2-furyl)glycolates, respectively, by the reactions outlined in Scheme 1 for the D-series.[16] Related approaches were used

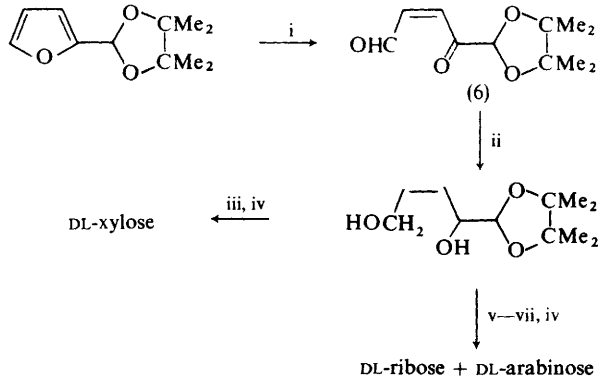


Reagents: i, H_3O^+; ii, $HC(OMe)_3$–BF_3; iii, $NaBH_4$; iv, *m*-$ClC_6H_4CO_3H$; v, H_2–Pd

Scheme 1

to synthesize derivatives of L-mannopyranose and 3-amino-3-deoxy-L-glucopyranose (L-kanosamine)[17] (see also Chapter 8). DL-Ribose, DL-arabinose, and DL-xylose have been synthesized from 2-furaldehyde by way of the unstable

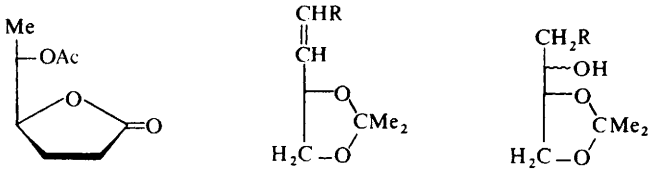
[15] O. Achmatowicz, jun, and G. Grynkiewicz, *Carbohydrate Res.*, 1977, **54**, 193.
[16] O. Achmatowicz, jun, and R. Bielski, *Carbohydrate Res.*, 1977, **55**, 165.
[17] O. Achmatowicz, jun, and R. Bielski, *Roczniki Chem.*, 1977, **51**, 1389.



Reagents: i, Br_2–H_2O; ii, $NaBH_4$; iii, $(CH_2CO_3H)_2$; iv, H_3O^+; v, BzCl–py; vi, OsO_4; vii, MeONa–MeOH

Scheme 2

endialone (6) (Scheme 2); protection of the formyl group against both acidic and basic conditions was achieved by formation of the 4,4,5,5-tetramethyl-1,3-dioxolan derivative from 2-furaldehyde.[18] In the synthesis of 2,3,6-trideoxyhexoses from such lactones as (7), reduction with di-isoamylborane gives ring-expanded products



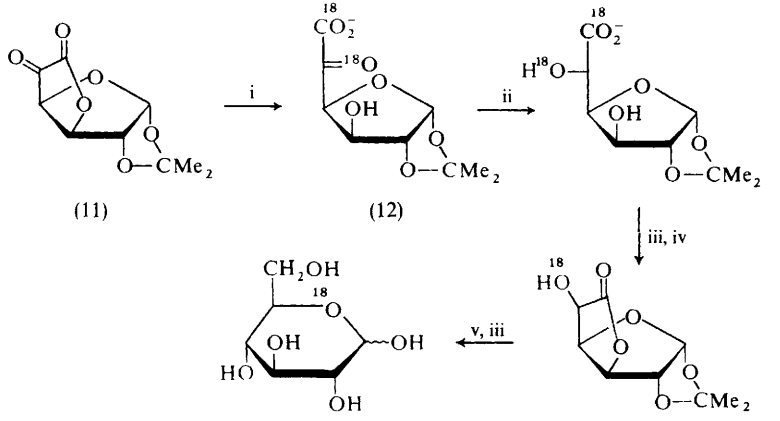
(7) (8) R = CO_2Et, COMe, or CN (9) R = COMe (10) R = CN

when the aglycone and the side-chain of the intermediate *O*-dialkylboryl furanosides are *cis* oriented and the substituents at C-4 and C-5 have the *threo* configuration.[19] D-*glycero*-L-*gluco*-Heptose and D-*glycero*-L-*manno*-heptose have been prepared by way of condensation of D-galactose with nitromethane.[20] Epimerization of each heptose in the presence of molybdate gave a 4:1-mixture in which D-*glycero*-L-*gluco*-heptose predominated. The nitromethane route has also been used to prepare D-*glycero*-D-*gulo*-heptose and D-*glycero*-D-*ido*-heptose from D-glucose.[21] D-*glycero*-D-*gulo*-Heptose and D-*glycero*-L-*manno*-heptose on treatment with nitromethane, *etc.*, gave D-*erythro*-L-*galacto*-, D-*erythro*-L-*talo*-, D-*threo*-L-*galacto*-, and D-*threo*-L-*talo*-octoses.[21a]

Condensation of 2,3-*O*-isopropylidene-D-glyceraldehyde with, for example, malonic, acetoacetic, and cyanoacetic acids gave unsaturated products (8) that could be converted into the 2-ulose (9) and the nitrile (10).[22] D-Erythrose has been

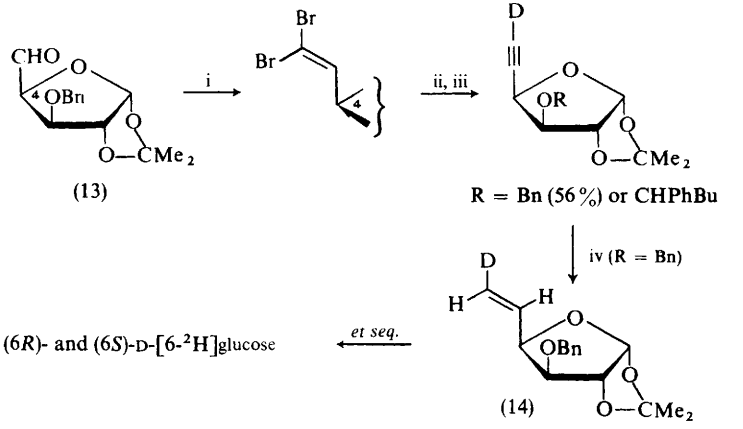
[18] R. Bognár and P. Herczegh, *Carbohydrate Res.*, 1976, **52**, 11.
[19] R. Knollmann, N. Jersch, I. Dyong, A. de Bruyn, and M. Anteunis, *Chem. Ber.*, 1977, **110**, 2729.
[20] V. Bilik and L. Petrus, *Chem. Zvesti*, 1976, **30**, 359 (*Chem. Abs.*, 1977, **87**, 136 187*v*).
[21] V. Bilik, L. Petrus, and J. Zemek, *Chem. Zvesti*, 1976, **30**, 693 (*Chem. Abs.*, 1977, **87**, 184 806*t*).
[21a] V. Bilik, L. Petrus, and J. Alfoldi, *Chem. Zvesti*, 1976, **30**, 698 (*Chem. Abs.*, 1977, **87**, 184 807*n*).
[22] F. J. Lopez Aparicio and F. J. Lopez Herrera, *An. Quim.*, 1976, **72**, 931 (*Chem. Abs.*, 1977, **87**, 184 815*v*).

purified by way of its 4-nitrophenylhydrazone[23] and procedures have been reported for the preparation of D-mannose from doum-palm kernels.[24,25] D-[5-^{18}O]Glucose, which was required for a study of the biosynthesis of *myo*-inositol, has been synthesized essentially free from L-idose *via* reduction (sodium borohydride) of the keto-acid (12) derived from 1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranurono-5-ulose-6,3-lactone (11) (Scheme 3)[26] (see Vol. 9, p. 118).



Reagents: i, $H_2{}^{18}O$–$NaHCO_3$; ii, $NaBH_4$–$H_2{}^{18}O$; iii, H_3O^+; iv, reflux in *p*-dioxan; v, $NaBH_4$

Scheme 3



Reagents: i, CBr_4–Ph_3P–Zn; ii, BuLi; iii, D_2O; iv, $CrSO_4$–DMF–H_2O

Scheme 4

[23] K. Linek, R. Sandtnerova, and V. Bilik, *Chem. Zvesti*, 1976, **30**, 564 (*Chem. Abs.*, 1977, **87**, 152 477*v*).
[24] M. A. E. Sallam, *Carbohydrate Res.*, 1977, **53**, 134.
[25] M. A. E. Sallam, *Acta Chem. Scand.*, 1977, **B31**, 181.
[26] W. R. Sherman, A. Rasheed, L. A. Mauck, and J. Wiecko, *J. Biol. Chem.*, 1977, **252**, 5672.

Derivatives of D-[2-^{2}H]glucose can be conveniently prepared by reduction (sodium borodeuteride) of methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-*arabino*-hexopyranosid-2-ulose,[27] while stereospecific syntheses of (6*R*)- and (6*S*)-D-[6-^{2}H]glucose from the aldehyde (13) (Scheme 4) have been described.[28] The key intermediate is the monodeuteriated alkene (14), which afforded the (6*S*)-isomer on *cis*-hydroxylation with osmium tetroxide and removal of the protecting groups or the (6*R*)-isomer on treatment with 3-chloroperbenzoic acid, separation of the resulting epoxides, and base-catalysed ring-opening of the D-*gluco* epoxide, etc.,

Physical Measurements

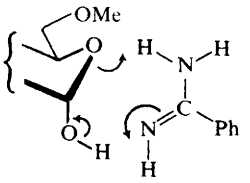
The effect of added electrolytes (sodium and potassium halides of progressively increasing molecular volume) in the concentration range 0.125—3 M on the viscosity behaviour of an aqueous sucrose solution (292 mM) between 25 and 40 °C has been investigated.[29] Conductance data on the interaction of the sodium salts of several low-carbon aliphatic acids with sucrose in water and in formamide solutions have been reported and interpreted in terms of the effects that hydrocarbon chains have on hydrogen bonding in saturated solutions of sucrose.[30] Conductance data have also been reported for the interaction of sucrose with symmetrical tetra-alkylammonium halides in formamide[31] and in water[32] in the temperature range 25—70 °C.

The ratio of α- and β-lactose crystallizing from various solvents has been measured by g.l.c.[33] and an image-analysing computer has been used to measure changes in the distribution of the crystal size during growth in lactose solutions of differing supersaturation.[34] The melting point of sucrose has been found to increase with the rate of heating and with the pH of the solution from which the sample crystallized.[35] Single crystals of sucrose growing in the presence of alkali metal ions or alkaline-earth metal ions have reproducible crystal habits.[36] Lattice energies have been calculated for *meso*-erythritol, α-D-glucopyranose, pentaerythritol, and glycerol, and they are in reasonable agreement with the sublimation energies obtained experimentally.[37] The acid dissociation constants calculated for raffinose and melezitose in sodium perchlorate solution at 25 °C are similar, both trisaccharides behaving as very weak triprotic acids.[38]

A study of the rate of mutarotation of α-D-glucopyranose in DMF in the presence of both pyridine and phenols indicated that their catalytic effect is greatest when they are present in equimolar concentrations, suggesting that the catalytic

[27] A. H. Haines, *Carbohydrate Res.*, 1977, **58**, 212.
[28] K. Kakinuma, *Tetrahedron Letters*, 1977, 4413.
[29] Vishnu and V. P. Misra, *Carbohydrate Res.*, 1977, **59**, 35.
[30] Vishnu and A. K. Singh, *Carbohydrate Res.*, 1977, **54**, 149.
[31] Vishnu and A. K. Singh, *Carbohydrate Res.*, 1977, **58**, 317.
[32] Vishnu and A. K. Singh, *Carbohydrate Res.*, 1977, **58**, 307.
[33] A. Olano, R. A. Bernhard, and T. A. Nickerson, *J. Food Sci.*, 1977, **42**, 1066 (*Chem. Abs.*, 1977, **87**, 125 423*t*).
[34] P. Valle-Vega and T. A. Nickerson, *J. Food Sci.*, 1977, **42**, 1069 (*Chem. Abs.*, 1977, **87**, 125 424*u*).
[35] Z. Bohmerova and A. Smelik, *Termanal* 1976, *Celostatna Konf. Term. Anal.*, [*Pr.*], *7th*, 1976, 0-43-0-48 (*Chem. Abs.*, 1977, **86**, 121 650*w*).
[36] G. Mantovani, C. A. Accorsi, and G. Vaccari, *Z. zuckerind.*, 1977, **27**, 286 (*Chem. Abs.*, 1977, **87**, 184 805*s*).
[37] G. Nemethy and H. A. Scheraga, *J. Phys. Chem.*, 1977, **81**, 928.
[38] F. Coccioli and M. Vicedomini, *Ann. Chim.* (*Italy*), 1976, **66**, 269 (*Chem. Abs.*, 1977, **87**, 39 753*u*).

process involves a termolecular complex.[39] Catalysis of the mutarotation of α- or β-D-glucopyranose in water by such salts as iron(III) chloride and nickel(II) chloride[40] and by carboxylate anions in DMF, DMSO, or aqueous DMSO[41] has also been studied and discussed. The enthalpy for the mutarotation of D-glucopyranose in water containing THF and t-butanol exhibited a complex dependence on the composition of the solvent.[42] The effect of the ternary solvent system on the rate of mutarotation differs from that found in water–DMF–DMSO. Benzamidine catalysed the mutarotation of 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranose more effectively than other compounds of comparable basicity.[43] The efficiency of benzamidine in promoting *anti*-dehydrohalogenations suggested that the catalytic effect of benzamidine may not be due to a concerted bifunctional mechanism, as depicted in (15), but rather to the formation of a glycosyloxy–benzamidinium ion



(15)

pair. Differences between the behaviours of benzamidine and other tautomeric catalysts (*e.g.* 2-pyridone) were discussed. A c.d. study of the mutarotation of D-glucose, D-ribose, and D-fructose is referred to in Chapter 21.[44]

Transitions observed by differential thermal analysis at and above the melting points of various mono-, di-, and poly-saccharides have been associated with the breaking of inter- and intra-molecular hydrogen bonds, respectively.[45] The thermal stabilities of a series of mono-*O*-methyl-D-glucoses and methyl D-glucopyranosides varied appreciably, depending on the position of the substituent: methyl β- and α-D-glucopyranosides are more stable than other monomethylated derivatives, among which 4-*O*-methyl-D-glucopyranose is the least thermally stable.[46] The partial molar volumes of a number of mono-, di-, tri-, oligo-, and poly-saccharides have been determined in water at 25 °C.[47] Measurements of the enthalpy of dilution of one- and two-solute aqueous mixtures of ethylene glycol, pentaerythritol, D-glucose, sucrose, and various amides have been used to calculate the pairwise enthalpy of interaction of each compound with the others.[48] The diffusion of

[39] K. Fujimori, *Austral. J. Chem.*, 1977, **30**, 685.
[40] S. Kirschner, R. V. Moraski, and C. Dragulescu, *J. Indian Chem. Soc.*, 1977, **54**, 29 (*Chem. Abs.*, 1977, **87**, 168 297*x*).
[41] B. G. Cox and R. Natarajan, *J.C.S. Perkin II*, 1977, 2021.
[42] G. Livingstone, F. Franks, and L. J. Aspinall, *J. Solution Chem.*, 1977, **6**, 203 (*Chem. Abs.*, 1977, **87**, 136 177*s*).
[43] V. Fiandanese and F. Naso, *J.C.S. Perkin II*, 1977, 1047.
[44] G. D. Maier, J. W. Kusiak, and J. M. Bailey, *Carbohydrate Res.*, 1977, **53**, 1.
[45] K. Nagata, *Kogai Shigen Kenkyusho Iho*, 1973, **3**, 17 (*Chem. Abs.*, 1977, **86**, 106 911*p*).
[46] M. Kosik, V. Reiser, and R. Kováč, in 'Thermal Analysis: Proceedings of the 4th International Conference,' 1974, **2**, 229 (*Chem. Abs.*, 1977, **87**, 136 194*v*).
[47] F. Shahidi, P. G. Farrell, and J. T. Edward, *J. Solution Chem.*, 1976, **5**, 807 (*Chem. Abs.*, 1977, **86**, 121 652*y*).
[48] J. J. Savage and R. H. Wood, *J. Solution Chem.*, 1976, **5**, 733 (*Chem. Abs.*, 1977, **86**, 120 567*n*).

amino-acids, D-glucose, maltose, and maltotriose in solutions of dextran and derivatized dextrans (*e.g.* DEAE-dextran) has been examined as an aid to the preparation of immobilized enzymes and to an understanding of enzyme kinetics.[49]

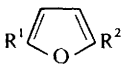
The α-furanose (0.6%) and β-furanose (0.3%) forms of D-mannose[50] and the β-furanose form (0.14%) of D-glucose[51] have been detected in aqueous solutions by taking advantage of the high resolving power of ^{13}C n.m.r. spectroscopy. The tautomeric forms adopted by ketoses and their biologically important phosphoric esters at equilibrium have also been discussed.[52] The proportion of carbonyl form present in the equilibrium mixture of each of 23 aldoses and 10 ketoses has been estimated from the c.d. spectrum obtained with a highly sensitive circular dichrometer.[53] An attempt to calculate the probable specific rotations of α- and β-D-fructofuranose is mentioned in Chapter 21.[54]

Reactions

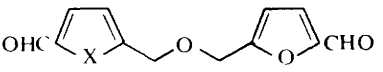
Affinity columns that could be used in the purification of lectins have been prepared by reductive amination of disaccharides (*e.g.* lactose, melibiose, maltose, and di-*N*-acetylchitobiose) with sodium cyanoborohydride in the presence of aminoethylated polyacrylamide gels at pH 9.[55] An L-asparaginase from *E. coli* was similarly glycosylated by reductive coupling with lactose or *N*-acetylneuraminyllactose.[55a] The amounts of D-mannose, D-fructose, and D-gluco-oligosaccharides formed in the acid-catalysed reversion of D-glucose depended on the acid used.[56] The highest yields of di- and oligo-saccharides (isomaltose, maltose, gentiobiose, cellobiose, isomalto-tetraose, -pentaose, and -heptaose) were obtained with 10% solutions of D-glucose in 0.01—0.5 M-HCl.[57] 5-Hydroxymethyl-2-furaldehyde and 1,6-anhydro-β-D-glucopyranose, as well as D-glucobioses, were among the acid-reversion products obtained from D-glucose in alcoholic solvents,[58] and 1,6-anhydro-β-D-glucopyranose appeared to be one of the first products formed when D-glucose reacted with acids in 1:1-mixtures of water and an alcohol.[59] The ratio (0.60—0.74) of (1→4)- and (1→6)-linked disaccharides formed by acid-catalysed reversion of D-glucose in water was found to be virtually independent of the acid used and of the concentrations of the acid and D-glucose. The dehydration of D-fructose has been studied in depth. Analytical procedures for the determination of D-fructose and its dehydration products, *viz.* 5-hydroxymethyl-2-furaldehyde,

[49] R. Namikawa, H. Okazaki, K. Nakanishi, R. Matsuno, and T. Kamikubo, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 1003.
[50] D. J. Wilbur, C. Williams, and A. Allerhand, *J. Amer. Chem. Soc.*, 1977, **99**, 5450.
[51] C. Williams and A. Allerhand, *Carbohydrate Res.*, 1977, **56**, 173.
[52] G. R. Gray, *Accounts Chem. Res.*, 1976, **9**, 418.
[53] L. D. Hayward and S. J. Angyal, *Carbohydrate Res.*, 1977, **53**, 13.
[54] R. S. Shallenberger, C. Y. Lee, T. E. Acree, J. Barnard, and M. G. Lindley, *Carbohydrate Res.*, 1977, **58**, 209.
[55] R. J. Baues and G. R. Gray, *J. Biol. Chem.*, 1977, **252**, 57.
[55a] J. W. Marsh, J. Denis, and J. C. Wriston, jun., *J. Biol. Chem.*, 1977, **252**, 7678.
[56] A. Soler, M. C. Martinez Sanchez, and P. A. Garcia Ruiz, *Ion (Spain)*, 1976, **36**, 527 (*Chem. Abs.*, 1977, **86**, 140 355*v*).
[57] A. Soler, P. A. Garcia Ruiz, and M. C. Martinez Sanchez, *An. Quim.*, 1976, **72**, 957 (*Chem. Abs.*, 1977, **87**, 184 836*e*).
[58] A. Soler, P. A. Garcia Ruiz, and M. C. Martinez Sanchez, *An. Quim.*, 1976, **72**, 961 (*Chem. Abs.*, 1977, **87**, 168 310*w*).
[59] M. C. Martinez Sanchez, A. Soler, and P. A. Garcia Ruiz, *An. Quim.*, 1977, **73**, 910 (*Chem. Abs.*, 1977, **87**, 184 845*e*).

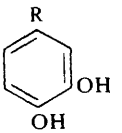
levulinic acid (formed by rehydration of 5-hydroxymethyl-2-furaldehyde), and 'humin', were developed [60] and applied in studies of the influence of the initial and catalyst concentrations on the dehydration of D-fructose at 95 °C in 0.5–2M-HCl.[61] The influence of the amount of water in the reaction mixture[62] and of pH[63] on the rates of formation and the yields of products in the acid-catalysed dehydration of D-fructose were also examined. These studies culminated in the development of a continuous process for the manufacture of 5-hydroxymethyl-2-furaldehyde from D-fructose in yields of 65—85%.[64] The Maillard reaction of D-glucose and methylamine in slightly acidic, aqueous solution yielded D-fructose and diverse furans, pyrroles, phenols, and enols, including (16)—(22).[65]

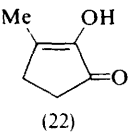


(16) $R^1 = CH_2OH$, $R^2 = H$
(17) $R^1 = CO_2H$, $R^2 = H$
(18) $R^1 = CH_2OH$, $R^2 = CHO$
(19) $R^1 = CH_2OH$, $R^2 = CO_2H$



(20) X = NMe or O





(22)

(21) R = CHO or Ac

A simple kinetic model for the isomerization of D-glucose to D-fructose in aqueous, alkaline media has been developed; the apparent activation energy, related to the hydroxy ion-independent rate constants, of the forward and reverse reactions was deduced to be *ca.* 121 kJ mol^{-1}.[66] The tautomeric equilibria of D-glucose and D-fructose in water or pyridine or both have been studied as a function of temperature and concentration by polarimetry,[67] by g.l.c. of the TMS ethers,[68] and by ^1H and ^{13}C n.m.r. spectroscopy.[69] The acid-catalysed isomerization of D-xylose to D-*threo*-pentulose has been shown to involve a hydride-transfer from C-2 to C-1,[70] analogous to that observed in the acid-catalysed isomerization of hexoses (see Vol. 9, p. 8). These results were discussed in relation to the mode of action of isomerases. Kinetic studies of the acid hydrolysis of lactose at pH 3.8 have shown that the rate of caramelization of D-galactose is much lower than that of D-glucose.[71] Maltol and isomaltol were among the products identified (g.l.c.–m.s.) on heating sucrose in water at 120 °C, although the yields of the various degradation products were not reported.[72] The polymeric product formed on dry pyrolysis of D-glucose has been

[60] B. F. M. Kuster and L. M. Tebbens, *Carbohydrate Res.*, 1977, **54**, 159.
[61] B. F. M. Kuster and H. S. van der Baan, *Carbohydrate Res.*, 1977, **54**, 165.
[62] B. F. M. Kuster, *Carbohydrate Res.*, 1977, **54**, 177.
[63] B. F. M. Kuster and H. M. G. Temmink, *Carbohydrate Res.*, 1977, **54**, 185.
[64] B. F. M. Kuster and H. J. C. van der Steen, *Stärke*, 1977, **29**, 99 (*Chem. Abs.*, 1977, **87**, 53 501*s*).
[65] K. Olsson, P.-Å. Pernemalm, T. Popoff, and O. Theander, *Acta Chem. Scand.*, 1977, **B31**, 469.
[66] C. Kooyman, K. Vellenga, and H. G. J. de Wilt, *Carbohydrate Res.*, 1977, **54**, 33.
[67] L. Hyvonen, P. Varo, and P. Koivistoinen, *J. Food Sci.*, 1977, **42**, 652 (*Chem. Abs.*, 1977, **87**, 136 179*u*).
[68] L. Hyvonen, P. Varo, and P. Koivistoinen, *J. Food Sci.*, 1977, **42**, 654 (*Chem. Abs.*, 1977, **87**, 136 180*n*).
[69] L. Hyvonen, P. Varo, and P. Koivistoinen, *J. Food Sci.*, 1977, **42**, 657 (*Chem. Abs.*, 1977, **87**, 136 181*p*).
[70] S. Ramchander and M. S. Feather, *Arch. Biochem. Biophys.*, 1977, **178**, 576.
[71] P. Barbetti and I. Chiappini, *Ind. Aliment. (Italy)*, 1976, **15**, 63 (*Chem. Abs.*, 1977, **86**, 5708*x*).
[72] H. Ito, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 1307.

shown to consist of furanyl residues.[73] Slow pyrolysis of D-glucose under nitrogen or hydrogen at 300—1000 °C gave furan and furan derivatives, while aromatic hydrocarbons, alcohols (under hydrogen), and carbonyl compounds were also produced at 1000 °C.[74,75] The proportions of tautomeric forms present in equilibrium mixtures of D-fructose at different temperatures and in melts, and variations in the sweetness of aqueous solutions of D-fructose with temperature have been investigated.[76]

Further studies of the formose reaction have been reported. Alkaline-earth metal hydroxides initiated zero-order reactions at intermediate conversions of formaldehyde, and the formation of glyceraldehyde or tetroses and pentoses, *etc.*, from formaldehyde in the presence of calcium hydroxide depended on whether or not glycolaldehyde was present.[77] Self-condensation of formaldehyde in the presence of alkaline-earth metal hydroxides has also been studied in the absence[78] and in the presence[79] of a co-catalyst such as D-glucose and in the presence of glycolaldehyde.[80] Self-condensation of formaldehyde in the presence of lead(II) oxide appears to involve a soluble complex in which the lead atom co-ordinates with the carbonyl oxygen atom of formaldehyde.[80a] The catalytic functions of calcium ion species in a homogeneous formose reaction[81] and the distribution of products in a photochemical formose reaction[82] have been investigated.

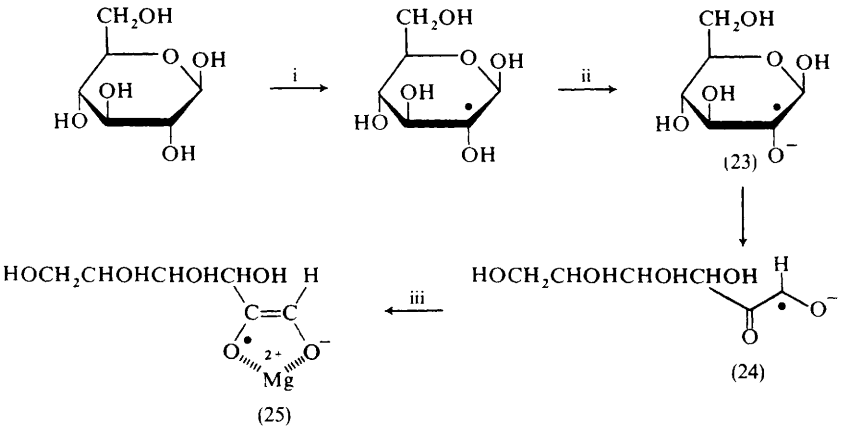
Hydroxy radicals reacted with D-ribose in deoxygenated or oxygenated aqueous solutions to give a number of products that included D-ribonic acid, D-*erythro*-pentosuloses, *ribo*-pentodialdose, deoxypentuloses, erythrose, and glyceraldehyde.[83] The attack of hydroxy radicals (generated by γ-radiolysis of water) involves the abstraction of carbon-bonded hydrogen atoms from C-1 (20%), C-2 and C-4 (*ca.* 35%), C-3 (*ca.* 20%), and C-5 (*ca.* 25%) of D-ribose. Scission of the hemiacetal bond and of C—H and C—C bonds next to it was typically observed on γ-irradiation of crystalline D-glucose and D-fructose.[84] 6-Deoxy-2,5-hexodiulose was formed by a chain reaction on γ-irradiation of D-fructose. 2-Deoxy-D-*arabino*-hexono-1,4-lactone, D-*xylo*-hexos-5-ulose, 3-deoxy-D-*erythro*-hexosulose, and D-*arabino*-hexosulose were among the products identified following γ-radiolysis of D-glucose in deoxygenated, aqueous solution.[85] The yields of volatile products,[86] and the influence of bivalent metal ions (*e.g.* Zn^{2+} and Cu^{2+}) on the yields of

[73] V. Prey, W. Eichberger, and H. Gruber, *Stärke*, 1977, **29**, 60 (*Chem. Abs.*, 1977, **87**, 23 626*u*).
[74] V. Prey and H. Gruber, *Stärke*, 1977, **29**, 96 (*Chem. Abs.*, 1977, **87**, 39 746*u*).
[75] V. Prey and H. Gruber, *Stärke*, 1977, **29**, 135 (*Chem. Abs.*, 1977, **87**, 136 183*r*).
[76] W. Mauch and E. O. Farhoudi, *Z. zuckerind.*, 1976, **26**, 767 (*Chem. Abs.*, 1977, **86**, 155 870*h*).
[77] T. I. Khomenko, O. A. Golovina, M. M. Sakharov, O. V. Krylov, R. D. Partridge, and A. H. Weiss, *J. Catalysis*, 1976, **45**, 356 (*Chem. Abs.*, 1977, **86**, 140 350*q*).
[78] A. H. Weiss, *Kinetika i Kataliz*, 1977, **18**, 539 (*Chem. Abs.*, 1977, **87**, 168 292*s*).
[79] O. A. Golovina and T. I. Khomenko, *Kinetika i Kataliz*, 1977, **18**, 556 (*Chem. Abs.*, 1977, **87**, 168 293*t*).
[80] R. D. Partridge, T. I. Khomenko, O. A. Golovina, and M. M. Sakharov, *Kinetika i Kataliz*, 1977, **18**, 557 (*Chem. Abs.*, 1977, **87**, 168 294*u*).
[80a] Ya. B. Gorokhovatskii, N. P. Evmenenko, and V. F. Gaevskii, *Kinetika i Kataliz*, 1977, **18**, 558 (*Chem. Abs.*, 1977, **87**, 168 295*v*).
[81] Y. Shigemasa, T. Fujitani, C. Sakazawa, and T. Matsuura, *Bull. Chem. Soc. Japan*, 1977, **50**, 1527.
[82] Y. Shigemasa, Y. Matsuda, C. Sakazawa, and T. Matsuura, *Bull. Chem. Soc. Japan*, 1977, **50**, 222.
[83] C. von Sonntag and M. Dizdaroglu, *Carbohydrate Res.*, 1977, **58**, 21.
[84] M. Dizdaroglu, D. Henneberg, K. Neuwald, G. Schomburg, and C. von Sonntag, *Z. Naturforsch.*, 1977, **32b**, 213 (*Chem. Abs.*, 1977, **87**, 136 166*n*).
[85] S. Kawakishi, Y. Kito, and M. Namiki, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 951.
[86] A. A. Ivko, S. I. Gol'din, N. T. Bondarenko, S. V. Markevich, and V. A. Sharpatyi, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1977, 88 (*Chem. Abs.*, 1977, **86**, 171 753*c*).

volatile and non-volatile products,[87] obtained on γ-radiolysis of D-glucose in deoxygenated, aqueous solution have been investigated. The influence of nitrous oxide on the yields of D-gluconic acid and other acidic products formed on γ-radiolysis of D-glucose in aqueous solution is referred to in Chapter 15.[88]

In a study of the oxidation of D-mannose and D-galactose with an excess of sodium periodate, the amount of oxidant consumed (determined by potentiometric titration) was found to be less than quantitative for D-galactose after 90 min at pH 8—10.[89] The influence of pH and the concentration of oxidant on the oxidation of D-fructose with periodate ion has also been studied.[89a]

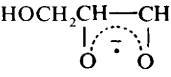
Kinetic studies of the oxidation of D-ribose with vanadium(V),[90] of D-arabinose[91] and D-glucose[92] with peroxydisulphate ions ($S_2O_8^{2-}$), and of D-xylose with Cu^{II}, Fe^{III}, Ce^{IV}, Hg^{II}, Hg^{I}, and Ag^{I}[93] have been reported. The oxidation of D-glucose, D-galactose, D-mannose, and D-fructose with Ti^{III}—H_2O_2 in neutral and basic solutions has been studied by e.s.r. measurements, which were made 20–50 ms after mixing solutions of the reactants.[94] Each hexose gave a characteristic and complex spectrum during oxidation at pH 4.5, while an intense e.s.r. signal observed during oxidation of the hexoses at pH ≥ 7 was attributed to the *cis*-semidione (24) derived from, for example, (23) (Scheme 5). Signals attributable to the chelate (25) and to the reductone anion (26), arising from retro-aldol cleavage of (24), were also observed in the presence of magnesium sulphate and at pH > 8, respectively. E.s.r. spectro-



Reagents: i, $HO^{\bullet}$; ii, HO^-; iii, Mg^{2+}

Scheme 5

[87] G. Haesen, G. Stehlik, and E. Maes, *Monatsh.*, 1977, **108**, 79 (*Chem. Abs.*, 1977, **87**, 136 167*p*).
[88] H. Esterbauer, J. Schubert, E. B. Sanders, and C. C. Sweeley, *Z. Naturforsch.*, 1977, **32b**, 315 (*Chem. Abs.*, 1977, **87**, 136 171*k*).
[89] Z. Dusic, *Arh. Farm.*, 1976, **26**, 129 (*Chem. Abs.*, 1977, **86**, 50 314*b*).
[89a] Z. Dusic, *Mikrochim. Acta*, 1976, **2**, 497 (*Chem. Abs.*, 1977, **87**, 6270*f*).
[90] R. P. Bhatnagar and A. G. Fadnis, *J. Indian Chem. Soc.*, 1976, **53**, 999 (*Chem. Abs.*, 1977, **87**, 136 169*r*).
[91] W. C. Vasudeva and S. N. Srivastava, *Libyan J. Sci.*, 1976, **6B**, 27 (*Chem. Abs.*, 1977, **87**, 184 802*p*).
[92] K. P. Sarbhai and L. K. Saxena, *Agra Univ. J. Res. Sci.*, 1975, **24**, 175 (*Chem. Abs.*, 1977, **87**, 136 186*u*).
[93] V. I. Krupenskii, I. I. Korol'kov, and N. P. Mikush, *Izvest. Vyssh. Uchebn. Zaved., Lesn. Zhur.*, 1976, **19**, 135 (*Chem. Abs.*, 1977, **87**, 136 189*x*).
[94] P. R. West, G. W. Schnarr, and L. Sitwell, *Tetrahedron Letters*, 1977, 3869.



(26)

scopy has been used to study the free radicals formed when hydrogen atoms react with sucrose, lactose, maltose, cellobiose, and αα-trehalose in sulphuric acid glasses at 110 K.[95] The adsorption constants for, and the kinetics of, the electrochemical oxidation of D-glucose to D-gluconic acid on a smooth platinum surface in neutral and acidic solutions have been determined.[96]

The photo-oxidation–reduction of a D-glucose–ferric ion complex has been studied using light of wavelengths between 298 and 405 nm.[97] The main oxidation products were identified as glyoxal, pyruvaldehyde, and hydroxypyruvaldehyde, which are also formed on thermal oxidation of D-glucose by Fe^{3+} ions. A study of the photolysis of mono-, di-, and oligo-saccharides and methyl glycopyranosides has shown that pentoses are degraded more readily than hexoses and that xylobiose is degraded more readily than xylotriose.[98] The C—O and O—H bonds have been shown to be more susceptible to cleavage than C—H bonds when D-fructose is irradiated with 30 keV electrons.[99]

The formation of pyrazines when pentoses and hexoses and their deoxy derivatives react with ammonia has been investigated.[100] Epimers, diastereoisomers, and enantiomers produced similar distributions of pyrazines, while the pyrazines formed from the deoxy derivatives depend on the position of the deoxy group. A method for separating mixtures of D- and L-glucose is based on the selective transport of D-glucose across a membrane containing hexokinase and phosphatase, which is made asymmetric by a pH-gradient.[101] In the presence of ATP, D-glucose is phosphorylated on entering the membrane and is regenerated on leaving the membrane, whereas L-glucose diffuses passively through the membrane. An enzymic method for assaying the anomers of D-glucopyranose is mentioned in Chapter 22.[102]

D-Arabinose has been obtained in yields $\leqslant 94\%$ following oxidative decarboxylation of D-glucono-1,5-lactone with cerium(IV) sulphate in aqueous sulphuric acid solution.[103] Kinetic studies indicated that an intermediate sugar–cerium(IV) complex undergoes unimolecular decomposition to a radical product, which reacts further with cerium(IV) to give the lower aldose. The oxidation of D-glucose to D-arabinonate by molecular oxygen in basic solution[104] and that of 4,6-*O*-alkyl-(aryl)idene-D-galactose and -glucose by silver carbonate–Celite[105] are discussed in Chapters 15 and 5, respectively.

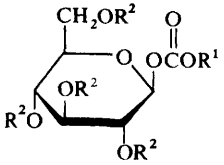
[95] G. V. Abagyan and A. S. Apresyan, *Armyan. khim. Zhur.*, 1976, **29**, 749 (*Chem. Abs.*, 1977, **86**, 106 933*x*).
[96] E. Skou, *Electrochim. Acta*, 1977, **22**, 313 (*Chem. Abs.*, 1977, **87**, 118 026*n*).
[97] G. J. Smith and I. J. Miller, *Experientia*, 1977, **33**, 1269.
[98] J.-Y. Peng, K. Minami, and T. Yoshimoto, *Mokuzai Gakkaishi*, 1976, **22**, 511 (*Chem. Abs.*, 1977, **86**, 73 015*u*).
[99] M. Parikh, J. T. Hall, and P. K. Hansma, *Phys. Rev.* (*A*), 1976, **14**, 1437.
[100] T. Shibamoto and R. A. Bernhard, *J. Agric. Food Chem.*, 1977, **25**, 609 (*Chem. Abs.*, 1977, **87**, 6275*n*).
[101] E. Selegny, J. C. Vincent, and M. Lancon, *Tetrahedron*, 1977, **33**, 3171.
[102] J. Okuda, I. Miwa, K. Maeda, and K. Tokui, *Carbohydrate Res.*, 1977, **58**, 267.
[103] L. F. Sala, A. F. Cirelli, and R. M. de Lederkremer, *J.C.S. Perkin II*, 1977, 685.
[104] N. I. Kirillova, I. S. Tul'chinskaya, A. A. Shereshevskii, and V. M. Berezovskii, *Zhur. priklad. Khim.*, 1976, **49**, 2504 (*Chem. Abs.*, 1977, **86**, 106 941*y*).
[105] S. Morgenlie, *Acta Chem. Scand.*, 1977, **B31**, 63.

3
Glycosides

O-Glycosides

Synthesis of Monosaccharide Derivatives.—New approaches to the synthesis of glycosides, including the alkoxylation of *N*-furanosyl-lactams, the reaction of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose with cyclic amide acetals derived from vicinal diols (see Vol. 10, p. 16), the Lewis acid-catalysed alcoholysis of sugars with an activated anomeric centre (see Vol. 10, p. 11), and the glycosidation of glycopyranosyl halides catalysed by silver trifluoromethanesulphonate (triflate), have been reviewed.[106] A general review of carbonyl compounds has also dealt with some aspects of glycoside synthesis.[107]

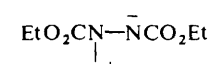
A novel approach to the synthesis of glycopyranosides has involved the pyrolysis of acylated glycopyranosyl carbonates.[108] Pyrolysis of (27; R^1 = Me or Ph,



(27)

R^2 = Ac or Bz) at 160—190 °C gave, after chromatography, modest yields of methyl or phenyl 2,3,4,6-tetra-*O*-acetyl(benzoyl)-β-D-glucopyranoside, whereas the α-anomers of (27) were not susceptible to decarboxylation. Fusion of (27; R^1 = Ph, R^2 = Ac) with 2-chloro- or 2-nitro-phenol at 130—140 °C provided satisfactory preparations of the substituted-phenyl β-glycosides in yields of 65% and 95%, respectively. These reactions proceed by way of 1,2-orthoester intermediates.

The betaines (28), which are formed *in situ* from tervalent organophosphorus compounds and diethyl azodicarboxylate, have also been used in the synthesis of glycosides from sugar derivatives having a free anomeric hydroxy-group.[109]

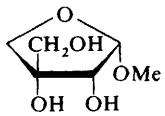


(28) R_3 = Ph_3 or $MePh_2$

[106] S. Hanessian and J. Banoub, *Amer. Chem. Soc. Symposium Ser.*, 1976, 39, Synthetic Methods Carbohydrates Symposium, 36.
[107] B. Capon, *Org. Reaction Mech.*, 1975, 1.
[108] Y. Ishido, S. Inaba, A. Matsuno, T. Yoshino, and H. Umezawa, *J.C.S. Perkin I*, 1977, 1382.
[109] W. A. Szarek, H. C. Jarrell, and J. K. N. Jones, *Carbohydrate Res.*, 1977, **57**, C13.

2,3:5,6-Di-*O*-isopropylidene-α-D-mannofuranose, for example, reacted with the betaine (28; R = Ph) and cyclohexanol in the presence of mercury(II) chloride to give cyclohexyl 2,3:5,6-di-*O*-isopropylidene-α- and -β-D-mannofuranosides in the ratio of *ca.* 7:1. In the reactions of (28) with 2,3,4,6-tetra-*O*-alkyl-D-glucopyranoses, the ratio of α:β-glycopyranosides formed is influenced by the mercury(II) halide used and the nature of the protecting groups. The procedure is mild and circumvents the need for glycosyl halides. Other novel glycosidation procedures are referred to later in this section.

Angyal's group has extended its studies on the Fischer glycosidation of sugars in the presence of metal cations, which can be used to alter the position of the anomeric equilibrium.[110] All the methyl glycosides of D-tagatose, D-psicose, D-apiose, and D-erythrose were synthesized by Fischer glycosidation in the absence or in the presence of calcium chloride; addition of the salt sometimes improved the yield of the furanosides. Metal complexing did not offer any advantage in the synthesis of the methyl D-tagatosides, although small amounts of each of the four methyl D-tagatosides could be separated on a cation-exchange resin in its calcium form. In the absence of calcium chloride, D-psicose reacted with acidified methanol to give the α- and β-furanosides in the ratio of 45:55, whereas in the presence of two molar equivalents of calcium chloride the ratio was 80:20. Conventional Fischer glycosidation of D-apiose furnished the methyl α-D-*erythro*-, β-D-*erythro*-, β-L-*threo*-, and α-L-*threo*-furanosides in a ratio of 20:54:8:17, whereas a ratio of 45:38:7:9 was found following the addition of two molar equivalents of calcium chloride to the



(29)

methanolysis mixture—hence, methyl α-D-*erythro*-apiofuranoside (29) can be prepared and separated by means of metal complexing. Not unexpectedly, the proportion of α-glycoside in an equilibrium mixture of methyl D-erythrofuranosides was greatly increased by the presence of two molar equivalents of calcium chloride. Conditions for the kinetically controlled formation of the α- and β-furanosides have been reported in a study of the acid-catalysed methanolysis of D-fructose.[110a] Methyl α-D-fructofuranoside could be isolated following removal of the β-glycoside and unreacted D-fructose by yeast fermentation.

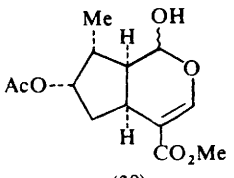
The reaction of 2-*O*-acetyl-3,4,6-tri-*O*-methyl-α-D-glucopyranosyl bromide and cyclohexanol in benzene–nitromethane in the presence of mercury(II) cyanide displayed a first-order dependence on the concentrations of the glycosyl bromide and mercury(II) cyanide, and the rate of the reaction was independent of the concentration of cyclohexanol.[111] The proportion of β-glycoside in the initial products increased as the concentration of cyclohexanol was increased and the β-glycoside appears to be formed directly from carboxonium ion intermediates and by selective

[110] S. J. Angyal, C. L. Bodkin, J. A. Mills, and P. M. Pojer, *Austral. J. Chem.*, 1977, **30**, 1259.
[110a] T. Kiersznicki and W. Szeja, *Roczniki Chem.*, 1977, **51**, 29.
[111] J. E. Wallace and L. R. Schroeder, *J.C.S. Perkin II*, 1977, 795.

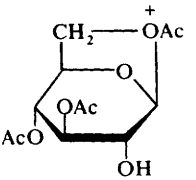
rearrangement of an intermediate 1,2-orthoester. The reactions of 2,3,4,6-tetra-*O*-acetyl-β-D-gluco- and -galacto-pyranosyl bromides with alcohols, including sterically hindered alcohols such as t-butanol, were facilitated in the presence of silver salicylate and gave β-glycosides, sometimes mixed with the respective 1,2-orthoester and 2,3,4,6-tetra-*O*-acetyl-1-*O*-salicyloyl-D-glycopyranose.[112]

3-*O*-α-D-Glucopyranosyl-1,2-di-*O*-stearoyl-L-glycerol, a D-glucosyl diglyceride, has been synthesized by way of treatment of 2-*O*-allyl-3,4-di-*O*-benzyl-6-*O*-(4-nitrobenzoyl)-D-glucopyranosyl chloride with 1,2-di-*O*-(but-2-enyl)-L-glycerol.[113,114] The Koenigs–Knorr reaction has also been used in the synthesis of α-L-rhamnopyranosyl or β-D-glucopyranosyl derivatives of strophanthidine[115,116] and other cardenolides[117] and gibberellic acids.[118,119] Koenigs–Knorr reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with the iridoid (30) gave a dimer (45%) of the iridoid and a mixture of the α- and β-glycosides.[120] However, the boron trifluoride-catalysed reaction of the iridoid (30) with 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose gave the α-glycoside, which is considered to be formed by way of the bicyclic ion (31), and only 5% of the dimer.

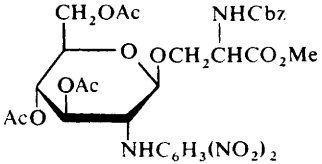
In the condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl bromide with *N*-benzyloxycarbonyl-L-serine methyl ester in the presence of mercury(II) cyanide only the β-glycoside (32) could be isolated and



(30)



(31)

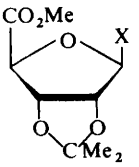


(32)

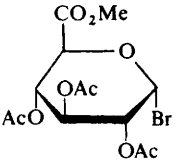
subsequent attempts to remove the *N*-(2,4-dinitrophenyl) group from (32) with a basic resin caused β-elimination of the glycosyl residue.[121] After removal of the *N*-benzyloxycarbonyl group from (32), the free amino-group could be condensed with *N*-protected 4-nitrophenyl esters of glycine, L-alanine, phenyl-L-alanine, and L-leucine to give the corresponding *N*-(benzyloxycarbonylamino-acid)-3-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-β-D-glucopyranosyl]-L-serine methyl esters in yields of 12–34%. The α-anomer was formed preferentially when 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitroanilino)-α-D-galactopyranosyl bromide condensed with *N*-toluene-*p*-sulphonyl-L-serine methyl ester in the presence of

[112] W. A. R. van Heeswijk, H. G. J. Visser, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1977, **58**, 494.
[113] R. Gigg, A. A. E. Penglis, and R. Conant, *J.C.S. Perkin I*, 1977, 2014.
[114] P. A. Gent and R. Gigg, *Chem. Phys. Lipids*, 1976, **17**, 111.
[115] U. Stache, W. Fritsch, W. Haede, and K. Radscheit, *Annalen*, 1977, 1461.
[116] N. Sh. Pal'yants, M. B. Gorovits, and N. K. Abubakirov, *Khim. prirod. Soedinenii*, 1976, 765 (*Chem. Abs.*, 1977, **86**, 121 702*q*).
[117] H. P. Albrecht, *Annalen*, 1977, 1429.
[118] G. Schneider, O. Miersch, and H.-W. Liebisch, *Tetrahedron*, 1977, 405.
[119] G. Schneider, G. Sembdner, and K. Schreiber, *Tetrahedron*, 1977, **33**, 1391.
[120] L.-F. Tietze, U. Niemeyer, and P. Marx, *Tetrahedron Letters*, 1977, 3441.
[121] H. G. Garg and R. W. Jeanloz, *Carbohydrate Res.*, 1976, **52**, 246.

mercury(II) cyanide as the acid acceptor and was transformed into *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-*N*-toluene-*p*-sulphonyl-L-serine.[122] The 2-aminoethyl β-glycosides of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose, di-*N*-acetylchitobiose penta-acetate, and cellobiose hepta-acetate have been prepared and coupled by the cyanuric trichloride procedure to bovine serum albumin.[123] (α-D-Ribofuranosid)uronates were obtained preferentially in reactions of the halides (33) with alcohols, presumably owing to participation by the neighbouring ester group.[124] α-D-Ribofuranosides can be obtained by reduction of the (α-D-ribofuranosid)uronates, and disaccharide derivatives containing two D-ribofuranosyl residues in α-(1→5)-linkage can also be synthesized by reaction of alkyl 2,3-*O*-isopropylidene-β-D-ribofuranosides with the halides (33), *etc.* The α-D-glucopyranuronosyl bromide (34) has been used in the synthesis of *O*-β-D-glucopyrano-



(33) X = Cl or Br

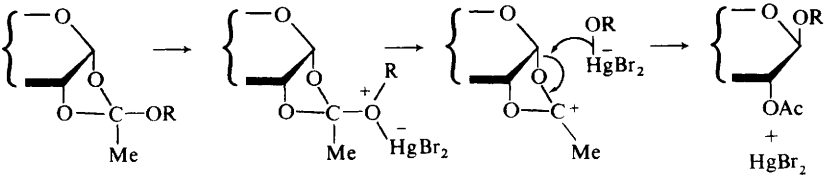


(34)

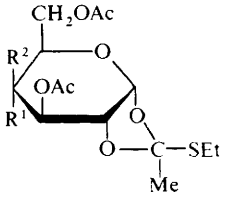
syluronic acid derivatives of 5-fluorouracil,[125] phenolphthalein,[126] and the cardenolide periplogenin.[127] β-D-Glucuronides of such cardenolides as digoxigenin, digoxin, and digitoxin have been prepared by platinum-catalysed oxidation of the primary hydroxy-group of the corresponding β-D-glucopyranoside.[128]

Kochetkov's group has studied the acid-catalysed isomerization of 3,4,6-tri-*O*-acetyl(methyl)-α-D-glucopyranosyl 1,2-(cyclohexyl orthoacetate) to the α- and β-glycosides under a variety of conditions.[129] The use of mercury(II) bromide in nitromethane favoured the formation of β-glycopyranosides from α-D-glucopyranose 1,2-orthoacetates and provided a convenient synthesis of (1→6)-β-D-linked disaccharides.[130] The mechanism suggested for isomerization is shown in Scheme 6. In related work, Garegg and Kvarnström have examined the influence of steric factors and the basicity of the aglycone on the outcome of orthoester glycosidation in the presence of mercury(II) bromide.[131] Steric factors may inhibit electrophilic attack by the catalyst at a bulky alkoxy-group (*cf.* Scheme 6), leading to increased proportions of 1,2-*cis*-glycopyranosides. The 1,2-thio-orthoesters (35)—(37) have also been investigated as glycosylating reagents.[132] They reacted with 1,2,3,4-

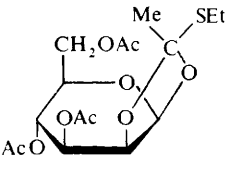
[122] R. Kaifu and T. Osawa, *Carbohydrate Res.*, 1977, **58**, 235.
[123] R. R. King, F. P. Cooper, and C. T. Bishop, *Carbohydrate Res.*, 1977, **55**, 83.
[124] R. R. Schmidt and P. Hermentin, *Angew. Chem. Internat. Edn.*, 1977, **16**, 48.
[125] M. Kaneko, H. Tanaka, M. Kimura, and B. Shimizu, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 2458.
[126] T. Nambara, N. Takizawa, J. Goto, and K. Shimada, *Chem. and Pharm. Bull (Japan)*, 1976, **24**, 2869.
[127] N. Sh. Pal'yants and N. K. Abubakirov, *Khim. prirod. Soedinenii*, 1977, 72 (*Chem. Abs.*, 1977, **87**, 85 198*q*).
[128] R. Petersen, H. Flasch, and N. Heinz, *Arzneim.-Forsch.*, 1977, **27**, 642 (*Chem. Abs.*, 1977, **87**, 33 543*d*).
[129] A. F. Bochkov, V. I. Betaneli, and N. K. Kochetkov, *Bioorg. Khim.*, 1976, **2**, 927 (*Chem. Abs.*, 1977, **86**, 5698*u*).
[130] A. F. Bochkov, V. I. Betaneli, and N. K. Kochetkov, *Bioorg. Khim.*, 1977, **3**, 39.
[131] P. J. Garegg and I. Kvarnström, *Acta Chem. Scand.*, 1977, **B31**, 509.
[132] N. K. Kochetkov, L. V. Backinowsky, and Y. E. Tsvetkov, *Tetrahedron Letters*, 1977, 3681.



Scheme 6



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



(37)

tetra-*O*-acetyl-6-*O*-trityl-β-D-glucopyranose in dichloromethane in the presence of triphenylmethylium perchlorate to give, with inversion of configuration at the anomeric centre, (1→6)-linked disaccharide derivatives in yields of 49—69%, together with some of the corresponding ethyl 1-thio-D-glycopyranosides. No α-linked disaccharides could be detected among the products from (35). The orthoester procedure has been used to prepare glyceryl β-D-gluco-, β-D-galacto-,[133] and α-D-manno-pyranosides,[133a] and *O*-β-D-fucopyranosyl and di-*O*-(α-L-rhamnopyranosyl) derivatives of periplogenin and strophanthidiol, respectively.[134]

High yields of β-D-ribofuranosides and β-D-glucopyranosides have been obtained by treating 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose and β-D-glucopyranose penta-acetate, respectively, in dichloromethane with equimolar quantities of an alcohol and tin(IV) chloride at or below room temperature.[135] Several alkyl and cyclohexyl β-D-ribofuranosides were prepared by this method, which was extended to the synthesis of the protected disaccharide 6-*O*-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose. Tin(IV) chloride also catalysed the formation of aryl β-D-ribofuranosides from β-D-ribofuranosyl tetra-acetate.[136] An acetylated precursor has also been used in

[133] A. P. Kaplun, V. I. Shvets, and R. P. Evstigneeva, *Bioorg. Khim.*, 1977, **3**, 222 (*Chem. Abs.*, 1977, **86**, 171 781*k*).

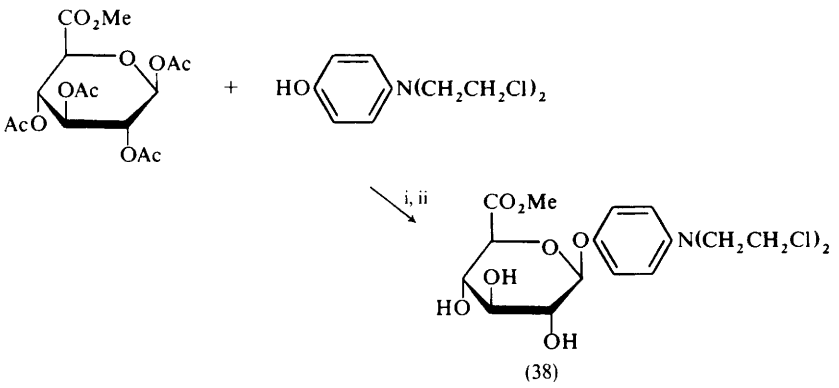
[133a] A. P. Kaplun, V. E. Kalugin, V. I. Shvets, and R. P. Evstigneeva, *Trudy Khim. i khim. Tekhnol.*, 1975, **5**, 57 (*Chem. Abs.*, 1977, **86**, 90 196*w*).

[134] N. Sh. Pal'yants and N. K. Abubakirov, *Khim. prirod. Soedinenii*, 1977, 125 (*Chem. Abs.*, 1977, **87**, 102 592*h*).

[135] S. Hanessian and J. Banoub, *Carbohydrate Res.*, 1977, **59**, 261.

[136] W. Z. Antkowiak, W. J. Krzyzosiak, J. Biernat, J. Ciesiolka, and P. Gornicki, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1977, **25**, 173 (*Chem. Abs.*, 1977, **87**, 202 012*n*).

a synthesis (Scheme 7) of methyl {4-[*NN*-bis-(2-chloroethyl)amino]phenyl β-D-glucopyranosid}uronate (38).[137]



(38)

Reagents: i, TsOH at 158—163 °C; ii, MeONa–MeOH

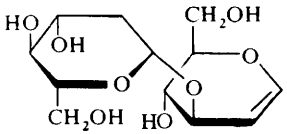
Scheme 7

The betaines (28; R = Ph, Bu, or Et) have been used to prepare aryl glycosides from a number of sugars containing an unsubstituted hydroxy-group at the anomeric centre.[138] For example, phenyl 2,3:5,6-di-*O*-isopropylidene-α- and -β-D-mannofuranosides were obtained in ratios of 3:7, 8:2, and 6:4 when 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose was treated with phenol, diethyl azodicarboxylate, and triphenyl- or tributyl- or triethyl-phosphine, respectively. A striking property of this glycosylating system is its ability to transfer a phenoxy residue to an unprotected sugar such as D-mannose, which rapidly gives phenyl α-D-mannopyranoside (>80% yield) on brief treatment with (28; R = Bu) and phenol in DMF at room temperature. Glycosidation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose has been achieved with an alcohol (*e.g.* cyclohexanol) in dichloromethane in the presence of cobalt(II) bromide and methanesulphonic acid.[139] Similar proportions of α- and β-D-glucopyranosides were formed, possibly by way of the glycosyl bromide.

The use of a deuteriated substrate has revealed that the β-D-galactosidase from *E. coli* catalyses a *trans* addition of glycerol to D-galactal in forming 1-deoxyglycerol-1-yl 2-deoxy-β-D-*lyxo*-hexopyranoside.[140] By contrast, the acid-catalysed reaction of phenol with fully acetylated D-galactal, which yields phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-α-D-*lyxo*-hexopyranoside, was shown to involve both a *cis* and a *trans* addition. The by-product in the hydration of D-glucal catalysed by emulsin β-D-glucosidase has been identified as the β-(1→3)-linked unsaturated disaccharide (39), which, in view of the high regiospecificity of the enzymic reaction, is believed to be formed by transfer of an allylic D-pseudoglucal cation to HO-1 of the hydrated

[137] K. Schwabe, B. Tschiersch, and D. Arnot, *Pharmazie*, 1977, **32**, 52 (*Chem. Abs.*, 1977, **86**, 145 880*x*).
[138] G. Grynkiewicz, *Carbohydrate Res.*, 1977, **53**, C11.
[139] S. Koto, N. Morishima, and S. Zen, *Chem. Letters*, 1976, 1109.
[140] J. Lehmann and B. Zieger, *Carbohydrate Res.*, 1977, **58**, 73.

product 2-deoxy-β-D-*arabino*-hexopyranose (*i.e.* aglycone transfer).[141] Other examples of the use of glycals in the synthesis of 2-deoxyglycosides are covered in Chapter 11.

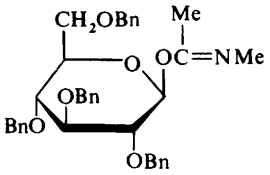


(39)

The well-tested route for preparing less readily accessible β-D-mannopyranosides by reduction of β-D-*arabino*-hexopyranosid-2-uloses (prepared by oxidation of β-D-glucopyranosides) has been adopted in the synthesis of 3-*O*-benzyl and 3-*O*-methyl derivatives of benzyl 4,6-*O*-benzylidene-β-D-mannopyranoside[142] and of 6-aminohexyl β-D-mannopyranoside.[143] A related inversion of configuration at C-4 of methyl 6-*O*-benzoyl-2,3-*O*-isopropylidene-α-D-mannopyranoside, which was used to prepare the corresponding α-D-talopyranoside, is mentioned in Chapter 5.

Synthesis of Disaccharides and their Derivatives.—Approaches to the synthesis of sucrose and related disaccharides have been discussed in a short review.[144]

An important development in the synthesis of 1,2-*cis*-related disaccharides has involved the use of the imidate (40), which forms in high yield when 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride is treated with *N*-methylacetamide in the



(40)

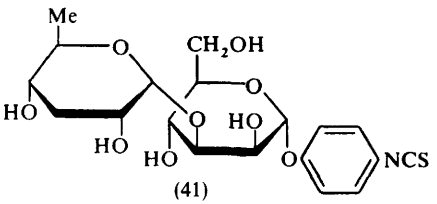
presence of silver oxide, ethyldi-isopropylamine, and molecular sieves.[145] Acid-catalysed reaction of the imidate (40) with methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside in benzene gave methyl 2,2′,3,3′,4′,6,6′-hepta-*O*-benzyl-α-maltoside in a yield of 85%. Disaccharide derivatives containing non-reducing α-D-glucopyranosyl, α-D-galactopyranosyl, and α-D-fucopyranosyl residues variously linked to D-glucose and D-galactose were also prepared in high yield from (40) and related imidates.

An efficient and general method for the synthesis of 1,2-*trans*-related disaccharide derivatives consists of treating an acetylated glycosyl halide with a sugar derivative

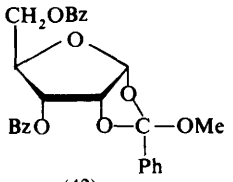
[141] J. Lehmann and E. Schröter, *Carbohydrate Res.*, 1977, **58**, 65.
[142] E. E. Lee, G. Keaveney, and P. S. O'Colla, *Carbohydrate Res.*, 1977, **59**, 268.
[143] M. A. E. Shaban, D. K. Podolsky, and R. W. Jeanloz, *Carbohydrate Res.*, 1976, **52**, 129.
[144] A. Klemer, *Forsch. Landes Nordrhein-Westfalen*, 1975, 2507 (*Chem. Abs.*, 1977, **87**, 184 785*k*).
[145] J.-R. Pougny, J.-C. Jacquinet, M. Nassr, D. Duchet, M.-L. Milat, and P. Sinaÿ, *J. Amer. Chem. Soc.*, 1977, **99**, 6762.

in the presence of silver triflate as the catalyst and 1,1,3,3-tetramethylurea as the proton-acceptor.[146] Glycosylations using 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and suitably protected sugars gave (1→2)-, (1→3)-, (1→4)-, and (1→6)-β-D-linked disaccharide derivatives of high anomeric purity and in preparatively significant yields. Glycosylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of silver triflate and 1,1,3,3-tetramethylurea gave, after chromatography, methyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-α-D-glucopyranoside in a yield of 47%,[146] which is a slight improvement on that obtained by a conventional Koenigs–Knorr reaction in which the ratio of 2- to 3-*O*-substitution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside was found to be 3:1.[147] A Koenigs–Knorr reaction between 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and 2,3-di-*O*-acetyl-1,6-anhydro-β-D-glucopyranose gave a mixture of maltose and cellobiose derivatives.[148] New syntheses of 3-*O*-β-D-galactopyranosyl-D-galactose, a component of the specific carbohydrate–protein linkage region of several connective-tissue proteoglycans, are based on condensation of either 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose[149] or 2-*O*-acetyl-1,6-anhydro-β-D-galactopyranose[150] with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in a Koenigs–Knorr reaction. Other disaccharides or their derivatives to be synthesized by way of Koenigs–Knorr reactions include 4-*O*-α-L-rhamnopyranosyl-D-glucopyranose,[151] 4-*O*-α- and -β-D-xylopyranosyl-D-xylopyranose hexaacetates,[152] and 4-isothiocyanatophenyl 3-*O*-(3,6-dideoxy-α-D-*ribo*-hexopyranosyl)-α-D-mannopyranoside (41).[153]

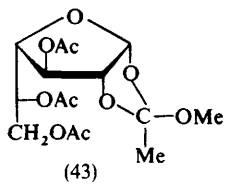
Reaction of the 1,2-(methyl orthobenzoate) (42) with 1,2,3,4-tetra-*O*-benzyl-L-ribitol and removal of the protecting groups from the product gave 1-*O*-β-D-



(41)

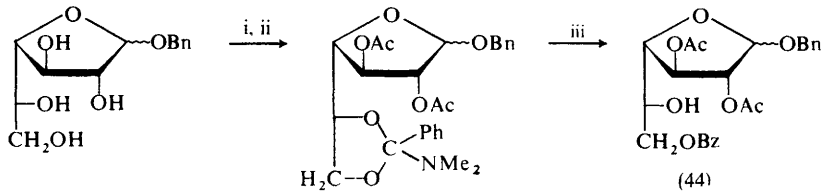


(42)



(43)

[146] S. Hanessian and J. Banoub, *Carbohydrate Res.*, 1977, **53**, C13.
[147] K. Takeo, *Carbohydrate Res.*, 1977, **59**, 258.
[148] G. Excoffier, D. Gagnaire, and F. Taravel, *Compt. rend.*, 1977, **284**, *C*, 389.
[149] L. Benzing-Nguyen and L. Rodén, *Carbohydrate Res.*, 1977, **53**, 123.
[150] M. E. Chacon Fuertes and M. Martin Lomas, *An. Quim.*, 1977, **73**, 146 (*Chem. Abs.*, 1977, **87**, 201 937*f*).
[151] F. Imperato, *Carbohydrate Res.*, 1977, **58**, 217.
[152] J.-P. Utille and P. J. A. Vottero, *Carbohydrate Res.*, 1977, **53**, 259.
[153] P. J. Garegg and B. Gotthammar, *Carbohydrate Res.*, 1977, **58**, 345.

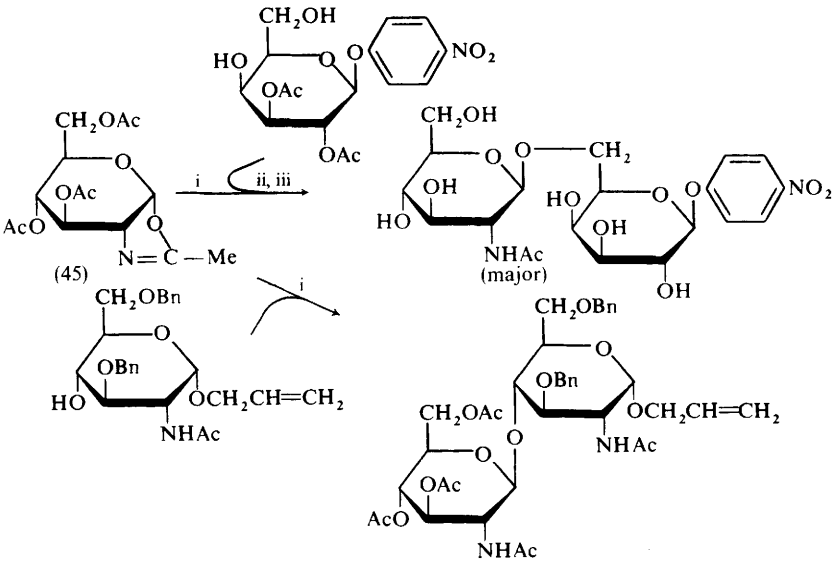


Reagents: i, $PhC(OMe)_2NMe_2$–$CHCl_3$; ii, Ac_2O–py; iii, H_3O^+

Scheme 8

ribofuranosyl-D-ribitol,[154] while 5-*O*-β-D-galactofuranosyl-D-galactofuranose has been synthesized by condensation of benzyl 2,3-di-*O*-acetyl-6-*O*-benzoyl-αβ-D-galactofuranoside (44) (Scheme 8) with the 1,2-(methyl orthoacetate) (43) in nitromethane in the presence of mercury(II) bromide, followed by removal of the protecting groups.[155]

It has been noted that the ratio of β-D-glucopyranose to α-D-glucopyranose in acid solution coincides with the ratio of α- to β-linked disaccharides formed on acid-catalysed reversion of D-glucose.[156] However, the suggestion that the glycosidic bonds are therefore formed with inversion of configuration seems unlikely, since this would require equilibration of D-glucose to be much slower than its glycosidation in acid solution.



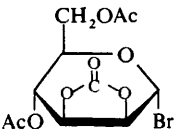
Reagents: i, TsOH–$MeNO_2$–PhMe; ii, Ac_2O–py; iii, Et_3N–MeOH

Scheme 9

[154] P. J. Garegg, B. Lindberg, and B. Samuelsson, *Carbohydrate Res.*, 1977, **58**, 219.
[155] W. A. R. van Heeswijk, H. G. J. Visser, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1977, **59**, 81.
[156] M. C. Martinez Sanchez, P. A. Garcia Ruiz, and A. Soler, *An. Quim.*, 1977, **73**, 908 (*Chem. Abs.*, 1977, **87**, 201 944*f*).

The oxazoline (45) has been used in the synthesis of 4-nitrophenyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-galactopyranoside[157] and of derivatives of allyl di-*N*-acetyl-α-chitobioside,[158] as shown in Scheme 9. A similar sequence of reactions following condensation of the crude oxazoline derived by acetolysis of methyl 2-acetamido-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2-deoxy-α-D-glucopyranoside with 4-nitrophenyl 2,3-di-*O*-acetyl-β-D-galactopyranoside furnished 4-nitrophenyl *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-β-D-galactopyranoside.[157] β-Linked disaccharides were formed when 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide reacted with appropriately protected sugars (*e.g.* methyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside) in the presence of the silver triflate–collidine complex: the corresponding α-halides underwent replacement with inversion of configuration, indicating that these reactions proceed by way of a cationic intermediate.[159] The synthesis of streptobiosamine derivatives is referred to in Chapter 18.

The Koenigs–Knorr reaction of 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide (46) with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-



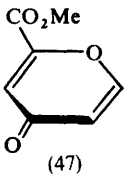
(46)

glucopyranoside gave the α-(1→3)-linked disaccharide derivative rather than the expected β-anomer, so that in order to prepare 2-acetamido-2-deoxy-3-*O*-β-D-mannopyranosyl-D-glucopyranose it was necessary to invert the configuration at C-2′ of benzyl 2-acetamido-3-*O*-(3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside by oxidation (DMSO–acetic anhydride) and reduction (sodium borohydride).[160] Condensation of the α-D-mannopyranosyl bromide (46) with 2-amino-2-*N*,3-*O*-carbonyl-5,6-*O*-isopropylidene-D-glucose diethyl acetal also gave the α-linked disaccharide derivative, necessitating the synthesis of 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucopyranose to be effected by way of a 4-*O*-β-D-glucopyranosyl analogue.[161]

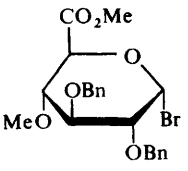
The acetylated 1-bromo-1-deoxy-α-D-glucopyranuronate (34) reacted with each of methyl 2,4,6-, 2,3,6-, and 2,3,4-tri-*O*-methyl-α-D-glucopyranoside and with methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-glucopyranoside in the presence of silver carbonate to give high yields of crystalline aldobiouronic acid derivatives, which on deacetylation (and hydrogenolysis) and methylation afforded a series of fully methylated derivatives of laminaribiouronic, cellobiouronic, and gentiobiouronic acids and the (1→2)-linked analogue.[162] Methylation with methyl iodide

[157] K. L. Matta and J. J. Barlow, *Carbohydrate Res.*, 1977, **53**, 209.
[158] C. D. Warren and R. W. Jeanloz, *Carbohydrate Res.*, 1977, **53**, 67.
[159] R. U. Lemieux, T. Takeda, and B. Y. Chung, *Amer. Chem. Soc. Symposium Ser.*, 1976, 39, Synthetic Methods Carbohydrates Symposium, 90 (*Chem. Abs.*, 1977, **87**, 23 651*y*).
[160] M. A. E. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1976, **52**, 103.
[161] M. A. E. Shaban and R. W. Jeanloz, *Carbohydrate Res.*, 1976, **52**, 115.
[162] P. Kováč, J. Hirsch, and V. Kováčik, *Carbohydrate Res.*, 1977, **58**, 327.

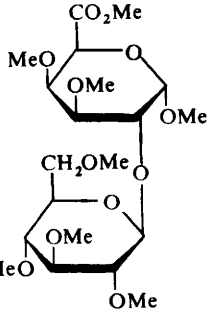
and silver oxide in DMF was invariably accompanied by β-elimination, with the formation of olefinic disaccharides, which were also obtained by β-elimination reactions on the precursor acetates followed by methylation of the deacetylated products. However, the latter conversions also resulted in cleavage of the interglycosidic linkage and the formation of 6-methoxycarbonyl-4-pyrone (47). A Koenigs-Knorr reaction of the bromide (48) with methyl 3,4-di-*O*-acetyl-β-D-xylopyranoside afforded α- and β-(1→2)-linked aldobiouronic acid derivatives in the ratio of 1.4:1.[162a] In contrast to the poor stereoselectivity of this reaction, the corresponding αβ-chlorides gave the α- and β-(1→2)-linked aldobiouronic acid derivatives in the ratio of 18:1. The fully methylated pseudoaldobiouronic acid derivative (49) has been synthesized by condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-



(47)



(48)



(49)

glucopyranosyl bromide with methyl (methyl 3,4-*O*-isopropylidene-α-D-galactopyranosid)uronate and methylation of the deprotected product.[163] Analogous glycosylation of methyl (methyl 2-*O*-methyl-α-D-galactopyranosid)uronate yielded a mixture of the β-(1→3)- and β-(1→4)-linked pseudoaldobiouronic acids, with the former predominating, which were transformed by conventional procedures into methyl [methyl 2,4-di-*O*-methyl-3-*O*-(2,3,4,6-tetra-*O*-methyl-β-D-glucopyranosyl)-α-D-galactopyranosid]uronate and the (1→4)-linked analogue.[164] A related approach to the synthesis of heparin-related pseudoaldobiouronic acids is summarized in Scheme 10.[165]

Koenigs–Knorr reactions or the orthoester procedure with disaccharide derivatives have been used in syntheses of 7-*O*-β-laminaribiosyl-formononetin[166] and 3-*O*-(6-*O*-α-L-rhamnopyranosyl-β-D-galactopyranosyl)quercetin[167] and in the glycosylation of cholest-5-en-3-ol with the disaccharide 1,2-orthoester (50).[168] A

[162a] P. Kováč and R. Palovčík, *Carbohydrate Res.*, 1977, **54**, C11.

[163] J. Hirsch, P. Kováč, V. Kováčik, and V. Mihalov, *Chem. Zvesti*, 1976, **30**, 674 (*Chem. Abs.*, 1977, **87**, 168 306*z*).

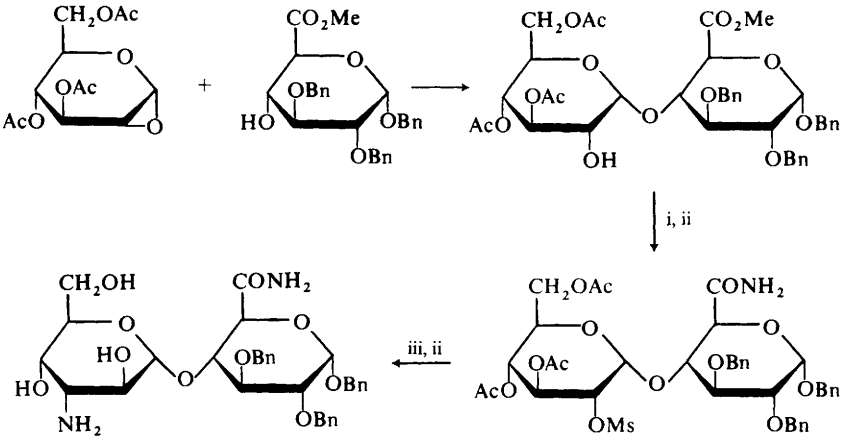
[164] J. Hirsch, P. Kováč, and V. Kováčik, *Carbohydrate Res.*, 1977, **56**, 391.

[165] J. Kiss and P. Taschner, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 101.

[166] H. Ohashi and H. Imamura, *Gifu Daigaku Nogakubu Kenkyu Hokoku*, 1976, **39**, 79 (*Chem. Abs.*, 1977, **87**, 202 006*p*).

[167] L. Farkas, A. Kalman, M. Nogradi, and B. Vermes, *Acta Chim. Acad. Sci. Hung.*, 1976, **91**, 445 (*Chem. Abs.*, 1977, **87**, 85 196*n*).

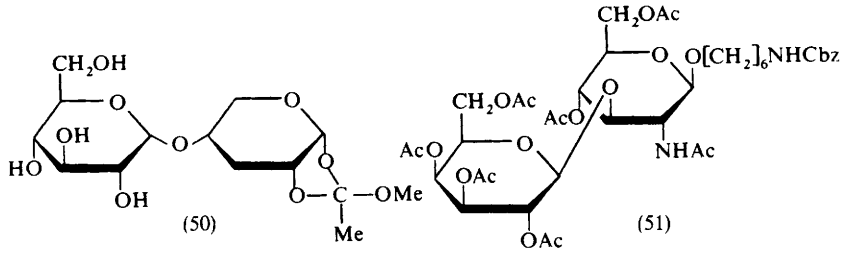
[168] N. I. Uvarova, G. I. Oshitok, A. M. Tolkach, and G. B. Elyakov, *Khim. prirod. Soedinenii*, 1977, 13 (*Chem. Abs.*, 1977, **87**, 152 476*u*).



Reagents: i, MsCl–py; ii, NH_3; iii, MeONa–MeOH

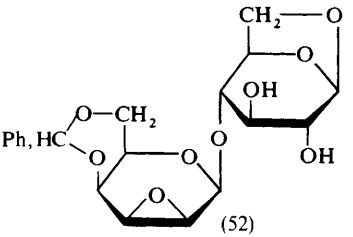
Scheme 10

synthesis of *O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-fucopyranosyl-L-threonine, a disaccharide amino-acid isolated from human urine, has been achieved by condensation of perbenzylated 3-*O*-β-D-glucopyranosyl-α-L-fucopyranosyl bromide with *N*-benzyloxycarbonyl-L-threonine benzyl ester in the presence of tetraethylammonium bromide and molecular sieves, followed by catalytic hydrogenolysis of the product.[169] 8-Carboxyoctyl β-glycosides of cellobiose and di-*N*-acetylchitobiose have been prepared and coupled to bovine serum albumin by the mixed-anhydride reaction.[123] The conjugates functioned as artificial antigens and gave rise to precipitating antibodies in rabbits. A simple synthesis of disaccharide oxazolines has been developed and used in the synthesis of the acetylated 6-(benzyloxycarbonylamino)-hexyl glycoside (51).[170]



Lactose has been converted into 4-*O*-β-D-idopyranosyl-D-glucopyranose by way of the 1,6:2′,3′-dianhydro derivative (52),[171] and a 2,3,6,6′-tetradeoxy derivative of maltose has been synthesized by conventional transformations on methyl 3-*O*-methanesulphonyl-6,6′-di-*O*-toluene-*p*-sulphonyl-β-maltoside tetra-acetate, al-

[169] P. J. Garegg and T. Norberg, *Carbohydrate Res.*, 1976, **52**, 235.
[170] K. L. Matta and J. J. Barlow, *Carbohydrate Res.*, 1977, **53**, 47.
[171] T. Chiba and S. Tejima, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 1049.



(52)

though the final product was not adequately characterized.[172] Under properly controlled conditions, fully (trimethylsilyl)ated αα-trehalose could be converted into 2,2',3,3',4,4',6'-hepta-*O*-(trimethylsilyl)-αα-trehalose, which on (4-nitrobenzoyl)ation, hydrogenation, and removal of the protecting groups furnished 6-*O*-(4-aminobenzoyl)-αα-trehalose.[173] The free amino-group was used to couple the αα-trehalose derivative to bovine serum albumin, yielding a conjugate analogous to the bacterial 'cord factors'. A related series of reactions with 2,2',3,3',4,4'-hexa-*O*-(trimethylsilyl)-αα-trehalose was used to link both the 6- and 6'-positions of αα-trehalose to bovine serum albumin *via* 4-aminobenzoic acid.

Synthesis of Tri-, Tetra-, and Penta-saccharides.—The following trisaccharides and derivatives of trisaccharides have been synthesized during the past year: *O*-β-D-glucopyranosyl-(1→3)-[*O*-β-D-glucopyranosyl-(1→2)]-D-glucopyranose,[174] *O*-β-D-galactopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→6)]-D-galactopyranose deca-acetate,[175] methyl *O*-β-D-xylopyranosyl-(1→4)-[*O*-(methyl 4-*O*-methyl-α-D-glucopyranosyluronate)-(1→2)]-β-D-xylopyranoside,[176] *O*-α-L-rhamnopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→6)-D-galactopyranose nona-acetate,[177] *O*-β-D-glucopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-D-galactopyranose,[178] *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-D-glucopyranose,[179] 4-nitrophenyl *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-β-D-galactopyranoside,[157] *O*-β-D-galactopyranosyl-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-D-galactopyranose (lacto-*N*-triose I),[180] *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→3)-D-galactopyranose, *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-D-glucopyranose (the blood-group A type-1 determinant), *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-deoxy-D-glucopyranose (the blood-group A type-2 determinant),[181] 3,4-di-*O*-(2-

[172] N. K. Sharma, J. Norula, S. Mall, and R. Varadarajan, *J. Inst. Chemists (India)*, 1976, **48**, 129 (*Chem. Abs.*, 1977, **86**, 16 853*s*).

[173] W. J. Gensler and I. Alam, *J. Org. Chem.*, 1977, **42**, 130.

[174] A. Klemer, E. Buhe, F. Gundlach, and G. Uhlemann, *Forsch. Landes Nordrhein-Westaflen*, 1974, 2393 (*Chem. Abs.*, 1977, **87**, 201 936*e*).

[175] A. Lipták and P. Nánási, *Tetrahedron Letters*, 1977, 921.

[176] P. Kováč, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 165.

[177] H. Wagner, A. Lipták, and P. Nánási, *Acta Chim. Acad. Sci., Hung.*, 1976, **89**, 405 (*Chem. Abs.*, 1977, **86**, 106 927*y*).

[178] N. K. Kochetkov, B. A. Dmitriev, A. V. Nikolaev, and N. E. Bairamova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1977, 1609 (*Chem. Abs.*, 1977, **87**, 201 939*h*).

[179] R. Adachi and T. Suami, *Bull. Chem. Soc. Japan*, 1977, **50**, 1901.

[180] C. Augé and A. Veyrières, *J. C. S. Perkin I*, 1977, 1343.

[181] H. Paulsen, W. Stenzel, and C. Kólař, *Tetrahedron Letters*, 1977, 2785.

acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose,[182] *O*-α-D-mannopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→3)-D-galactopyranose,[182a] 3-*O*-β-gentiotriosyl-kaempferol and -quercitin,[183] *O*-β-D-galactopyranosyl-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-D-mannopyranose.[184]

Among the tetra- and penta-saccharides to be synthesized were (53),[174] (54) (the repeating-unit of the O-specific polysaccharide from *Salmonella senftenberg*),[185] a derivative of (55),[186] (56) (in connection with immunological studies of the blood-group substances),[187] and (57) (one of the core oligosaccharides of human blood-group substances).[188]

β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc*p*
6
↑
1
β-D-Glc*p*

(53)

β-D-Man*p*-(1→4)-α-L-Rha*p*-(1→3)-D-Gal*p*
6
↑
1
α-D-Glc*p*

(54)

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-D-Glc*N*Ac*p*
3
↑
1
α-L-Fuc*p*

(56)

α-D-Glc*p*-(1→6)-D-Gal*p*
3
↑
1
β-D-Glc*p*-(1→4)-α-L-Rha*p*

(55)

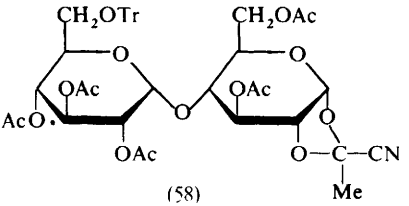
β-D-Gal*p*-(1→4)-β-D-Glc*N*Ac*p*-(1→6)-D-Gal*p*
3
↑
1
β-D-Gal*p*-(1→3)-β-D-Glc*N*Ac*p*

(57)

Synthesis of Higher Saccharides.—A series of end-labelled isomaltose oligosaccharides has been prepared by making use of the ability of *Arthrobacter globiformis exo*-dextranase to transfer isomaltosyl residues from dextran to D-[^{14}C]glucose to give end-labelled trisaccharides.[189] Isomalto-oligosaccharides of higher DP were then synthesized by the transfer of D-glucosyl residues from sucrose to the non-reducing end of [^{14}C]isomaltotriose with a D-glucosyltransferase from *Streptococcus mutans*. Isomaltose, panose, and nigerose were the principal saccharides produced by the action of glucoamylase and of the D-glucosyltransferase from *Aspergillus niger* on D-glucose and maltose, the extents of their respective conversion into new oligosaccharides being 2.2 and 1.9 % with glucoamylase, and 5.7 and 33 % with

[182] C. Augé and A. Veyrières, *Carbohydrate Res.*, 1977, **54**, 45.
[182a] N. K. Kochetkov, E. M. Klimov, and V. I. Torgov, *Bull. Acad. Sci. U.S.S.R.*, 1976, **25**, 154.
[183] L. Farkas, B. Vermes, N. Mihaly, and A. Kalman, *Phytochemistry*, 1976, **15**, 1184.
[184] R. Kaifu and T. Osawa, *Carbohydrate Res.*, 1976, **52**, 179.
[185] N. K. Kochetkov, N. N. Malysheva, V. I. Torgov, and E. M. Klimov, *Carbohydrate Res.*, 1977, **54**, 269.
[186] N. K. Kochetkov, N. N. Malysheva, V. I. Torgov, and E. M. Klimov, *Izvest. Akad. Nauk S.S.S.R. Ser. khim.*, 1977, 654 (*Chem. Abs.*, 1977, **87**, 53 506*x*).
[187] J.-C. Jacquinet and P. Sinaÿ, *J. Org. Chem.*, 1977, **42**, 720.
[188] C. Augé, S. David, and A. Veyrières, *J.C.S. Chem. Comm.*, 1977, 449.
[189] G. J. Walker, *Carbohydrate Res.*, 1977, **53**, 263.

the D-glucosyltransferase.[190] The transferase, unlike glucoamylase, also synthesized oligosaccharides having α-(1→6)-linked D-glucopyranosyl residues at the non-reducing ends from malto-oligosaccharides of higher molecular weight. Polycondensation of 3-*O*-acetyl-α-D-xylopyranose 1,2,4-orthoacetate (using triphenylmethylium perchlorate),[191] of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (using phosphorus pentafluoride),[192] and of the acetal (58) (using triphenylmethyl-

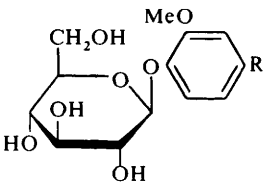


(58)

ium perchlorate)[193] has been used in syntheses of stereoregular polysaccharides. Chemical studies indicated that the D-glucan (33% yield) obtained from polycondensation of (58) has a regular structure containing alternate α-(1→4)- and β-(1→6)-linked D-glucopyranosyl residues. Polycondensation of formose has been studied under a variety of conditions (*e.g.* as a melt with phosphorous acid or with acidic reagents in DMSO and DMF).[194]

Hydrolysis and Related Reactions.—Aspects of the hydrolysis of glycosides have been dealt with by Capon in his review of carbonyl compounds[107]

The rate constants have been measured for the hydrolysis of several alkyl and aryl β-D-xylofuranosides in aqueous perchloric acid at various temperatures.[195] The effects of varying the structure of the aglycone on the rate of hydrolysis were interpreted in terms of two concurrent mechanisms. It was suggested that D-xylofuranosides having a strongly electron-attracting aglycone react mainly by protonation on the glycosidic oxygen atom followed by rate-limiting heterolysis to form a cyclic oxocarbonium ion, whereas those having a strongly electron-repelling aglycone favour initial rapid protonation on the ring-oxygen atom followed by rate-limiting, unimolecular cleavage of the five-membered ring. Kinetic studies have suggested that the acid-catalysed hydrolysis of the aryl β-D-glucopyranosides (59) proceed by an A-1 mechanism rather than with the participation of water as a



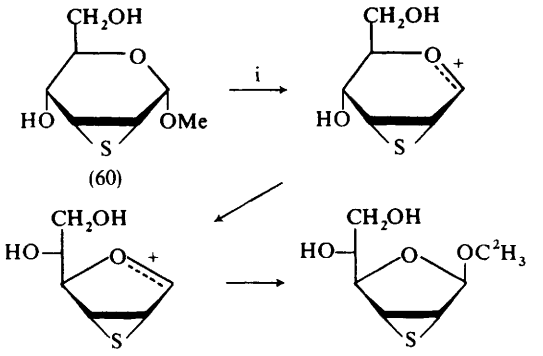
(59) R = CHO, CH=CHMe, $CH_2CH=CH_2$, or CH_2COMe

[190] J. H. Pazur, A. Cepure, S. Okada, and L. S. Forsberg, *Carbohydrate Res.*, 1977, **58**, 193.
[191] A. F. Bochkov and A. V. Rodionov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 2789 (*Chem. Abs.*, 1977, **86**, 106 934*y*).
[192] T. Uryu, H. Tachikawa, K. Ohaku, K. Terui, and K. Matsuzaki, *Makromol. Chem.*, 1977, **178**, 1929.
[193] N. K. Kochetkov and I. V. Obruchnikov, *Tetrahedron Letters*, 1977, 57.
[194] T. Mizuno, T. Wada, K. Miyoshi, and Y. Hibi, *Shizuoka Daigaku Nogakubu Kenkyu Hokoku*, 1976, **26**, 33 (*Chem. Abs.*, 1977, **87**, 168 307*a*).
[195] H. Lönnberg, A. Kankaanperä, and K. Haapakka, *Carbohydrate Res.*, 1977, **56**, 277.

nucleophilic reagent in the transition state.[196] The A-1 hydrolyses of β-D-galactopyranosyl derivatives with nitrogen-, oxygen-, and fluorine-bonded aglycones have been measured and, at constant basicity, have been shown to adopt the order of rates F > O > N.[197] The observed order of leaving abilities is concluded to represent the 'intrinsic leaving-group abilities' of these elements from carbon in water.

Kinetic studies of the hydrolyses of a series of mono- and di-nitrophenyl α-L-arabinopyranosides and β-D-galactopyranosides by *E. coli* β-D-galactosidase have indicated that the hydroxymethyl-group of the D-galactopyranosides assists only in binding and does not destabilize the substrate–enzyme complex.[198] Other studies with β-D-galactosidase are mentioned in Chapter 9.

Methyl 2,3-dideoxy-2,3-epithio-α-D-allopyranoside (60) gave trideuteriomethyl 2,3-dideoxy-2,3-epithio-β-D-allofuranoside on treatment with a cation-exchange resin in [$^2H_4$]methanol.[199] The pyranoside → furanoside isomerization, caused by the inductive effect of the S-substituent at C-2, was explained by a mechanism involving the cyclic cationic intermediates shown in Scheme 11. The effect of the S-



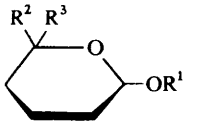
Reagent: i, $C^2H_3O^2H$–*aci*-resin

Scheme 11

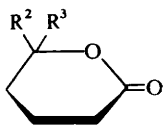
substituent at C-2 is clear from the observations that methyl 2-*S*-benzyl-2-thio-α-D-altropyranoside gave methyl 2-*S*-benzyl-2-thio-αβ-D-altrofuranoside on acid-catalysed methanolysis, whereas the 3-thio analogue was unaffected.

A neat method for cleaving acid-sensitive triterpenoid and steroid aglycones from L-arabinopyranosides and D-galactopyranosides involved oxidation (DMSO–acetic anhydride) of the corresponding 3,4-*O*-isopropylidene derivative to the glycopyranosidulose, which can be cleaved on u.v.-irradiation in t-butanol.[200] Photolysis of the model acetals (61) gave, depending on the substituents R^2 and R^3, either the lactones (62) or the esters (63) (R^2 and/or $R^3 \neq H$),[201] while photolysis of the oxoalkoxytetrahydropyrans (64) gave the lactones (62; $R^2 = R^3$

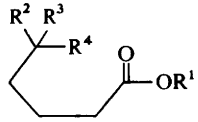
[196] B. Kosikova and D. Joniak, *Cellulose Chem. Technol.*, 1976, **10**, 695.
[197] C. C. Jones and M. L. Sinnott, *J.C.S. Chem. Comm.*, 1977, 766.
[198] P. Marshall, C. G. Reed, M. F. Sinnott, and I. J. L. Souchard, *J.C.S. Perkin II*, 1977, 1198.
[199] T. Yamaguchi and M. Kojima, *Carbohydrate Res.*, 1977, **59**, 129.
[200] I. Kitagawa, K. S. Im, and Y. Fujimoto, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 800.
[201] C. Bernasconi, L. Cottier, and G. Descotes, *Bull. Soc. chim. France*, 1977, 101.



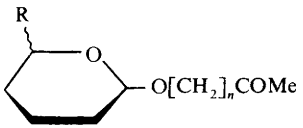
(61) R^1 = $CH_2CH{=}CH_2$ or $CH_2C{\equiv}CH$; R^2 = R^3 = Me or R^2, R^3 = OMe,H



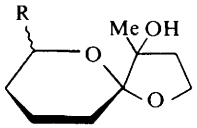
(62)



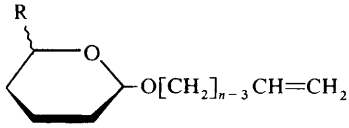
(63) R^4 = H or OH



(64) R = H or OMe



(65)



(66)

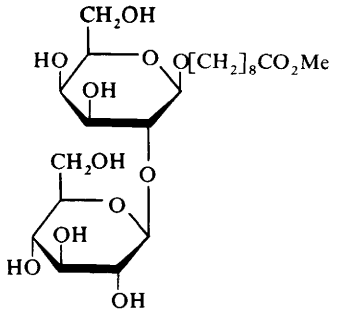
= H or R^2 = H, R^3 = OMe) with n = 1, the hydroxydioxospirans (65) with n = 2, and the alkene (66), by Norrish type II cleavage, with $n > 2$.[202]

A novel procedure for deuterium-labelling of glycosides is noted in Chapter 14.

Natural Products.—Among the natural glycosides to be isolated and characterized during the past year are 2-methylbut-3-en-2-yl β-D-glucopyranoside (from *Ferula loscosii*),[203] 5′-*O*-β-D-glucopyranosylpyridoxine (the bound form of vitamin B_6 from rice bran),[204] and 6′-(4-hydroxy-3-methoxycinnamoyl)catalpol (a β-glucopyranoside from *Picrorhiza kurrooa*).[205] Among the phenolic glycosides isolated from the needles of *Pinus sylvestris* L. were *O*-β-D-glucopyranosyl and *O*-α-L-rhamnopyranosyl derivatives of 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol, two *O*-β-D-glucopyranosyl derivatives and an *O*-α-L-arabinofuranosyl derivative of (+)-isolariciresinol, and 3′-*O*-β-D-glucopyranosyl derivatives of quercetin and 2,3-dihydroquercetin.[206]

A sarsasapogenin glycoside, filiferin A, from the seeds of *Yucca filifera* has been identified as 3-*O*-(2-*O*-β-D-xylopyranosyl-β-D-galactopyranosyl)sarsasapogenin, whereas filiferin B is identical to timosaponin A-III, previously shown to be 3-*O*-(2-*O*-β-D-glucopyranosyl-β-D-galactopyranosyl)sarsasapogenin.[207] 8-Methoxycarbonyloctyl 2-*O*-β-D-glucopyranosyl-β-D-galactopyranoside (67) was synthesized to serve as a model for interpreting the ^{13}C n.m.r. spectrum of filiferin B. Chemical or spectroscopic methods or both have identified a new luteolin tetraglycoside from the moss *Hedwigia ciliata* as 4′-*O*-(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-7-*O*-(2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranosyl)luteolin,[208] and the tetrasaccharide chain of the principal saponin from the roots and rhizomes of *Primula elatior* (L.) Schreber as (68).[209] Enzymic degradation of avenacosides A

[202] C. Bernasconi, L. Cottier, and G. Descotes, *Bull. Soc. chim. France*, 1977, 107.
[203] M. Pinar and M. Martin-Lomas, *Phytochemistry*, 1977, **16**, 281.
[204] K. Yasumoto, H. Tsuji, K. Iwami, and H. Mitsuda, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 1061.
[205] K. Weinges and K. Künstler, *Annalen*, 1977, 1053.
[206] T. Popoff and O. Theander, *Acta Chem. Scand.*, 1977, **B31**, 329.
[207] R. U. Lemieux, R. M. Ratcliffe, B. Arreguin, A. R. de Vivar, and M. J. Castillo, *Carbohydrate Res.*, 1977, **55**, 113.
[208] B.-G. Österdahl and G. Lindberg, *Acta Chem. Scand.*, 1977, **B31**, 293.
[209] R. Tschesche and W. Wiemann, *Chem. Ber.*, 1977, **110**, 2407.



(67)

β-D-Glc*p*-(1→6)-β-D-Gal*p*-(1→4)-D-GlcUA*p*
2
↑
1
α-L-Rha*p*

(68)

β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-β-D-Glc*p*-nuatigenin
4
↑
1
α-L-Rha*p*

(69)

and B from oats (*Avena sativa*) removed from each the single D-glucopyranosyl residue at C-26, yielding products [*e.g.* (69)] that showed both haemolytic and antibiotic activities.[210]

S-Glycosides

The catabolism and biological properties of the glucosinolates have been reviewed.[211]

An efficient synthesis of 4-methylphenyl 1,2-*trans*-1-thioglycopyranosides has involved isomerization of a mixture of the peracetylated *endo*- and *exo*-1,2-thioorthoesters with deactivated Raney nickel and a trace of 4-methylthiophenol in toluene.[212] 1,2,3,5-Tetra-*O*-acetyl-β-D-ribofuranose reacted with thiophenol in the presence of tin(IV) chloride to give a mixture of *S*-glycosides containing principally phenyl 2,3,5-tri-*O*-acetyl-1-thio-β-D-ribofuranoside.[136]

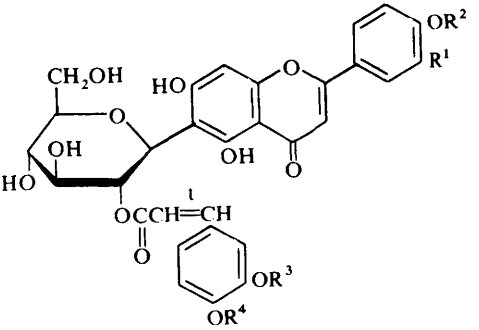
An interesting photochemical rearrangement of an *S*-glycoside having a heterocyclic aglycone to a *C*-glycoside is mentioned in the next section. The conversion of acylated phenyl 1-thiohexopyranosides into the corresponding phenyl 1-thiohex-1-enopyranosid-3-uloses is discussed in Chapter 12, and other references to *S*-glycosides will be found in Chapters 10 and 21.

C-Glycosides

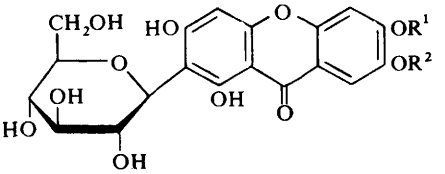
A review of *C*-glycosylflavones has appeared.[213]

Three new *C*-(2-*O*-cinnamoyl-β-D-glucopyranosyl)flavones [*e.g.* (70)] have been isolated from the leaves of *Gentiana punctata* L,[214] while the leaves of *G. asclepiadea*

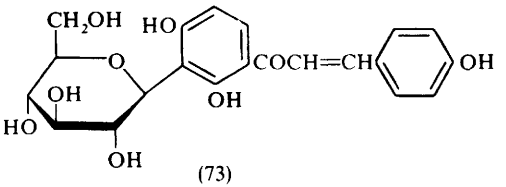
[210] R. Tschesche and W. Wiemann, *Chem. Ber.*, 1977, **110**, 2416.
[211] M. Benn, *Pure Appl. Chem.*, 1977, **49**, 197.
[212] G. Magnusson, *J. Org. Chem.*, 1977, **42**, 913.
[213] J. Chopin and M. L. Bouillant, *Flavonoids*, 1975, **2**, 631 (*Chem. Abs.*, 1977, **86**, 30 007*u*).
[214] M. D. Luong and A. Jacot-Guillarmod, *Helv. Chim. Acta*, 1977, **60**, 2099.



(70) R^1 = OH, R^2 = β-D-Glc*p*, R^3 = R^4 = H



(71) R^1 = H, R^2 = β-D-Glc*p*
(72) R^1 = β-D-Glc*p*, R^2 = H



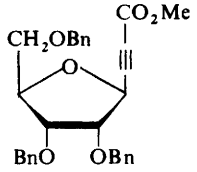
(73)

L have yielded the first di-*O*-(β-D-glucopyranosyl) derivatives of *C*-glycosylflavones[214a] and the 6- and 7-*O*-β-D-glucopyranosyl derivatives (71) and (72) of mangiferin (a *C*-glycosylxanthone).[215] Di-*C*-glycosides of apigenin,[216,217] luteolin,[216] and tricetin[216] and more complex di-*C*-glycosylflavones[218] have been isolated from plant sources. The 3′-*C*-β-D-glucopyranosylchalcone (73) and 8-*C*-β-D-glucopyranosyl-4′,5,7-trihydroxy-6-*C*-β-D-xylopyranosylflavone (vicenin 1) have been isolated from the wood of *Cladrastis platycarpa*.[219] 6-*C*-β-Neohesperidosylacacetin and a small amount of the 8-*C*-isomer have been synthesized by reaction of the acetobromo derivative of neohesperidose (2-*O*-α-L-rhamnopyranosyl-D-glucopyranose) and 4′-methoxy-5,7-dihydroxyflavone (acacetin) in the presence of

[214a] M. Goetz and A. Jacot-Guillarmod, *Helv. Chim. Acta*, 1977, **60**, 1322.
[215] M. Goetz and A. Jacot-Guillarmod, *Helv. Chim. Acta*, 1977, **60**, 2104.
[216] R. Mues and H. D. Zinsmeister, *Phytochemistry*, 1976, **15**, 1757.
[217] T. K. Chumbalov and R. A. Zhubaeva, *Khim. prirod. Soedinenii*, 1976, 661 (*Chem. Abs.*, 1977, **86**, 103 079*n*).
[218] M. Zielinska-Stasiek and S. Gill, *Roczniki Chem.*, 1977, **51**, 921.
[219] H. Ohashi, M. Goto, and H. Imamura, *Phytochemistry*, 1977, **16**, 1106.

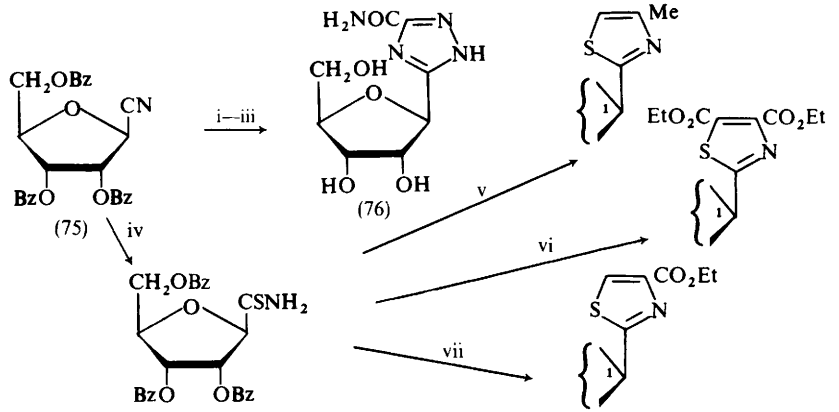
lithium methoxide; the isomers were found to be identical with isomargariten and margariten, respectively, isolated from *Fortunella margarita*.[220]

The principal product of the reaction of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide with mercury(II) or copper(I) phenylacetylide has been shown to possess the β-configuration by ^{1}H n.m.r. examination of isopropylidenated and acylated derivatives ($J_{1,2}$ = 0 Hz) of the debenzoylated product and by chemical degradation of the side-chain to give known 2,5-anhydro-D-allonic acid derivatives.[221] Details have appeared on the synthesis of methyl 3-(2,3,5-tri-*O*-benzyl-α- and -β-D-ribofuranosyl)propiolates [*e.g.* (74)] by reaction of 2,3,5-tri-*O*-benzyl-D-ribo-



(74)

furanosyl bromide with the silver salt of methyl propiolate (see Vol. 9, p. 26) and of the reactions of these esters and β-D-ribofuranosylethyne with several 1,3-dipoles to give pyrazole, triazole, and pyrazolone derivatives.[222] A number of the products of dipolar addition were converted into new D-ribofuranosyl-triazoles and -pyrazoles [*e.g.* 4(5)-methoxycarbonyl-5(4)-α-D-ribofuranosyl-1,2,3-triazole and 4-β-D-ribofuranosylpyrazole] by hydrogenolysis. 2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl cyanide (75) has been converted into the *C*-nucleoside analogue (76) of virazole (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide)[223] and into some *C*-bonded

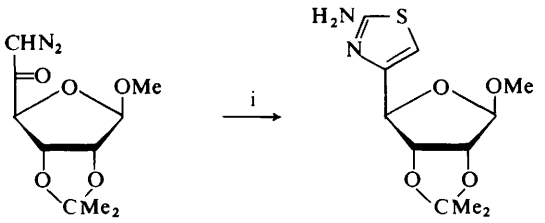


Reagents: i, MeONa–MeOH; ii, $H_2NCOCONHNH_2$; iii, heat; iv, H_2S; v, $ClCH_2COMe$; vi, $EtO_2CCHClCOCO_2Et$; vii, $BrCH_2COCO_2Et$

Scheme 12

[220] J. Chopin, A. Besset, and M.-L. Bouillant, *Compt. rend.*, 1977, **284**, *C*, 1007.
[221] K. Arakawa, T. Miyasaka, and N. Hamamichi, *Chem. Letters*, 1976, 1119.
[222] J. G. Buchanan, A. R. Edgar, M. J. Power, and G. C. Williams, *Carbohydrate Res.*, 1977, **55**, 225.

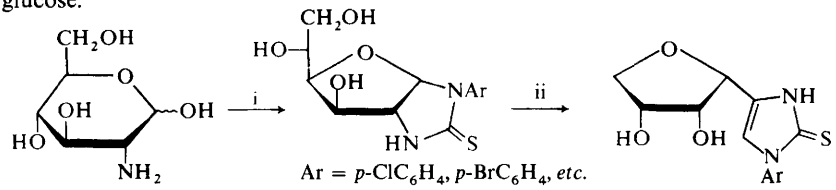
thiazole analogues (Scheme 12).[224] A 'reverse' *C*-nucleoside derivative containing a *C*-bonded thiazole residue was also prepared by the reaction shown in Scheme 13.[224]



Reagent: i, H_2NCSNH_2

Scheme 13

Reaction of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl, 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl, and 2,3,4-tri-*O*-acetyl-β-D-ribopyranosyl cyanides with 2-aminothiophenol in warm ethanol gave the corresponding acylated 2-β-D-glycopyranosylbenzothiazoles, which furnished crystalline *C*-glycosyl derivatives of benzothiazole on deacetylation.[225] The glycosyl cyanides also reacted with sodium azide and ammonium chloride in hot DMF to give acylated 5-β-D-glycopyranosyltetrazoles. The reactions outlined in Scheme 14 have been used to obtain 4-α-D-erythrofuranosyl-1-halophenylimidazoline-2-thiones from 2-amino-2-deoxy-D-glucose.[226]



Reagents: i, ArNCS; ii, CF_3CO_2H at 75—80 °C

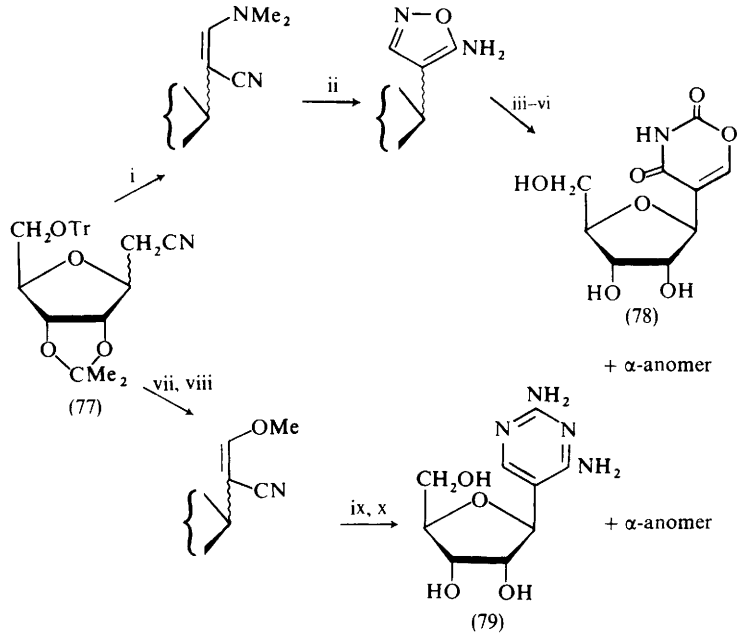
Scheme 14

The protected β-D-ribofuranosylacetonitrile (77) has been converted in the *C*-nucleoside antibiotic oxazinomycin (78),[227] 2,4-diamino-5-β-D-ribofuranosylpyrimidine (79) (Scheme 15), and 5-β-D-ribofuranosyl-2-thiocytosine (2-thiopseudocytidine).[228]

An interesting photochemical rearrangement of a protected 4-(1-thio-β-D-ribofuranosyl)pyrimidine to a pyrimidine *C*-nucleoside, which could be further transformed into β-pseudouridine, is illustrated in Scheme 16.[229]

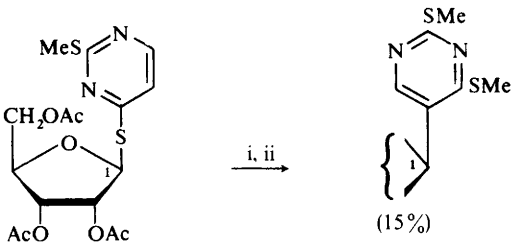
An antifungal antibiotic (W-7783) produced by the soil-inhabiting myxobacteriale *Polyangium cellulosum* var. *fulvum* has been assigned structure (80) on the basis of chemical and spectroscopic evidence.[230] The detailed stereochemistry of this antibiotic was confirmed by *X*-ray crystallography.

[224] M. Fuertes, T. García-López, G. García-Muñoz, and M. Stud, *J. Org. Chem.*, 1976, **41**, 4074.
[225] I. Farkas, I. F. Szabó, and R. Bognár, *Carbohydrate Res.*, 1977, **56**, 404.
[226] J. Fernandez-Bolanos and J. Fuentes Mota, *An. Quim.*, 1976, **72**, 79 (*Chem. Abs.*, 1977, **86**, 121 657*d*).
[227] S. de Bernardo and M. Weigele, *J. Org. Chem.*, 1977, **42**, 109.
[228] K. Chu, U. Reichman, K. A. Watanabe, and J. J. Fox, *J. Org. Chem.*, 1977, **42**, 711.
[229] J.-L. Fourrey, G. Henry, and P. Jouin, *J. Amer. Chem. Soc.*, 1977, **99**, 6753.
[230] D. T. Connor, R. C. Greenough, and M. von Strandtmann, *J. Org. Chem.*, 1977, **42**, 3664.



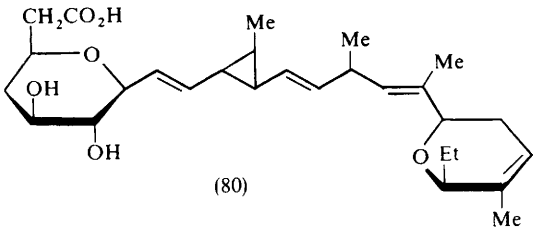
Reagents: i, $Bu^tOCH(NMe_2)_2$: ii, NH_2OH; iii, H_2–Pt; iv, H_3O^+; v, $(C_3H_3N_2)CO$; vi, aq. CF_3CO_2H; vii, HCO_2Et–NaH; viii, MeI–DMF; ix, $(NH_2)_2C{=}NH$–EtONa; x, HCl–MeOH

Scheme 15

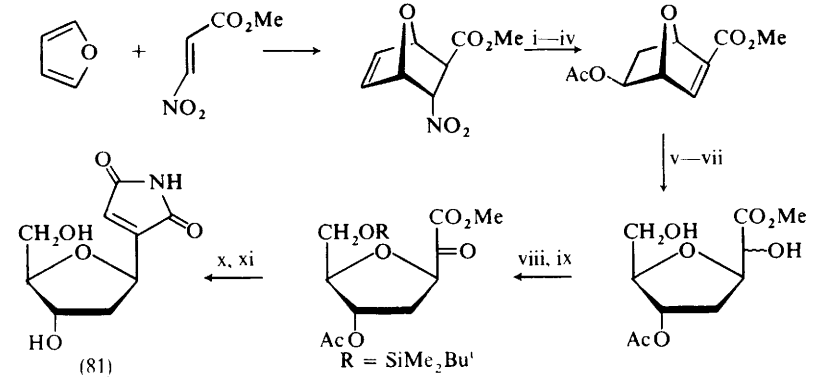


Reagents: i, *hν*; ii, MeI

Scheme 16

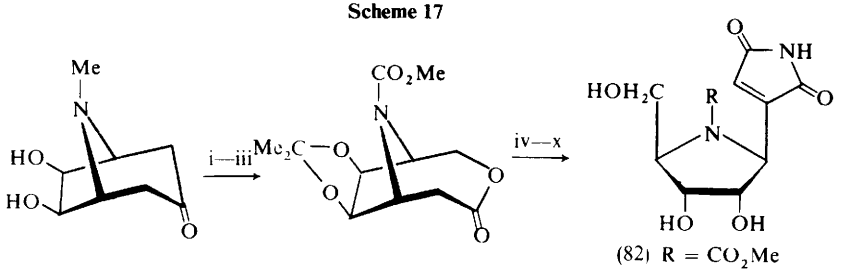


In continuing their studies on the synthesis of racemic *C*-nucleosides, Just and co-workers have reported the synthesis of DL-2′-deoxyshowdomycin (81) from furan and methyl β-nitroacrylate (Scheme 17),[231] of a nitrogen analogue (82) of



Reagents: i, B_2H_6; ii, Et_3NO; iii, Ac_2O–TsOH; iv, DBU; v, O_3; vi, Me_2S; vii, $(Bu^tO)_3LiAlH$; viii, Bu^tMe_2SiCl; ix, DMSO–Ac_2O; x, $Ph_3P{=}CHCONH_2$; xi, H^+

Scheme 17



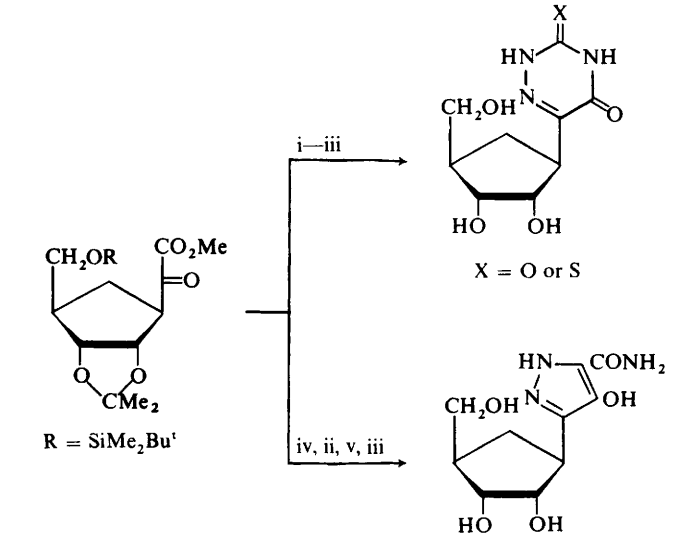
Reagents: i, Me_2CO–H^+; ii, $ClCO_2Me$; iii, *m*-$ClC_6H_4CO_3H$; iv, MeONa–MeOH; v, Bu^tMe_2SiCl–DMF; vi, Pr^i_2NLi–CO_2; vii, HCHO–Et_2NH; viii, O_3–Me_2S; ix, $Ph_3P{=}CHCONH_2$; x, aq. CF_3CO_2H

Scheme 18

DL-showdomycin from teloidinone (Scheme 18),[232] and of racemic carbocyclic analogues of 6-azapseudouridine, 2-thio-6-azapseudouridine, and pyrazofurin by way of cyclization of the *syn*- and *anti*-semicarbazones, thiosemicarbazones, and ethoxycarbonylmethylhydrazones, respectively, of a cyclopentylglyoxylate derivative (Scheme 19).[233]

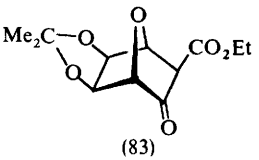
1,3-Diethoxycarbonylallene, an active dienophile and ethoxycarbonylketen equivalent, has been used to prepare (83), which is a key intermediate for projected syntheses of antibiotic *C*-nucleosides.[234] Treatment of such 2,3-dideoxyald-2-enonic acid esters as (84) with sodium methoxide yielded 3,6-anhydro-2-deoxyhexonic acid esters [*e.g.* (85)] from an internal Michael reaction on the deacetylated derivative.[235]

[231] G. Just and M.-I. Lim, *Canad. J. Chem.*, 1977, **55**, 2993.
[232] G. Just and G. P. Donnini, *Canad. J. Chem.*, 1977, **55**, 2998.
[233] G. Just and S. Kim, *Canad. J. Chem.*, 1977, **55**, 427.
[234] A. P. Kozikowski, W. C. Floyd, and M. P. Kuniak, *J.C.S. Chem. Comm.*, 1977, 582.
[235] I. Dyong, R. Knollmann, W. Hohenbrink, and H. Bendlin, *Chem. Ber.*, 1977, **110**, 1175.

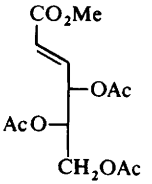


Reagents: i, $NH_2NHCONH_2$ or $NH_2NHCSNH_2$; ii, MeONa–MeOH; iii, 50% CF_3CO_2H; iv, $NH_2NHCH_2CO_2Et$; v, NH_3–MeOH

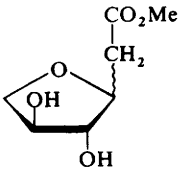
Scheme 19



(83)



(84)



(85)

4
Ethers and Anhydro-sugars

Ethers

Methyl Ethers.—A practical guide to the methylation analysis of carbohydrates has been published.[236]

Lewis-acid-catalysed methylation of methyl α-L-rhamnopyranoside and its 4-*O*-methyl derivative in methanol with diazomethane has been used to prepare several mono- and di-*O*-methyl derivatives.[237] Use of catalytic amounts of tin(II) chloride dihydrate and titanium(IV) chloride in the methylation of methyl α-L-rhamnopyranoside gave methyl 3-*O*-methyl-α-L-rhamnopyranoside preferentially, whereas use of cerium(III) chloride favoured the formation of the corresponding 2-*O*-methyl derivative. Methylation of methyl 4-*O*-methyl-α-L-rhamnopyranoside gave a mixture of methyl 3,4- and 2,4-di-*O*-methyl-α-L-rhamnopyranosides in the approximate ratios of 2:1, 1:2, and 4:1 with catalytic amounts of tin(II) chloride dihydrate, cerium(III) chloride, and titanium(IV) chloride, respectively. Methylation of benzyl 4,6-*O*-benzylidene-β-D-talopyranoside with diazomethane in the presence of catalytic amounts of tin(II) chloride dihydrate gave benzyl 4,6-*O*-benzylidene-3-*O*-methyl-β-D-talopyranoside in a yield of 89%.[238] 2-*O*-Methyl-, 3-*O*-methyl-, and 2,3-di-*O*-methyl-D-talose were also prepared in high yield *via* methylation of benzyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-talopyranoside, the corresponding 2-*O*-benzoyl derivative, and methyl 4,6-*O*-benzylidene-β-D-talopyranoside, respectively, with diazomethane in the presence of boron trifluoride etherate, *etc.* Jarý's group has examined the partial methylation of methyl 4,6-dideoxy-α-D-*arabino*-hexopyranoside (both hydroxy-groups in axial positions) using methyl iodide in acetonitrile in the presence of solid sodium hydroxide.[239] The relative rates for the formation of mono- and di-substituted products were determined and rationalized in terms of steric and electronic factors.

Methylation (methyl iodide–silver oxide) of methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (prepared by selective benzylation of the equatorial hydroxy-group of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside) followed by hydrogenolysis and hydrolysis with acid has provided a convenient synthesis of 2-*O*-methyl-D-mannose.[240] 3-*O*-Methyl- and 3,6-di-*O*-methyl-D-galactose have been synthesized by straightforward procedures from methyl β-D-galactopyrano-

[236] P. E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, and J. Lönngren, *Chem. Comm. Univ. Stockholm*, 1976, 1 (*Chem. Abs.*, 1977, **87**, 136 153*f*).
[237] R. Toman, S. Karácsonyi, and R. Palovčík, *Carbohydrate Res.*, 1977, **56**, 191.
[238] G. J. F. Chittenden, *Carbohydrate Res.*, 1976, **52**, 23.
[239] K. Kefurt, Z. Kefurtová, V. Ineman, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1977, **42**, 3180.
[240] H. C. Srivastava and V. K. Srivastava, *Carbohydrate Res.*, 1977, **58**, 227.

side.[241] In order to define the fragmentation pattern of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside more precisely, derivatives containing each of H-1—H-6,6′ replaced by deuterium have been synthesized by reduction of an appropriately protected keto-sugar (*e.g.* methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-*arabino*-hexopyranoside-2-ulose) with sodium borodeuteride, prior to conversion into the fully methylated derivative.[242] Details of the thermal degradation of mono-*O*-methyl-D-glucoses and of the mass spectrometry of methylated sugars are reported in Chapters 2 and 21, respectively.

The following *O*-methylaldoses have been isolated from, or identified as constituents of, natural products: 3-*O*-methylxylose (in the extracellular mucilage from the red alga *Rhodella maculata*),[243] 3-*O*-methyl-L-xylose (together with L-xylose and L-rhamnose from the lipopolysaccharide of *Pseudomonas maltophilia*),[244] 3-*O*-methyl-D-ribose (from the extracellular polysaccharide of a Cowpea strain of slow-growing *Rhizobium*, strain CB 756),[245] 2-*O*-methyl- and 2,4-di-*O*-methyl-L-rhamnose (in the antibiotics steffimycin and steffimycin B produced by *Streptomyces steffisburgensis*),[246] and 3-*O*-methyl-galactose and -mannose (from the haemocyanin of snails).[247]

Other Alkyl and Aryl Ethers.—Selective benzylation of O-3 of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside has been noted in the previous section,[240] while 2,3,4,5-tetra-*O*-benzyl-L-arabinose diethyl dithioacetal and 2,3,4,5-tetra-*O*-benzyl-D-arabinose dibenzyl and ethylene dithioacetals have been prepared by benzylation of the thioacetals using benzyl chloride and potassium hydroxide.[248] An improved synthesis of 2,4,6-tri-*O*-benzyl-D-galactopyranose has been accomplished by way of benzylation of allyl 3-*O*-allyl-2,6-di-*O*-benzyl-α-D-galactopyranoside, which was obtained by regiospecific allylation of the 3,4-*O*-dibutylstannylene derivative of allyl 2,6-di-*O*-benzyl-α-D-galactopyranoside[249] (see Vol. 10, p. 122).

Alkylation of metal chelates of sucrose with allyl halides and sodium bromoacetate in DMSO yielded mono-*O*-allylated and mono-*O*-carboxymethylated derivatives.[250] A new acidic monosaccharide from the O-specific lipopolysaccharide of *Shigella dysenteriae* type 3 has been identified as 4-*O*-[(*R*)-1-carboxyethyl]-D-glucopyranose on the basis of chemical and mass-spectral evidence and by its synthesis by alkylation of methyl 2,3,6-tri-*O*-benzyl-α(β)-D-glucopyranoside with (*S*)-2-chloropropionic acid, esterification (with diazomethane), hydrogenolysis, and acid hydrolysis.[251]

A convenient procedure for the tritylation of secondary hydroxy-groups of sugars is based on the use of triphenylmethylium perchlorate or tetrafluoroborate in

[241] A. I. Usov and S. V. Yarotskii, *Bioorg. Khim.*, 1977, **3**, 746 (*Chem. Abs.*, 1977, **87**, 202 001*h*).
[242] A. Ya. Ott and O. S. Chizhov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1977, 201 (*Chem. Abs.*, 1977, **86**, 140 401*g*).
[243] V. S. Fareed and E. Percival, *Carbohydrate Res.*, 1977, **53**, 276.
[244] F. Brown, D. J. Neal, and S. G. Wilkinson, *Biochem. J.*, 1977, **163**, 173.
[245] L. D. Kennedy, *Carbohydrate Res.*, 1976, **52**, 259.
[246] R. C. Kelly, I. Schletter, J. M. Koert, F. A. MacKellar, and P. F. Wiley, *J. Org.Chem.*,1977, **42**, 3591.
[247] R. L. Hall, E. J. Wood, J. P. Kamberling, G. J. Gerwig, and F. G. Vliegenthart, *Biochem. J.*, 1977, **165**, 173.
[248] J. Fernandez-Bolanos and S. Munoz Guerra, *An. Quim.*, 1977, **73**, 458 (*Chem. Abs.*, 1977, **87**, 152 478*w*).
[249] M. A. Nashed and L. Anderson, *Carbohydrate Res.*, 1977, **56**, 419.
[250] E. Avela, S. Aspelund, B. Holmbom, B. Melander, H. Jalonen, and C. Peltonen, *Amer. Chem. Soc. Symposium Ser.*, 1977, 41, Sucrochemistry Symposium, 1976, 62 (*Chem. Abs.*, 1977, **87**, 136 200*a*).
[251] N. K. Kochetkov, B. A. Dmitriev, and V. L. Lvov, *Carbohydrate Res.*, 1977, **54**, 253.

combination with 2,4,6-tri(t-butyl)pyridine (a sterically hindered acid-acceptor).[252] This tritylation procedure is applicable to such acid-labile derivatives as α-D-xylopyranose 1,2,4-orthoacetate and β-L-arabinofuranose 1,2,5-orthobenzoate, and only a small amount of detritylation occurred on hydrolysis of the orthoesters.

Monotritylation of raffinose afforded the 6′- and 6″-*O*-trityl derivatives, in yields of 1.7% and 9.4%, respectively, which were used to prepare deca-*O*-acetylraffinoses.[253] Raffinose reacted with three molar equivalents of trityl chloride in pyridine to give, among other products, 1′,6′,6″-tri-*O*-tritylraffinose in a yield of 20%.[254]

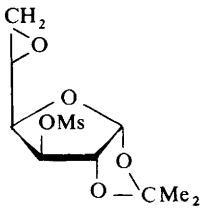
1,2:5,6-Di-*O*-isopropylidene-3-*O*-(3-nitrophenyl)-α-D-glucofuranose and 1,2:3,4-di-*O*-isopropylidene-6-*O*-(3-nitrophenyl)-α-D-galactopyranose have been synthesized by alkoxide displacement of a nitro-group from 1,3-dinitrobenzene.[255] The isopropylidene groups were then removed with acid to give the parent (3-nitrophenyl)ated hexoses, which might find application as radiocontrast agents.

Silyl Ethers.—Examples of the usefulness of t-butyldimethylsilyl ethers as protecting groups are nicely illustrated in Schemes 17 and 19. Methyl α-D-glucopyranoside reacted with a molar equivalent of t-butyldimethylsilyl chloride in pyridine to give the 6-*O*-(t-butyldimethylsilyl) derivative in virtually quantitative yield, and sucrose reacted with 3.5 molar equivalents of the silylating reagent to give almost exclusively 1′,6,6′-tri-*O*-(t-butyldimethylsilyl)sucrose and traces of mono- and di-substituted products.[256] In the presence of an excess of sucrose, a mixture of 6′-, 6,6′-di-, and 1′,6,6′-tri-*O*-(t-butyldimethylsilyl)sucroses was obtained.

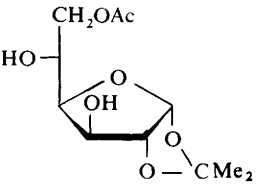
Octa-*O*-(trimethylsilyl)sucrose is a stable crystalline material that could have potential value as an internal g.l.c. standard.[257] The selective hydrolysis of octa-*O*-(trimethylsilyl)-αα-trehalose at one or both of the primary positions has been noted earlier.[173]

Intramolecular Ethers (Anhydro-sugars)

Oxirans.—A convenient synthesis of 5,6-anhydro-1,2-*O*-isopropylidene-3-*O*-methanesulphonyl-β-L-idofuranose (86) is available by methanesulphonylation of (87) and treatment of the resulting dimesylate with sodium isopropoxide.[258]



(86)

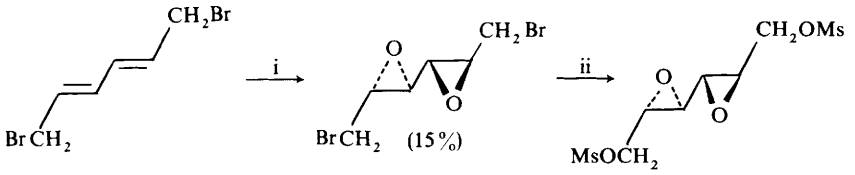


(87)

[252] Y. V. Wozney and N. K. Kochetkov, *Carbohydrate Res.*, 1977, **54**, 300.
[253] T. Suami, T. Otake, T. Ikeda, and R. Adachi, *Bull. Chem. Soc. Japan*, 1977, **50**, 1612.
[254] L. Hough, M. A. Salam, and E. Tarelli, *Carbohydrate Res.*, 1977, **57**, 97.
[255] F. L. Weitl, M. Sovak, and D. Keil, *J. Org. Chem.*, 1977, **42**, 2513.
[256] F. Franke and R. D. Guthrie, *Austral. J. Chem.*, 1977, **30**, 639.
[257] R. Bentley, *Carbohydrate Res.*, 1977, **59**, 274.
[258] N. A. Hughes and P. D. Tyson, *Carbohydrate Res.*, 1977, **57**, 317.

Reduction of the oxiran (86) with lithium aluminium hydride gave, depending on the reaction time, 6-deoxy-1,2-*O*-isopropylidene-β-L-idofuranose either as the only product or accompanied by its 3-mesylate (see also Chapter 10).

α, ω-Disubstituted derivatives of 2,3-anhydro-DL-threitol, 2,3-anhydroerythritol, and 2,3:4,5-dianhydro-galactitol and -allitol have been synthesized by epoxidation of appropriate alkenes and dienes.[259] For example, epoxidation of 1,6-dibromo-*trans,trans*-hexa-2,4-diene with 3-chloroperbenzoic acid gave 2,3:4,5-dianhydro-1,6-dibromo-1,6-dideoxygalactitol (15%), which afforded the 1,6-dimesylate when treated with silver methanesulphonate in acetonitrile (Scheme 20).

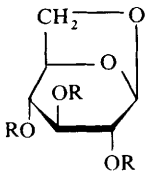


Reagents: i, *m*-$ClC_6H_4CO_3H$; ii, $MeSO_3Ag$–MeCN

Scheme 20

Other sugar oxirans are mentioned in Chapters 7, 9, and 10.

Other Anhydrides.—A D-glucosyltransferase from *Aspergillus niger* acted on maltose to give, among other products, 1,6-anhydro-β-D-glucopyranose (1%) by an intramolecular transfer reaction.[260] A series of oligomeric ethers (88) was



(88) R = $-[CH_2CHMeO]_n-H$

produced when 1,6-anhydro-β-D-glucopyranose reacted with propylene oxide under basic conditions.[261] Hydrolysis of methyl 2-*S*-benzyl(benzoyl)-4,6-*O*-benzylidene-2-thio-α-D-altropyranoside with acid gave the corresponding 1,6-anhydro-2-thio-β-D-altropyranoses.[262] Other references to 1,6-anhydrohexopyranoses will be found in Chapters 3, 7, 8, 11, 12, and 20.

Treatment of methyl 2,3-di-*O*-methyl-6-*O*-toluene-*p*-sulphonyl-α-D-galactopyranoside with hot aqueous, alcoholic sodium hydroxide afforded methyl 4,6-anhydro-2,3-di-*O*-methyl-α-D-galactopyranoside in a yield of 84%.[263] The

[259] G. Schneider, T. Horváth, and P. Sohár, *Carbohydrate Res.*, 1977, **56**, 43.
[260] J. H. Pazur, Y. Tominaga, and L. S. Forsberg, *Arch. Biochem. Biophys.*, 1977, **182**, 774.
[261] R. Pernikis, B. Apsite, B. Lazdina, N. Pundure, and J. Surna, *Latv. P.S.R. Zinat. Akad. Vestis. Kim. Ser.*, 1977, 340 (*Chem. Abs.*, 1977, **87**, 201 920v).
[262] J. Thiem, H.-J. Jürgens, and H. Paulsen, *Chem. Ber.*, 1977, **110**, 2834.
[263] C. R. Hall and T. D. Inch, *Carbohydrate Res.*, 1977, **53**, 254.

oxetane was not affected by treatment with boiling 6M-NaOH and it was only moderately affected by treatment with boiling 6M-HCl. 1′,4′:3,6:3′,6′-Trianhydrosucrose was obtained in high yield on treating 1′,6,6′-tri-*O*-mesitylenesulphonylsucrose with alkali; the axial hydroxy-groups at C-2 and C-4 of the trianhydrosucrose displayed considerable differences in reactivities towards acylation and etherification, permitting the preparation of the 4-*O*-acetyl and 4-*O*-methyl derivatives.[264]

The formation of 2,5-anhydropentose dibenzyl dithioacetals from pentose dibenzyl dithioacetals and that of 2,5-anhydro-D-allose derivatives *via* diazotization are discussed in Chapters 10 and 8, respectively.

[264] D. H. Ball, F. H. Bissett, and R. C. Chalk, *Carbohydrate Res.*, 1977, **55**, 149.

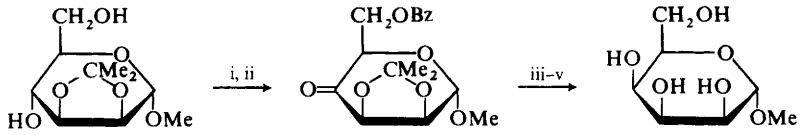
5
Acetals

Synthesis

Use of anhydrous iron(III) chloride to catalyse the acetonation of aldoses, alditols, and nucleosides has been recommended.[265, 265a] Work-up can be effected by neutralization with ammonium hydroxide and heating to coagulate iron(III) hydroxide before its removal by centrifugation or filtration. D-Glucose reacted with acetone in DMF containing a trace of toluene-*p*-sulphonic acid to give a mixture of 1,2-*O*-, 5,6-*O*-, and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranoses.[266] Acid-catalysed acetonation of D-glucose using 2,2-dimethoxypropane in DMF at room temperature gave 4,6-*O*-isopropylidene-D-glucopyranose (80%) and traces of 5,6-*O*-isopropylidene-D-glucofuranose and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, whereas the two latter derivatives and an acyclic di-*O*-isopropylidene derivative were the principal products when the reaction was conducted at 95°C.[267] This variation in the products with temperature presumably reflects differences in the pyranose–furanose equilibria of D-glucose at room temperature and at higher temperatures. Similar acetonation of D-xylose at 40–45°C gave a mixture of 3,5-*O*-isopropylidene-D-xylofuranose, 1,2:3,5-di-*O*-isopropylidene-α-D-xylofuranose, 1,2-*O*-isopropylidene-α-D-xylopyranose, and the acyclic derivatives 2,3:4,5-di-*O*-isopropylidene-1-*O*-methyl-(D-xylose aldehydrol) and 2,3:4,5-di-*O*-isopropylidene-(D-xylose aldehydrol), while acetonation of D-arabinose and D-ribose at room temperature gave virtually quantitative yields of 3,4-*O*-isopropylidene-β-D-arabinopyranose and 2,3-*O*-isopropylidene-D-ribofuranose, respectively.[268] D-Arabinose gave 1,2:3,4-di-*O*-isopropylidene-β-D-arabinopyranose (88%) when the reaction was conducted at 80°C. Methyl α-D-mannopyranoside and methyl α-D-mannofuranoside each reacted with a molar equivalent of 2,2-dimethoxypropane in DMF containing a trace of toluene-*p*-sulphonic acid to give the 4,6-*O*-isopropylidene derivative and the 5,6-*O*-isopropylidene derivative, respectively.[269] Acid-catalysed acetonation of methyl α-D-mannopyranoside under conditions of thermodynamic control, using concentrated sulphuric acid as the catalyst, furnished methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside, which can also be prepared by graded hydrolysis of methyl 2,3:4,6-di-*O*-isopropylidene-α-D-mannopyranoside. Successive benzoylation, oxidation, and reduction, *etc.*, of

[265] P. P. Singh, M. M. Gharia, F. Dasgupta, and H. C. Srivastava, *Tetrahedron Letters*, 1977, 439.
[265a] A. Malkiewicz, E. Sochacka, and B. Bochwic, *Roczniki Chem.*, 1977, **51**, 2041.
[266] M. Mosihuzzaman and M. R. Islam, *Dacca Univ. Stud.*, 1976, **24**, 15 (*Chem. Abs.*, 1977, **87**, 184 804*r*).
[267] M. Kiso and A. Hasegawa, *Carbohydrate Res.*, 1976, **52**, 87.
[268] M. Kiso and A. Hasegawa, *Carbohydrate Res.*, 1976, **52**, 95.
[269] M. E. Evans and F. W. Parrish, *Carbohydrate Res.*, 1977, **54**, 105.

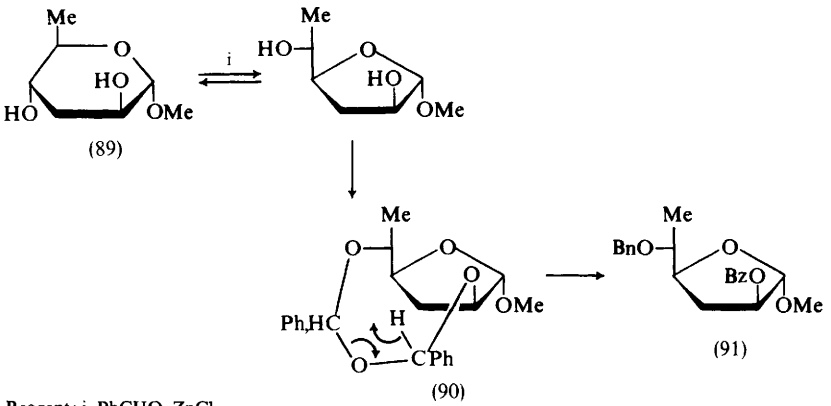
methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside was used to prepare methyl α-D-talopyranoside (Scheme 21).



Reagents: i, BzCl–py–CH_2Cl_2; ii, CrO_3–py–CH_2Cl_2; iii, $NaBH_4$; iv, MeONa–MeOH; v, H_3O^+

Scheme 21

Standard *O*-benzylidene derivatives have been prepared in yields of ⩽ 95% from methyl glycopyranosides using αα-diethoxytoluene in dry *p*-dioxan containing an acid resin.[270] Methyl 3,6-dideoxy-α-D-*arabino*-hexopyranoside (89) reacted with benzaldehyde in the presence of zinc chloride to give methyl 2-*O*-benzoyl-5-*O*-benzyl-3,6-dideoxy-α-D-*arabino*-hexofuranoside (91), presumably by ring-contraction and rearrangement of the intermediate (90), as shown in Scheme 22.[271]



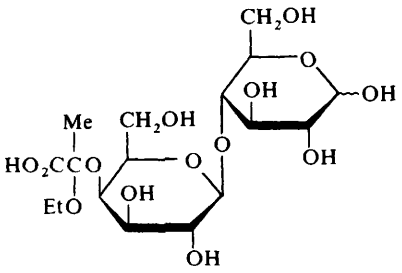
Reagent: i, $PhCHO–ZnCl_2$

Scheme 22

The acid-catalysed reaction of 1,2-*O*-trichloroethylidene-α-D-glucofuranose (α-chloralose) with butyraldehyde afforded diastereoisomeric 5,6-*O*-butylidene-1,2-*O*-trichloroethylidene-α-D-glucofuranoses, which were identified by chemical and spectroscopic evidence.[272] The 3,5-*O*-butylidene derivative was not formed, but it could be prepared by debenzoylation of the product obtained from the reaction of 6-*O*-benzoyl-1,2-*O*-trichloroethylidene-α-D-glucofuranose with butyraldehyde. The butylidene group of the 3,5-acetal was hydrolysed much more readily than that of the 5,6-acetals.

[270] A. Yu. Romanovich, A. F. Sviridov, and S. V. Yarotskii, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1977, 2160 (*Chem. Abs.*, 1977, **87**, 202 014*q*).
[271] J.-C. Florent, C. Monneret, and Q. Khuong-Huu, *Tetrahedron Letters*, 1977, 243.
[272] L. Yüceer, *Carbohydrate Res.*, 1977, **56**, 87.

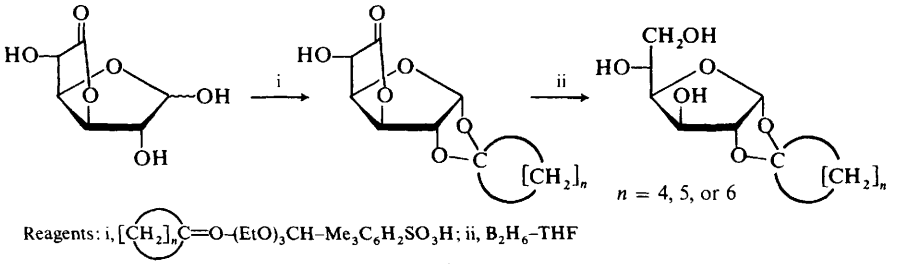
4′-*O*-(2-Ethoxypropion-2-yl)lactose (92), which was required as a model for a substrate inhibitor of viral neuraminidases, has been synthesized from benzyl 2,2′,3,3′,6,6′-hexa-*O*-benzoyl-β-lactoside by way of a transacetalation reaction with ethyl 2,2-diethoxypropionate.[273]



(92)

Reactions and Other Features

Studies of the acid-catalysed hydrolysis of 1,2-*O*-alkylidene-α-D-glucofuranoses (prepared as shown in Scheme 23) have indicated that the cyclopentylidene and



Reagents: i, $[CH_2]_nC{=}O$-$(EtO)_3CH$-$Me_3C_6H_2SO_3H$; ii, B_2H_6–THF

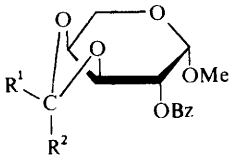
Scheme 23

cycloheptylidene groups are hydrolysed more rapidly than the cyclohexylidene group, which is slightly more stable than the isopropylidene group.[274] Hydrolysis was impeded by a nitrate group at O-5 or, more effectively, at O-3.

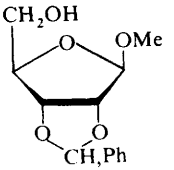
On heating, with benzaldehyde, methyl β-L-arabinopyranoside gave, after benzoylation, the 3,4-*O*-(*S*)-benzylidene derivative (93), whereas selective hydrolysis of a 1:1-mixture of the diastereoisomers gave the (*R*)-isomer (94).[275] The pure isomers (93) and (94) were converted into methyl 3,4-*O*-(*S*)- and -(*R*)-benzylidene-β-L-ribopyranoside, respectively, on successive debenzoylation, oxidation (DMSO–acetic anhydride), and reduction (sodium borohydride). Under anhydrous acid conditions, methyl 3,4-*O*-(*R*)-benzylidene-β-D-ribopyranoside rearranged to the

[273] I. E. Valashek, M. K. Shakhova, G. I. Samokhvalov, V. M. Royhel, and F. N. Reyzin, *Bioorg. Khim.*, 1976, **2**, 1551 (*Chem. Abs.*, 1977, **86**, 106 926*x*).
[274] W. A. R. van Heeswijk, J. B. Goedhart, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1977, **58**, 337.
[275] D. M. Clode, *Canad. J. Chem.*, 1977, **55**, 4066.

2,3-*O*-(*R*)-benzylidene derivative, which gave a pseudoequilibrium mixture containing the four pyranosidic acetals on further rearrangement.[276] At higher concentrations of acid, ring-contraction also occurred to give the diastereoisomers (95) as the only products. Similar results were obtained with methyl 3,4-*O*-(*S*)-benzylidene-β-D-ribopyranoside.



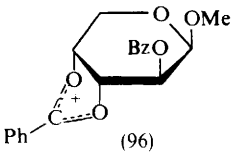
(93) R^1 = Ph, R^2 = H
(94) R^1 = H, R^2 = Ph



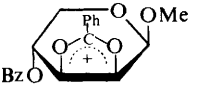
(95)

Regiospecific hydrogenolysis of the *exo*-isomer of benzyl 6-*O*-allyl-2-*O*-benzyl-3,4-*O*-benzylidene-α-D-galactopyranoside with the lithium aluminium hydride–aluminium chloride reagent gave benzyl 6-*O*-allyl-2,4-di-*O*-benzyl-α-D-galactopyranoside, which was required for the synthesis of branched oligosaccharides containing a 3,6-disubstituted D-galactopyranosyl residue.[277] The corresponding *endo*-isomer was similarly converted into benzyl 6-*O*-allyl-2,3-di-*O*-benzyl-α-D-galactopyranoside. Hydrogenolysis of benzyl 2,2′,3,3′,6-penta-*O*-benzyl-4′,6′-*O*-benzylidene-β-cellobioside, -maltoside, -allolactoside, and -lactoside with the lithium aluminium hydride–aluminium chloride reagent gave the corresponding 2,2′,3,3′,4′,6-hexa-*O*-benzyl derivatives (*i.e.* with HO-6′ unsubstituted) in yields of 55, 78, 90, and 71%, respectively, and, in the latter case, a small proportion of benzyl 2,2′,3,3′,6,6′-hexa-*O*-benzyl-β-lactoside.[278] The influence of the aglycone bonded to a D-galactopyranosyl residue on the direction of benzylidene ring-cleavage was discussed.

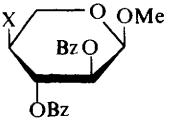
Treatment of methyl 2-*O*-benzoyl-3,4-*O*-benzylidene-β-D-arabinopyranoside with triphenylmethylium tetrafluoroborate in acetonitrile gave an equilibrium mixture of the benzoxonium ions (96) and (97), which reacted with chloride or iodide ions to give (98) and (99) in the ratio of 6:1 (X = Cl) or 1:2 (X = I), signifying differences in the reactivities of (96) and (97) towards different halide ions.[279] The



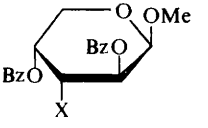
(96)



(97)



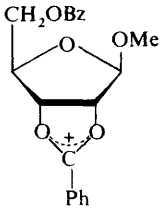
(98) X = Cl or I



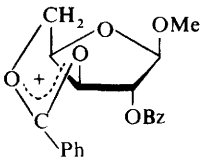
(99) X = Cl or I

[276] D. M. Clode, *Canad. J. Chem.*, 1977, **55**, 4071.
[277] P. Rollin and P. Sinaÿ, *Compt. rend.*, 1977, **284**, *C*, 65.
[278] A. Lipták, I. Jodál, and P. Nánási, *Carbohydrate Res.*, 1976, **52**, 17.
[279] S. Jacobsen, B. Nielsen, and C. Pedersen, *Acta Chem. Scand.*, 1977, **B31**, 359.

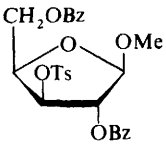
benzoxonium ions (96) and (97) also underwent *trans* opening with bromide, thiocyanate, toluene-*p*-sulphonate, and trifluoroacetate ions, whereas orthoacid derivatives were formed with fluoride, acetate, and cyanide ions, methanol, and acetamide. The orthoacid derivatives gave *cis*-hydroxybenzoates on hydrolysis. Methyl 5-*O*-benzoyl-2,3-*O*-benzylidene-β-D-ribofuranoside reacted with triphenylmethylium tetrafluoroborate in acetonitrile to give a mixture of the benzoxonium ions (100) and (101), in which the former preponderated.[280] The benzoxonium ions were opened with toluene-*p*-sulphonate ions to yield (102) and (103), whereas they gave orthoacid derivatives with acetate and azide ions.



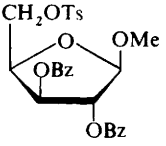
(100)



(101)



(102)



(103)

Oxidation of 4,6-*O*-alkylidene-D-galactopyranoses and -glucopyranoses in methanol with silver carbonate on Celite furnished 2,4-*O*-alkylidene-D-threoses and -erythroses, respectively.[105] However, oxidation of 4,6-*O*-alkylidene-D-galactoses in DMF–benzene with this reagent gave mainly 3,5-*O*-alkylidene-D-lyxo(furano)ses and, in some instances (*e.g.* with 4,6-*O*-ethylidene-D-galactopyranose), small proportions of 4,6-*O*-alkylidene-D-galactono-1,5-lactones, whereas similar oxidation of 4,6-*O*-alkylidene-D-glucopyranoses gave mainly 4,6-*O*-alkylidene-D-glucono-1,5-lactones with little or no degradation to 3,5-*O*-alkylidene-D-arabinoses. The causes of these differences, particularly conformational effects and the relative reactivities of the α- and β-anomers of the 4,6-*O*-alkylidene-D-hexopyranoses to oxidation in DMF–benzene, were discussed.

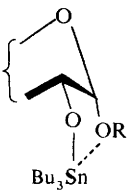
[280] S. Jacobsen and C. Pedersen, *Acta Chem. Scand.*, 1977, **B31**, 365.

6
Esters

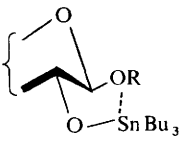
Carboxylic Esters

Chloroacetyl groups (see Vol. 4, p. 38) have been used as temporary protecting groups in the synthesis of a number of D-xylose derivatives, including 1,2,3-tri-*O*-acetyl-β-D-xylopyranose and 1,2-di-*O*-acetyl-α- and -β-D-xylofuranose.[281] The hydroxy-groups of nucleosides have been protected as crotonic and other substituted acrylic esters, which can be readily removed with hydrazine under mild conditions.[282] The claim that acetylation of D-ribose with hot acetic anhydride in the presence of barium carbonate gives only 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose has been refuted, since ^{13}C n.m.r. spectroscopy has shown D-ribo-furanose and -pyranose tetra-acetates are formed.[283]

As trialkylstannyl alkoxides form a co-ordination bond between a neighbouring oxygen atom and the tin atom under favourable conditions, trialkylstannylation of polyhydroxy-compounds with a limited amount of bis(tributylstannyl) oxide would be expected to occur regioselectively, allowing regioselective acylation with acid chlorides through enhancement of the nucleophilicity of the hydroxy-groups by trialkylstannylation.[284] This approach has been used in the regioselective benzoylation of methyl α-D-glucopyranoside to give methyl 2,6-di-*O*-benzoyl-α-D-glucopyranoside in a yield of 82%, and of methyl α-D-mannopyranoside and methyl β-D-galactopyranoside to give high yields of the respective 3,6-dibenzoates. Under identical reaction conditions, methyl α-D-galactopyranoside gave methyl 6-*O*-, 2,6- and 3,6-di-*O*-, and 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside in yields of 21, 10, 20, and 41%, respectively. The results suggest that formation of the trialkylstannyl alkoxide of type (104) is thermodynamically favoured over that of type (105). When this approach was used with sucrose, αα-trehalose, and cyclohexa-



(104)



(105)

[281] N. Héran, J.-P. Utille, and P. J. A. Vottero, *Carbohydrate Res.*, 1977, **53**, 268.
[282] R. Arentzen and C. B. Reese, *J.C.S. Chem. Comm.*, 1977, 270.
[283] S. C. Sharma and B. M. Lynch, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 347.
[284] T. Ogawa and M. Matsui, *Carbohydrate Res.*, 1977, **56**, C1.

amylose, 1'2,3,6,6'-penta-*O*-benzoylsucrose, 2,2',3,3',6,6'-hexa-*O*-benzoyl-αα-trehalose, and hexa(2-*O*-benzoyl)cyclohexa-amylose were obtained. Partial benzoylation of methyl 4,6-*O*-benzylidene-α- and -β-D-glucopyranoside and 1,5-anhydro-4,6-*O*-benzylidene-D-glucitol using benzoic anhydride and sodium benzoate in hot chloroform has been shown to give mainly the 2-benzoate (70%), a mixture of the 2- and 3-benzoates (26 and 29%, respectively), and the 2-benzoate (87%), respectively.[284a] Details of the partial acetylation of 1,6-anhydro-β-D-galactopyranose using acetic anhydride in pyridine have been reported[285] (see Vol. 9, p. 40). Of the two axial hydroxy-groups of methyl 3,6-anhydro-α-D-glucopyranoside, that at C-4 appears to be more reactive towards acylation.[264] Benzyl 2,2',3,3',6-penta-*O*-benzoyl-β-cellobioside has been prepared from cellobiose, *via* a 4',6'-*O*-benzylidene derivative, and converted into the 4',6'-dimesylate, which was used to synthesize 6'- and 4'-amino- and 4',6'-diamino-analogues of lactose.[286] Details of the selective benzoylation of methyl β-lactoside (Vol. 8, p. 45) have appeared,[287] while selective benzoylation of benzyl β-lactoside gave the heptabenzoate and a mixture (44%) of the 2,2',3',4',6,6'- and 2,2',3,3',6,6'-hexabenzoates.[288] Acetylation of L-fucose with acetic anhydride in pyridine at 0 °C gave an 86:14-mixture of the α- and β-pyranose tetra-acetates, whereas L-fucofuranose tetra-acetates were synthesized for the first time by acetolysis of methyl 2,3,5-tri-*O*-acetyl-β-L-fucofuranoside.[289] Improved preparations of 1,2,3,5-tetra-*O*-acetyl- and -benzoyl-αβ-D-xylofuranoses (from 1,2:3,5-di-*O*-isopropylidene-α-D-xylofuranose)[290] and of sucrose monoesters (*via* metal chelates)[250] have been reported.

The nature of the damage caused by high-energy radiation on some furoic and thenoic esters of the methyl D-glucopyranosides has been determined.[291] 5-Bromo-2-furoyl groups almost completely protected the methyl D-glucopyranosides from radiative degradation, while radiation-induced cleavage of the glycosidic bond and dehydrogenation reactions were less pronounced with the other esters than with methyl α-D-glucopyranoside. The stability of these fully esterified glycosides to radiation may arise from intramolecular transfer of energy to the ester groups, followed by dissipation of the energy as heat or light. The stereoselective synthesis of 1-*O*-acyl-α- and -β-D-glucopyranoses, *via* metallation of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose, has been discussed (see Vol. 10, p. 39), and the stability and products of acyl migration of 1-*O*-acyl-α-D-glucopyranoses have been examined.[292] Several 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl esters have been prepared by reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with abscisic, gibberellic, cumaric, indole-3-acetic, and salicylic acids, *etc.*[293] Syntheses of 1-*O*-[5-fluoro-2-

[284a] Y. Kondo, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 2089.

[285] M. E. Chacon Fuertes and M. Martin Lomas, *Anales de Quim.*, 1976, **72**, 964 (*Chem. Abs.*, 1977, **87**, 168 301*u*).

[286] R. G. Edwards, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1977, **55**, 129.

[287] R. S. Bhatt, L. Hough, and A. C. Richardson, *J.C.S. Perkin I*, 1977, 2001.

[288] I. E. Valashek, M. K. Shakhova, B. M. Zolotarev, and G. I. Samokhvalov, *Zhur. obshchei Khim.*, 1977, **47**, 460 (*Chem. Abs.*, 1977, **87**, 6279*r*).

[289] H. S. Prihar, J.-H. Tsai, S. R. Wanamaker, S. J. Duber, and E. J. Behrman, *Carbohydrate Res.*, 1977, **56**, 315.

[290] A Holý, *Coll. Czech. Chem. Comm.*, 1977, **42**, 902.

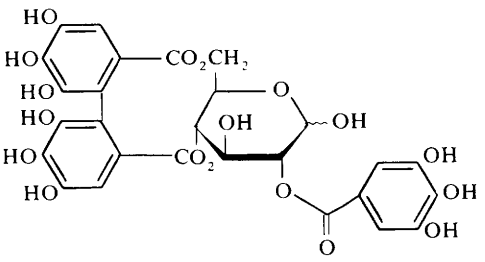
[291] J. A. Harris, J. C. Arthur, jun., O. Hinojosa, G. J. Boudreaux, and E. R. McCall, *Carbohydrate Res.*, 1977, **53**, 57.

[292] P. E. Pfeffer, G. G. Moore, P. D. Hoagland, and E. S. Rothman, *Amer. Chem. Soc. Symposium Ser.*, 1976, No. 39, Synthetic Methods Carbohydrate Symposium, 155 (*Chem. Abs.*, 1977, **86**, 190 367*w*).

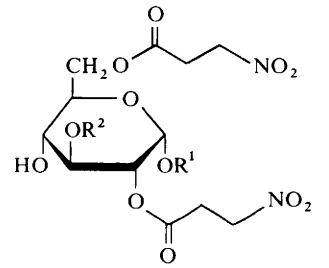
[293] H. Lehmann and H. R. Schuette, *J. prakt. Chem.*, 1977, **319**, 117 (*Chem. Abs.*, 1977, **86**, 171 779*r*).

methyl-1-(4-methylsulphinylbenzylidene)inden-3-yl]acetyl-β-D-glucopyranosyluronic acid[294] and of 1-*O*-alkanoyl-α-D-glucopyranoses, by reaction of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose with an appropriate fatty-acid derivative and hydrogenolysis of the product,[295] have been reported. Monoesters of sucrose possessing pesticidal activity have been obtained in yields of 41—72% by transesterification reactions with various chlorinated methyl phenoxyacetates in DMF.[295a] A solid-phase synthesis of methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside (86% yield) has been achieved by coupling methyl α-D-glucopyranoside to a polymer containing pendant chlorodiphenyl groups, benzoylation of the coupled glycoside, and acid treatment.[296]

Green coffee beans contain a D-glucopyranosyl ester (cofaryloside) which chemical and spectroscopic evidence identified as 1-*O*-(9β,16α,17-trihydroxykauran-18-oyl)-β-D-glucopyranose,[297] and 2′,3-di-*O*-decanoyl-2-*O*-octanoyl-6-*O*-succinoyl-αα-trehalose has been isolated from the lipids of *Mycobacterium paraffinicum*.[298] Hippomanin A, a toxic principle from *Hippomane mancinella*, has



(106)



(107) $R^1 = H, R^2 = COCH_2CH_2NO_2$
(108) $R^1 = COCH_2CH_2NO_2, R^2 = H$
(109) $R^1 = R^2 = H$

structure (106),[299] and structures (107)—(109) have been assigned to corollin, coronillin, and coronarian, respectively, from the crownvetch *Coronilla varia*, which is toxic to non-ruminants because of the presence of 3-nitropropionic acid.[300]

1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose reacted with dichlorocarbene to yield the 6-*O*-formyl derivative, whereas diacetals having unsubstituted secondary hydroxy-groups gave chloro-sugars[301] (see Chapter 7).

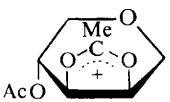
The preparation of methyl 4,6-*O*-benzylidene-3-*O*-selenobenzoyl-α-D-glucopyranoside has been reported by Barton's group in work concerned with the general synthesis of *O*-alkyl selenoesters by condensation of an alcohol with a di-*N*-substituted imidoyl chloride, using sodium hydrogenselenide to introduce selenium

[294] H. Jones, R. Bugianesi, and T. Y. Shen, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 369.
[295] Y. Nishikawa and K. Yoshimoto, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 624.
[295a] J. Arct and Z. Eckstein, *Roczniki Chem.*, 1977, **51**, 39.
[296] J. M. J. Frechet, *Polymer Prep., Amer. Chem. Soc. Div. Polymer Chem.*, 1975, **16**, 255.
[297] H. Richter, H. Obermann, and G. Spiteller, *Chem. Ber.*, 1977, **110**, 1963.
[298] S. G. Batrakov, B. V. Rosynov, T. V. Koronelli, R. A. Kozhuhova, and L. D. Bergelson, *Bioorg. Khim.*, 1977, **3**, 55.
[299] K. V. Rao, *Lloydia*, 1977, **40**, 169 (*Chem. Abs.*, 1977, **87**, 102 547x).
[300] B. G. Moyer, P. E. Pfeffer, J. L. Moniot, M. Shamma, and D. L. Gustine, *Phytochemistry*, 1977, **16**, 375.
[301] P. C. Cesare and B. Gross, *Carbohydrate Res.*, 1977, **58**, C1.

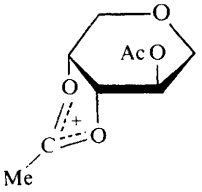
into the resulting salt.[302] The reduction of selenoesters with Raney nickel provides a new route to ethers that does not require the use of strongly basic conditions; thus, deselenization of methyl 4,6-*O*-benzylidene-3-*O*-selenobenzoyl-α-D-glucopyranoside afforded the corresponding benzyl ether in 65% yield. The use of tetrabutylammonium fluoride to catalyse the acylation of sterically hindered hydroxy-groups is referred to[303] in Chapter 19, while the synthesis of deoxy-sugars by photolysis of acetic and pivalic esters is noted[304] in Chapter 11.

Acyloxonium Ions and Orthoesters

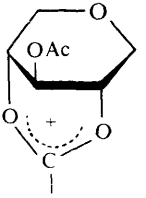
In continuing their studies on the rearrangements of acyloxonium ions in carbohydrate chemistry, Paulsen's group has examined the ions formed from 2,3,4-tri-*O*-acetyl-1,5-anhydro-D-arabinitol.[305] The acetoxonium ion (110) can undergo a 1,2-rearrangement to give the chiral ion (111) or a competitive 1,3-rearrangement



(110)

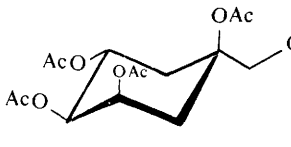


(111)

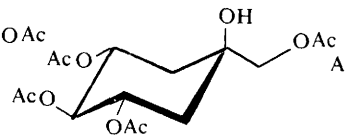


(112)

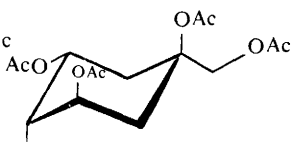
to give the *meso* ion (112). Complete racemization was observed with $CF_3SO_3^-$ or $SbCl_6^-$ as the counter anion, indicating the intervention of a 1,3-rearrangement, whereas racemization was much less extensive (12%) in liquid hydrogen fluoride. Similarly, treatment of (−)-quinicol penta-acetate (113) in dichloromethane with a large excess of antimony(v) chloride, followed by hydrolysis of the intermediate,



(113)



(114)

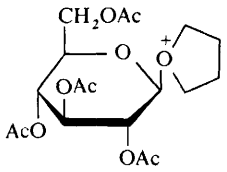


(115)

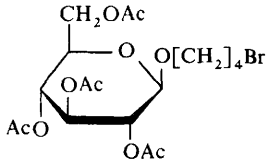
gave the *meso* tetra-acetate (114), whereas (113) gave some of the chiral penta-acetate (115) following treatment with anhydrous hydrogen fluoride, hydrolysis of the intermediate, and acetylation.[306] The work of Pedersen's group on dioxolanylium ions derived from methyl 2-*O*-benzoyl-3,4-*O*-benzylidene-β-D-arabinopyranoside[279] and methyl 5-*O*-benzoyl-2,3-*O*-benzylidene-β-D-ribofuranoside[280] is covered in Chapter 5.

[302] D. H. R. Barton, P.-E. Hansen, and K. Picker, *J.C.S. Perkin I*, 1977, 1723.
[303] S. L. Beaucage and K. K. Ogilvie, *Tetrahedron Letters*, 1977, 1691.
[304] P. M. Collins and V. R. Z. Munasinghe, *J.C.S. Chem. Comm.*, 1977, 927.
[305] H. Paulsen and O. Brauer, *Chem. Ber.*, 1977, **110**, 331.
[306] H. Paulsen and H. Höhne, *Carbohydrate Res.*, 1977, **58**, 484.

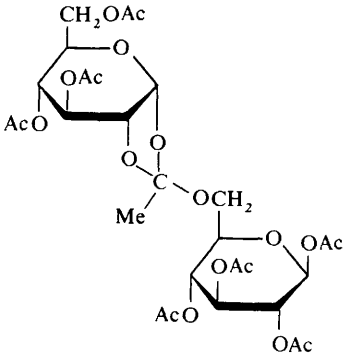
1,2-Orthoacetates have been obtained in good yields from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and alcohols (*e.g.* cholesterol) in THF in the presence of silver salicylate.[307] The presence of an intermediate oxonium ion (116), which loses THF in forming the acetoxonium ion intermediate, was inferred from



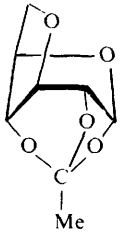
(116)



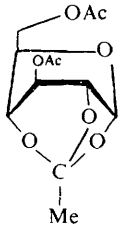
(117)



(118)



(119)

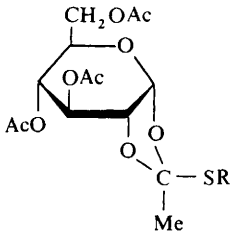


(120)

the isolation of 4-bromobutyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (117) as a by-product of the reaction and from semi-quantitative kinetic investigations. The 1,2-*O*-[1-(1,2,3,4-tetra-*O*-acetyl-6-deoxy-β-D-glucopyranos-6-yloxy)]ethylidene derivative (118) was obtained on fusion of the β-D-glucopyranosyl carbonates (27; R^1 = Ph or CH_2CCl_3, R^2 = Ac) with 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose at 160—170 °C, whereas related 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranosyl carbonates gave the expected disaccharide derivative.[308] The mercury(II) bromide-catalysed isomerization of the internal orthoesters (119) and (120) to the corresponding 2-*O*-acetyl-1,4-anhydropyranose has been studied.[309] Comb-like molecules having oligomeric side-chains have been prepared by condensation of peracetylated 1,2-(ethyl orthoacetate) derivatives of maltose, maltotetraose, and maltohexaose with 2,3-di-*O*-phenylcarbamoyl derivatives of amylose and cellulose.[310] Peracylated 1,2-(t-butyl orthoester) derivatives of mono- and di-saccharides gave the corresponding steroid or triterpenoid orthoesters by transesterification (*e.g.* with cholesterol) in the absence of a catalyst[310a] (see Vol. 9, p. 45).

[307] G. Wulff and W. Schmidt, *Carbohydrate Res.*, 1977, **53**, 33.
[308] Y. Ishido, S. Inaba, H. Komura, and A. Matsuno, *J.C.S. Chem. Comm.*, 1977, 90.
[309] A. F. Bochkov and Ya. V. Voznyi, *Zhur. org. Khim.*, 1977, **13**, 750 (*Chem. Abs.*, 1977, **87**, 53 504*v*).
[310] B. Pfannemüller, G. C. Richter, and E. Husemann, *Carbohydrate Res.*, 1977, **56**, 147.
[310a] N. I. Uvarova, N. F. Samoshina, G. I. Oshitok, and G. B. Elyakov, *Zhur. org. Khim.*, 1976, **12**, 1431.

Peracylated 1,2-thio-orthoesters derived from α-D-glucopyranose [*e.g.* (121)], methyl D-glucopyranuronate, and α-lactose gave the 1,2-(ethyl or isopropyl orthoester) derivatives when treated with Raney nickel in ethanol or propan-2-ol, respectively, although complex alcohols (*e.g.* t-butanol) failed to yield ortho-



(121) R = *p*-MeC_6H_4
(122) R = Et

esters.[311] However, the 1,2-thio-orthoacetate (122) reacted with 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose in the presence of mercury(II) acetate or bromide to give the 1,2-orthoacetate (118).[312] Such 1,2-thio-orthoacetates as (121) have been obtained from the reactions of peracetylated glycopyranosyl bromides with *p*-thiocresol in nitromethane in the presence of collidine.[313].

The use of 1,2-thio-orthoacetates as glycosylating agents[132] and the isomerization of (121), for example, into the 1,2-*trans*-1-thioglycopyranoside[212] are mentioned in Chapter 3.

Phosphates

A 9:1-mixture of the α- and β-pyranose 1-phosphates was formed on fusion of β-L-fucopyranose tetra-acetate with phosphoric acid at 50 °C for 1 min, whereas longer fusion times gave only the α-pyranose 1-phosphate.[289] Fusion of the L-fucofuranose tetra-acetates with phosphoric acid gave a mixture of L-fucofuranose 1-phosphates containing predominantly the β-anomer. Enzymic transformations have been used to synthesize α-D-[1-^{18}O]glucopyranose 1-phosphate and a corresponding [^{18}O]phosphate-labelled compound[314] and in the large-scale synthesis of D-glucose 6-phosphate from D-glucose.[315] Improved procedures for the preparation of 3′-deoxyadenosine (cordycepin) 5′-triphosphate from cordycepin have been reported.[316] 2-Deoxy-α-D-*arabino*-hexopyranose 1-phosphate and corresponding 3-, 4-, and 6-deoxy derivatives, each labelled with tritium in the deoxy group, have been prepared and their rates of acid hydrolysis have been determined.[317] The observed order of reactivity, *viz.* α-D-glucopyranose 1-phosphate <6-deoxy- <4-deoxy- <3-deoxy- <2-deoxy-α-D-glycopyranose 1-phosphate,

[311] G. Magnusson, *Carbohydrate Res.*, 1977, **56**, 188.
[312] N. K. Kochetkov, L. V. Bakinovskii, and Yu. E. Tsvetkov, *Doklady Akad. Nauk S.S.S.R.*, 1977, **235**, 589 (*Chem. Abs.*, 1977, **87**, 201 924z).
[313] G. Magnusson, *J. Org. Chem.*, 1976, **41**, 4110.
[314] F. C. Kokesh and Y. Kakuda, *Canad. J. Biochem.*, 1977, **55**, 548.
[315] A. Pollak, R. L. Baughn, and G. M. Whitesides, *J. Amer. Chem. Soc.*, 1977, **99**, 2366.
[316] M. Blandin, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 341.
[317] J. Zámocký, J. Zemek, Š. Kučár, and V. Soukupová, *Coll. Czech. Chem. Comm.*, 1977, **42**, 1379.

was interpreted in terms of electronic and steric (1,2-*gauche*) interactions in the ground state and in the activated complex through which the hydrolysis proceeds.

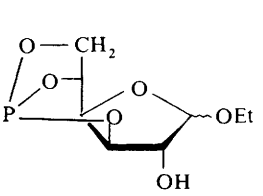
The release of orthophosphate was found to be the primary and dominant reaction on photolysis of α-D-glucopyranose 1-(dipotassium phosphate) at 254 nm in either oxygen-free or oxygen-saturated aqueous solutions.[318] The principal products formed in oxygen-free solution were deoxy-sugars and malondialdehyde, whereas carbon dioxide and monoxide, D-gluconate, D-arabinose, glyoxal, and glycolate were the main products formed in oxygenated solution. The primary photochemical process may be the release of orthophosphate from the D-glucopyranose 1-phosphate dianion, producing a glycosyl radical that undergoes further degradation. The most abundant products formed on photolysis of D-fructose 6-phosphate under similar conditions were carbon monoxide and orthophosphate, together with 2-deoxy-D-*glycero*-tetrose 4-phosphate, 2,4-dihydroxycyclobutyl phosphate, and, in oxygenated solution, D-glyceraldehyde 3-phosphate.[319] These results can be explained by assuming that the free carbonyl group of the sugar is excited, yielding carbon monoxide in the primary process *via* a Norrish type I reaction. The resulting phosphorylated radical loses water to give 2-deoxy-D-*glycero*-tetrose 4-phosphate, which is further degraded by Norrish type II reactions. It appears, therefore, that the photochemical reactions of sugar phosphates in aqueous solution depend markedly on the structural form(s) of the solvated compound, a small proportion of the free carbonyl form being sufficient to give the classical photoreactions of this group.

γ-Radiolysis of aqueous solutions of D-glucitol 6-phosphate saturated with nitrous oxide or argon gave L-gulose (*via* radiation-induced dephosphorylation) and 1-deoxy-L-*xylo*-hexulose (by elimination of phosphoric acid).[320] γ-Radiolysis of solutions of D-glucose 6-phosphate and D-glucitol 6-phosphate in deuterium oxide afforded 6-deoxy-D-*xylo*-[6-^{2}H]hexos-5-ulose (with one atom of deuterium incorporated) together with D-*gluco*-hexadialdose, and 1-deoxy-L-*xylo*-[1-^{2}H]hexulose (with one atom of deuterium incorporated) together with L-gulose, respectively. Possible mechanisms for radiation-induced dephosphorylation of the 6-phosphates were discussed in the light of these results.

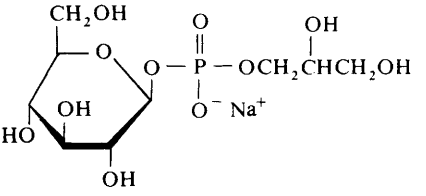
1,2-*O*-Isopropylidene-α-D-xylo- and -ribo-furanose each gave the two possible isomers of the corresponding cyclic 3,5-(phenyl phosphate) when treated with phenyl phosphorodichloridate, but only one isomer was formed on phosphorylation of the former compound with bis-(2,2,2-trichloroethyl) phosphorochloridate.[321] The synthesis of a new type of branched-chain sugar phosphate, *trans*-3-*C*-cyanomethylene-3-deoxy-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose 5-[bis-(2,2,2-trichloroethyl) phosphate], was also reported. 2,3,4,6-Tetra-*O*-acetyl(or benzoyl)-β-D-glucopyranose reacted with tris(dimethylamino)phosphine and carbon tetrachloride to give both anomeric oxyphosphonium chlorides (see Vol. 9, p. 48), which afforded 1,2-orthoesters with alcohols or a mixture of phenyl 1-thio-D-glucopyranosides with thiophenol.[322] Similar treatment of 2,3,4,6-tetra-

[318] M. Trachtman and M. Halmann, *J.C.S. Perkin II*, 1977, 132.
[319] C. Triantaphylides and R. Gerster, *J.C.S. Perkin II*, 1977, 1719.
[320] N. K. Kochetkov, L. I. Kudrjashov, M. A. Chlenov, and L. P. Grineva, *Carbohydrate Res.*, 1977, **53**, 109.
[321] J. M. J. Tronchet, J.-R. Neeser, and E. J. Charollais, *Helv. Chim. Acta*, 1977, **60**, 243.
[322] Y. Chapleur, B. Castro, and B. Gross, *Tetrahedron*, 1977, **33**, 1609.

O-acyl-β-D-mannopyranoses gave only the α-pyranose 1-(oxyphosphonium chlorides), which reacted with alcohols to give 1,2-orthoesters and with thiophenol to give mainly phenyl 2,3,4,6-tetra-*O*-acyl-1-thio-β-D-mannopyranosides.[323] The use of hexa-alkylphosphortriamides to prepare 3,5,6-phosphites (see Vol. 10, p. 41) has been extended to the alkyl hexofuranosides; for example, the anomers of the 3,5,6-phosphite (123) derived from ethyl αβ-D-glucofuranoside could be separated by chromatography on silica gel and then converted into the pure ethyl furanosides by methanolysis.[324] Methyl α-D-mannofuranoside and ethyl 1-thio-α-D-glucofuranoside also yielded 3,5,6-phosphites, whereas methyl α-D-galactofuranoside did not. The sodium salt of β-D-glucopyranosyl glycer-1-yl phosphate (124) has been



(123)



(124)

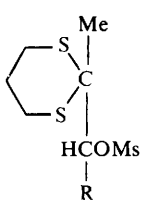
prepared by way of the orthoester procedure.[325] Phosphorylated hexoses (*e.g.* D-fructose 6-phosphate) and trioses (*e.g.* D-glyceraldehyde 3-phosphate) containing a free aldehydo- or keto-group have been shown to glycosylate haemoglobin A to the extent of 7—12% in a non-enzymic reaction, whereas no significant glycosylation of haemoglobin A occurred with D-glucose or D-fructose.[326] The preparation of a matrix from D-fructose 1,6-diphosphate and aminated agarose for the affinity chromatography of yeast aldolase is noted in Chapter 9.[327]

Reference is made in Chapter 2 to the use of n.m.r. spectroscopy to identify the tautomeric forms of ketose phosphates present in equilibrium mixtures,[52] while the formation of cyclic phosphites and related derivatives of D-mannitol[328] is dealt with in Chapter 17.

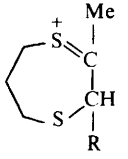
Sulphonates

Details have appeared on the behaviour of methyl 2,3-*O*-isopropylidene-4-*O*-toluene-*p*-sulphonyl-α-D-lyxo- and -β-L-ribo-pyranoside towards nucleophiles[329] (see Vol. 8, p. 53). The dithiepane derivatives (127) and (128) were formed *via* the intermediate (126), resulting from sulphur migration, when the mesylate (125) was treated with sodium azide in warm DMF[330] (see also Chapter 15). The reactions of

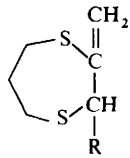
[323] Y. Chapleur, B. Castro, and B. Gross, *Tetrahedron*, 1977, **33**, 1615.
[324] E. Y. Nifant'ev, M. P. Koroteev, Z. K. Zhane, A. A. Borisenko, and N. K. Kochetkov, *Tetrahedron Letters*, 1977, 4125.
[325] L. V. Volkova, L. L. Danilov, V. L. Efimova, N. P. Danilova, and R. P. Evstigneeva, *Bioorg. Khim.*, 1977, **3**, 248 (*Chem. Abs.*, 1977, **86**, 190 365*u*).
[326] V. J. Stevens, H. Vlassara, A. Abati, and A. Cerami, *J. Biol. Chem.*, 1977, **252**, 2998.
[327] D. Lubini, M. J. Healy, and P. Christen, *Experientia*, 1977, **33**, 709.
[328] L. I. Gurarii, A. Kh. Plyamovatyi, R. P. Arshinova, E. T. Mukmenev, and B. A. Arbuzov, *Doklady Akad. Nauk S.S.S.R.*, 1977, **232**, 1304 (*Chem. Abs.*, 1977, **86**, 190 386*b*).
[329] J. S. Brimacombe, J. Minshall, and L. C. N. Tucker, *Carbohydrate Res.*, 1976, **52**, 31.
[330] A. Gateau-Olesker, A. M. Sepulchre, G. Vass, and S. D. Gero, *Tetrahedron*, 1977, **33**, 393.



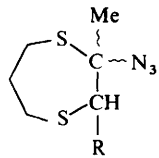
(125)



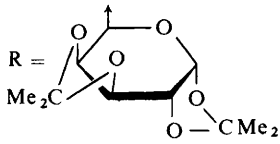
(126)



(127)



(128)



some 4,6-disulphonates of methyl 2,3-di-*O*-acyl(or methyl)-D-gluco- and -galacto-pyranosides with thiocyanate, thioacetate, and thiobenzoate anions have been studied under a variety of conditions.[331] In the D-glucopyranoside series, somewhat similar reactivity is shown by isomers differing only in anomeric configuration and there are only slight differences between the reactivities of a 2,3-dibenzoate and its 2,3-di-*O*-methyl analogue. In contrast to the situation in the β-D-galacto-pyranoside series, the presence of *O*-benzoyl groups in an α-D-galactopyranoside does not have an unfavourable effect on nucleophilic displacements at C-4. The reductions of sugar tosylates and mesylates with sodium hydrogen bis-(2-methoxy-ethoxy)aluminate occurred more rapidly than those with lithium aluminium hydride.[332] Secondary sulphonates gave the corresponding hydroxy derivatives on reduction, whereas primary sulphonates gave a mixture of hydroxy and deoxy derivatives by cleavage of the S—O or C—O bond, respectively; lithium aluminium hydride displayed a greater tendency to cleave a C—O bond than did sodium hydrogen bis-(2-methoxyethoxy)aluminate. Partial tosylation of 1,5-anhydro-D-glucitol gave the relative reactivity of the hydroxy-groups as HO-6 > HO-2 > HO-4 > HO-3.[333]

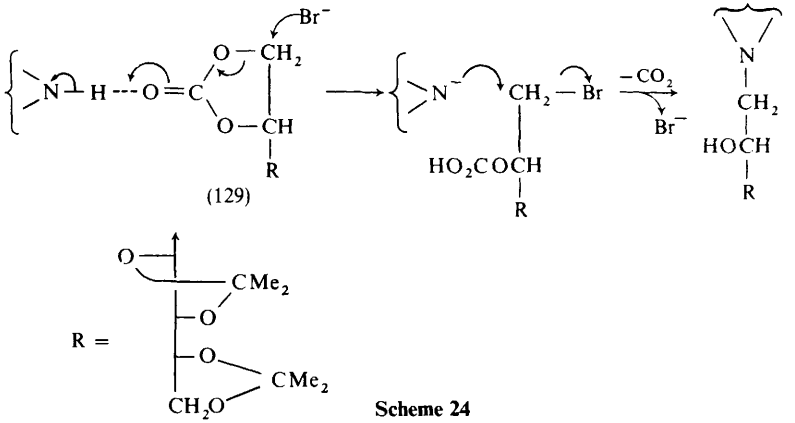
1′,6′,6″-Tri-*O*-mesitylenesulphonylraffinose has been obtained in a yield of 20% by treatment of raffinose with three molar equivalents of mesitylenesulphonyl chloride in pyridine,[254] while trimolar mesitylenesulphonylation of sucrose gave, without recourse to chromatography, 1′,6,6′-tri-*O*-mesitylenesulphonylsucrose in a yield of ⩾ 50%.[264] Monotosylation of suitably protected glycosides having two free hydroxy-groups (*e.g.* methyl 4,6-*O*-benzylidene-α-D-glucopyranoside) can be achieved using a phase-transfer procedure[334] (see Vol. 10, p. 25 for analogous monobenzylations). The HO-2 group of protected glycopyranosides is more reactive than HO-3 towards monotosylation, and the axial or equatorial disposition of the hydroxy-group appears to be relatively unimportant, as methyl 4,6-*O*-benzylidene-α-D-mannopyranoside afforded the 2-tosylate with the phase-transfer

[331] D. M. C. Hull, P. F. Orchard, and L. N. Owen, *Carbohydrate Res.*, 1977, **57**, 51.
[332] A. Zobáčová, V. Heřmánková, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1977, **42**, 2540.
[333] Y. Kondo, K. Yabuuchi, and S. Hirano, *Canad. J. Chem.*, 1977, **55**, 3820.
[334] P. J. Garegg, T. Iversen, and S. Oscarson, *Carbohydrate Res.*, 1977, **53**, C5.

procedure (*cf.* the formation of the 3-tosylate on partial tosylation with toluene-*p*-sulphonyl chloride in pyridine). Studies of the conversion of alkyl 4,6-*O*-benzylidene-2,3-di-*O*-sulphonylhexopyranosides into unsaturated sugar derivatives[335] and of the deoxygenation of sugar triflates[336] are dealt with in Chapters 12 and 11, respectively.

Other Ester Derivatives

Fusion of 5,6-*O*-carbonyl-1,2:3,4-di-*O*-isopropylidene-D-mannitol (129) with nitrogen heterocycles (*e.g.* pyrimidines, purines, and cyclic imines) in the presence of catalytic amounts of tetraethylammonium bromide gave the corresponding 1-



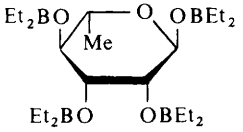
Scheme 24

deoxy-D-mannitol-1-yl derivatives by the pathway shown in Scheme 24.[337] 1,2,3,4-Tetra-*O*-acetyl-5,6-*O*-carbonyl-D-mannitol gave 2,3,5,6-tetra-*O*-acetyl-1,4-anhydro-D-mannitol under identical reaction conditions, *via* an intermediate 1,2,4-orthoacetate. The cyclic carbonate (129) reacted with benzoyl chloride and tetraethylammonium bromide to give 2-*O*-benzoyl-1-chloro-1-deoxy-3,4:5,6-di-*O*-isopropylidene-D-mannitol and with acid anhydrides to give 1,2-di-*O*-acyl derivatives of 3,4:5,6-di-*O*-isopropylidene-D-mannitol. Treatment of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose with dichlorophosphorylvinyl isocyanate ($Cl_2POCH{=}CHNCO$) furnished the corresponding 1-carbamate.[338] The conversions of sugar cyclic thiocarbonates[339] and dimethylthiocarbamates[340] into deoxysugars are reported in Chapter 11, while the formation of olefinic sugars from bis-(dithiocarbonates)[341] is dealt with in Chapter 12.

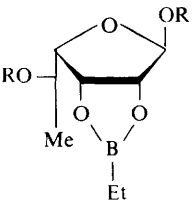
Diethylborylation of L-rhamnose with activated triethylborane gave the α-pyranose tetraboronate (130), which afforded a mixture of products, containing

[335] T. Yamazaki, K. Matsuda, M. Sugiyama, S. Seto, and N. Yamaoka, *J.C.S. Perkin I*, 1977, 1981.
[336] T. H. Haskell, P. W. K. Woo, and D. R. Watson, *J. Org. Chem.*, 1977, **42**, 1302.
[337] Y. Ishido, H. Komura, and T. Yoshino, *Carbohydrate Res.*, 1976, **52**, 49.
[338] I. S. Trokhimenko, *Zhur. obshchei Khim.*, 1977, **47**, 1039 (*Chem. Abs.*, 1977, **87**, 68 458*g*).
[339] D. H. R. Barton and R. Subramanian, *J.C.S. Perkin I*, 1977, 1718.
[340] R. H. Bell, D. Horton, D. M. Williams, and E. Winter-Mihaly, *Carbohydrate Res.*, 1977, **58**, 109.
[341] A. G. M. Barrett, D. H. R. Barton, R. Bielski, and S. W. McCombie, *J.C.S. Chem. Comm.*, 1977, 866.

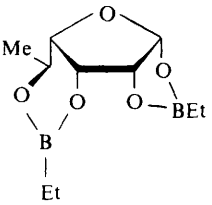
principally the ring-contracted derivative (131), following treatment with ethyldiborane and distillation.[342] The reaction of L-rhamnose with triethylboroxole gave, depending on the molar proportion of reagent used, either (132) or (133); acetylation of (132) and deborylation of the acetylated product afforded 1,5-di-*O*-acetyl-α-L-rhamnofuranose.



(130)



(131) R = BEt_2
(132) R = H



(133)

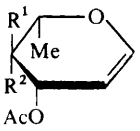
The use of mass spectrometry for identifying sulphated sugars is discussed[343] in Chapter 21, and the nitration of nucleosides and nucleotides with nitronium tetrafluoroborate is noted [344] in Chapter 19.

[342] W. V. Dahlhoff and R. Köster, *J. Org. Chem.*, 1977, **42**, 3151.
[343] A. I. Kalinovsky, N. I. Belogortseva, S. V. Moroz, A. F. Pavlenko, A. K. Dzizenko, and Y. S. Ovodov, *Carbohydrate Res.*, 1977, **58**, 473.
[344] G.-F. Huang and P. F. Torrence, *J. Org. Chem.*, 1977, **42**, 3821.

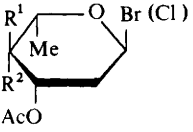
7
Halogenated Sugars

Glycosyl Halides

The stereochemistry of the addition of chlorine to 3,4-dihydro-2*H*-pyran has been found to be influenced by the polarity of the solvent in which the reaction is conducted.[345] Stereoselective *syn* addition occurred in non-polar solvents (*e.g.* n-pentane) to give a mixture of *cis*- and *trans*-2,3-dichlorotetrahydropyrans in the ratio of *ca.* 4:1, whereas the ratio was 1:2 in polar solvents (*e.g.* dichloromethane). *cis*-2,3-Dichlorotetrahydropyran yielded exclusively *trans*-products with various nucleophiles (N_3^-, MeO^-, PhS^-, and AcO^-) in DMF with inversion of configuration at C-2, whereas *trans*-2,3-dichlorotetrahydropyran yielded the *cis*-product with the thiophenate anion and a mixture of *cis*- and *trans*-products with the other anions. Hydrogen chloride and hydrogen bromide added to 3,4-di-*O*-acetyl-L-fucal (134) and -rhamnal (135) to give adducts (136) and (137), respectively, possessing the α-L configuration.[346]



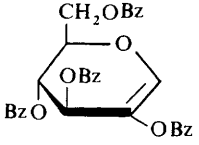
(134) $R^1 = H$, $R^2 = OAc$
(135) $R^1 = OAc$, $R^2 = H$



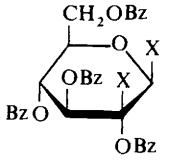
(136) $R^1 = H$, $R^2 = OAc$
(137) $R^1 = OAc$, $R^2 = H$

Further work on the chlorination and bromination of 1,5-anhydro-2,3,4,6-tetra-*O*-benzoyl-D-*arabino*-hex-1-enitol (138) has been reported.[347] Bromination of (138) in carbon tetrachloride yielded 2-bromohexopyranosyl bromide derivatives having the β-D-*gluco* (139; X = Br), α-D-*manno* (140; X = Br), and α-D-*gluco* configurations in the ratio of 6:3:1, while chlorination of (138) afforded only the *cis*-adducts (139; X = Cl) and (140; X = Cl). The stereochemistry at C-2 of the α-D-*manno*-dichloride (140; X = Cl) was established by *X*-ray crystallography and, like that of the analogous dibromide (140; X = Br), from chiroptical properties. The β-D-*gluco*-dibromide (139; X = Br) was hydrolysed to the α-D-hexopyranosulose tetrabenzoate (141) on contact with silica gel, whereas conversion of the α-D-*manno*-dibromide (140; X = Br) into the corresponding β-anomer (142) required silver nitrate in acetone.

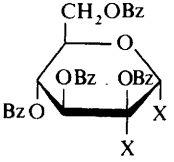
[345] T. E. Stone and G. D. Daves, *J. Org. Chem.*, 1977, **42**, 2151.
[346] H. S. El Khadem, D. L. Swartz, J. K. Nelson, and L. A. Berry, *Carbohydrate Res.*, 1977, **58**, 230.
[347] F. W. Lichtenthaler, T. Sakakibara, and E. Oeser, *Carbohydrate Res.*, 1977, **59**, 47.



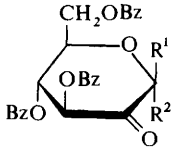
(138)



(139)

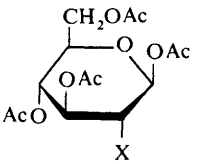


(140)



(141) R^1 = H, R^2 = OBz
(142) R^1 = OBz, R^2 = H

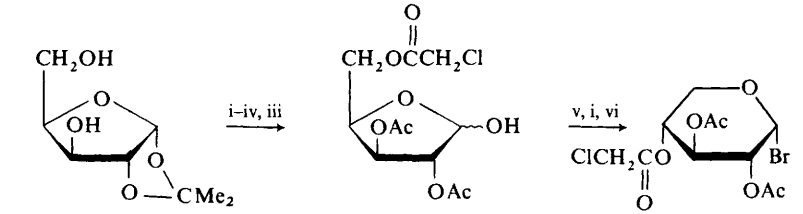
Methyl 1,2,3-tri-*O*-acetyl-4-*O*-methyl-β-D-glucopyranuronate gave a very good yield of the α-glycosyl chloride on treatment with dichloromethyl methyl ether in the presence of a catalytic amount of zinc chloride, whereas the β-glycosyl chloride was obtained in a reaction catalysed by boron trifluoride etherate.[348] The α-glycosyl chloride and bromide were obtained from methyl 4-*O*-methyl-1,2,3-tri-*O*-(4-nitrobenzoyl)-α-D-glucopyranuronate by treatment with dichloromethyl methyl ether in the presence of zinc chloride or with hydrogen bromide in acetic acid, respectively; these crystalline α-glycosyl halides are potential intermediates for the synthesis of oligosaccharides containing residues of 4-*O*-methyl-D-glucuronic acid. Boron trifluoride-catalysed reactions of dichloromethyl methyl ether with cellobiose β-octa-acetate and rutinose β-hepta-acetate have been used to prepare hepta-*O*-acetyl-β-cellobiosyl and hexa-*O*-acetyl-β-rutinosyl chlorides, respectively.[349] The view that these reactions proceed *via* a 1,2-acetoxonium ion intermediate is supported by the failure of the 2-trichloroacetate (143) and the 2-chloro derivative (144) to react with the reagent.



(143) X = $OCOCCl_3$
(144) X = Cl

2,3-Di-*O*-acetyl-4-*O*-chloroacetyl-α-D-xylopyranosyl bromide, a potential intermediate for the synthesis of oligosaccharides containing (1 → 4)-linked β-D-xylopyranosyl residues, has been obtained from 1,2-*O*-isopropylidene-α-D-xylofuranose by the reactions outlined in Scheme 25.[350]

[348] P. Kováč and R. Palovčik, *Carbohydrate Res.*, 1977, **56**, 399.
[349] I. Farkas, I. F. Szabó, L. Zolnay, and R. Bognár, *Carbohydrate Res.*, 1977, **58**, 478.
[350] J.-P. Utille and P. J. A. Vottero, *Carbohydrate Res.*, 1976, **52**, 241.



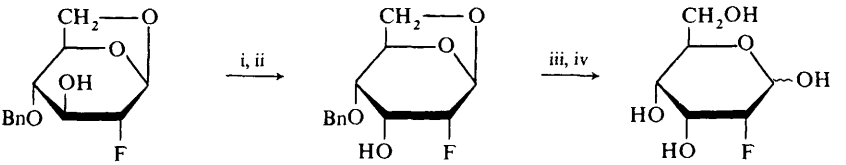
Reagents: i, $ClCH_2COCl$–*sym*-trimethylpyridine; ii, Ac_2O–py; iii, H^+; iv, AcCl–py; v, $(NH_2)_2CS$–H_2O; vi, HBr–AcOH

Scheme 25

D-Mannofuranosyl halide derivatives are referred to in the following section, while references to other glycosyl halides appear in Chapters 3, 10, 12, and 17.

Other Halogenated Derivatives

3,4,6-Tri-*O*-acetyl-D-glucal has been converted into 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-glucopyranosyl fluoride (35%) and the corresponding β-D-*manno*-difluoride (26%) by reaction with fluorine diluted with argon at −78 °C.[351] This method could be used to introduce a ^{18}F-label into 2-deoxy-2-fluoro-D-glucose, which is readily obtained by hydrolysis of the α-D-*gluco*-difluoride. The reactions shown in Scheme 26 have been used to synthesize 2-deoxy-2-fluoro-D-allose; a



Reagents: i, RuO_4; ii, $NaBH_4$; iii, H_2–Pd; iv, H_3O^+

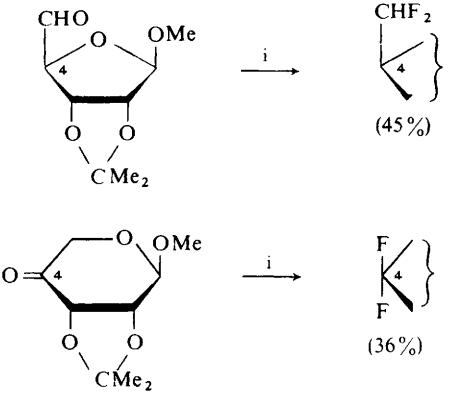
Scheme 26

related sequence of reactions on 1,6-anhydro-2,4-dideoxy-2,4-difluoro-β-D-glucopyranose gave 2,4-dideoxy-2,4-difluoro-D-allose.[352]

3-Deoxy-3-fluoro-D-glucose is toxic when injected into the adult locust species *Locusta migratoria* and *Schistocerca gregaria*.[353] 3-Deoxy-3-fluoro-D-glucitol was identified (by g.l.c.) as a metabolite of the fluorinated hexose in the haemolymph of the locusts. The growth of *E. coli* cells in the presence of D-glucose and lactose was inhibited by 4-deoxy-4-fluoro-D-glucose.[353a]

Diethylaminosulphur trifluoride can be used to introduce a CH_2F group into partially acetylated carbohydrate derivatives.[354] For example, 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose reacted with this fluorinating agent in diglyme at 60 °C to give the corresponding 6-deoxy-6-fluoro derivative in a yield of 80%. Diethylaminosulphur trifluoride also reacted with keto- and aldehydo-sugar

[351] T. Ido, C.-N. Wan, J. S. Fowler, and A. P. Wolf, *J. Org. Chem.*, 1977, **42**, 2341.
[352] J. Pacák, M. Braunová, D. Štropová, and M. Černý, *Coll. Czech. Chem. Comm.*, 1977, **42**, 120.
[353] A. Romaschin, N. F. Taylor, D. A. Smith, and D. Lopes, *Canad. J. Biochem.*, 1977, **55**, 369.
[353a] N. F. Taylor and L.-Y. Lonie, *Canad. J. Biochem.*, 1977, **55**, 911.
[354] M. Sharma and W. Korytnyk, *Tetrahedron Letters*, 1977, 573.

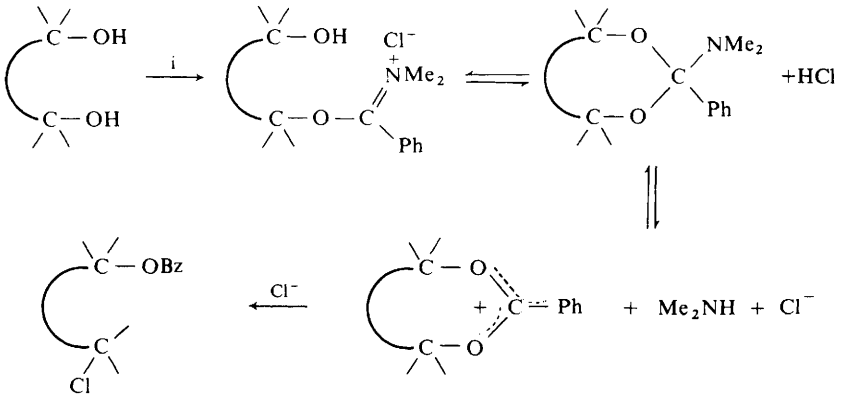


Reagent: i, Et_2NSF_3–PhH or CH_2Cl_2

Scheme 27

derivatives to give moderate yields of *gem*-difluorides (Scheme 27), although it decomposed 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose.[355]

Barton's group has described a simple preparation of chlorohydrin benzoates by reaction of a Vilsmeier salt with 1,2- and 1,3-diols.[356] Treatment of the diols at



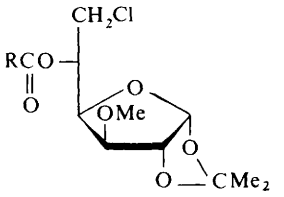
Reagent: i, $Me_2\overset{+}{N}=CPhCl$

Scheme 28

room temperature with the imidoyl chloride derived from *NN*-dimethylbenzamide afforded the corresponding chlorohydrin benzoates (Scheme 28) in excellent yields and regiospecifically; thus, 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose reacted with α-chlorobenzylidene-*NN*-dimethylammonium chloride to give 5-*O*-benzoyl-6-chloro-6-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose (145; R = Ph). A related salt, dichloromethylene-*NN*-dimethylammonium chloride

[355] R. A. Sharma, I. Kavai, Y. L. Fu, and M. Bobek, *Tetrahedron Letters*, 1977, 3433.
[356] T. G. Back, D. H. R. Barton, and B. L. Rao, *J.C.S. Perkin I*, 1977, 1715.

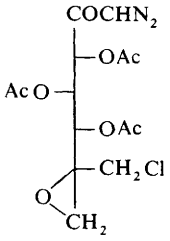
$[Me_2\overset{+}{N}{=}CCl_2Cl^-]$, reacted with 1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose in dichloromethane to give the 5-*O*-carbamoyl-6-chloro-6-deoxy derivative (145; R = NMe_2) in a yield of 85%, whereas 1,2-*O*-isopropylidene-α-D-glucofuranose gave 3,6-anhydro-5-*O*-(dimethylcarbamoyl)-1,2-*O*-isopropylidene-α-D-glucofuranose (72%) under identical conditions.[357] Under modified conditions, methyl α-D-gluco-, -galacto-, and -manno-pyranosides furnished the corresponding methyl 6-chloro-6-deoxy-4-*O*-(dimethylcarbamoyl)-α-D-hexopyranoside in yields of ⩽85%.



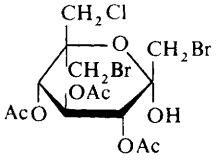
(145)

Dichlorocarbene (generated *in situ* from chloroform) reacted with 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose in a phase-transfer system to give the rearranged product 6-chloro-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose, whereas 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranosyl chloride was obtained from the reaction of dichlorocarbene with 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose.[301] The α-D-mannofuranosyl chloride derivative was used to prepare the corresponding methyl and benzyl α-glycofuranosides and in the synthesis of α-linked disaccharide derivatives.

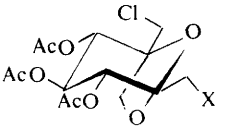
'1',4,6,6'-Tetrachloro-*galacto*-sucrose' and 1',6'-dichloro-1',6'-dideoxysucrose are intensely sweet, whereas 6,6'-dichloro-6,6'-dideoxysucrose is not as sweet as sucrose, suggesting that the substituent at C-1' is important in determining the sweetness of these sucrose derivatives.[358] Treatment of benzyl 2,2',3,3',6-penta-*O*-benzoyl-β-cellobioside with sulphuryl chloride initially gave the 6'-chloro derivative, which was slowly transformed into a mixture of the 4',6'-dichlorolactoside and an isomeric 3',6'-dichloro derivative, namely benzyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,4-di-*O*-benzoyl-3,6-dichloro-3,6-dideoxy-β-D-gulopyranosyl)-β-D-glucopyranoside, that probably arose by neighbouring-group participation by the 3'-benzoyloxy-group prior to the introduction of the 3'-chloro substituent.[286]



(146)



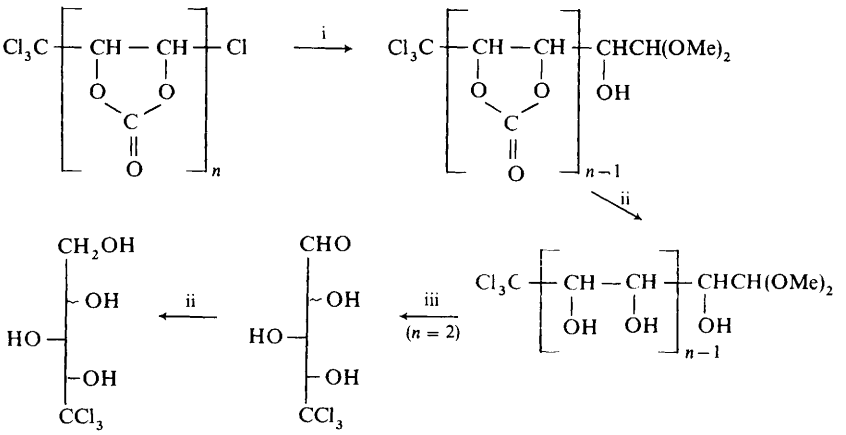
(147)



(148) X = Br
(149) X = N_3

[357] A. Klemer, R. Lemmes, and G. Nicolaus, *Annalen*, 1977, 177.
[358] L. Hough and S. P. Phadnis, *Nature*, 1976, **263**, 800.

2,3,4-Tri-*O*-acetylxylaryl dichloride reacted with diazomethane to give the expected bis(diazoketone) and a by-product having structure (146), which yielded the heptopyranulose derivative (147) on treatment with hydrogen bromide.[359] A mixture of the 2,7-anhydro derivatives (148) and (149) was obtained when (147) reacted with sodium azide in acetone.

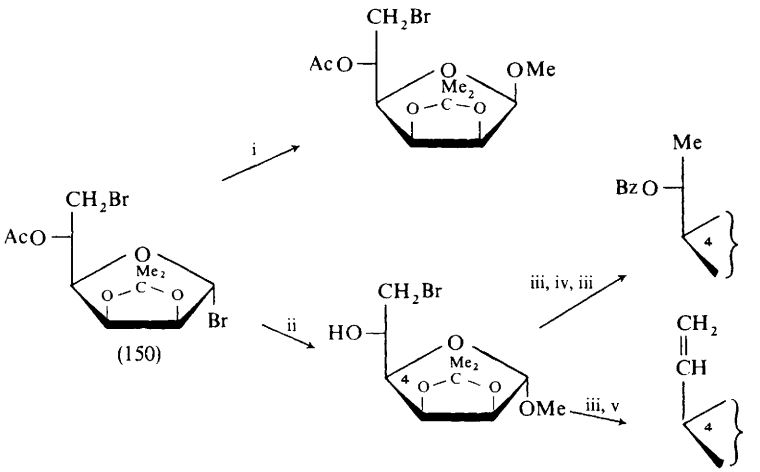


Reagents: i, MeOH; ii, $NaBH_4$; iii, H_3O^+

Scheme 29

Racemic xylose and lyxose derivatives having a terminal trichloromethyl group have been obtained by the reactions shown in Scheme 29 [360] (see also Vol. 10, p. 6).

Treatment of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose with hydrogen



Reagents: i, MeOH–Ag_2CO_3; ii, MeOH; iii, BzCl–py; iv, $LiAlH_4$; v, Zn–AcOH

Scheme 30

[359] G. J. Abruscato, D. E. Kiely, W. J. Cook, and C. E. Bugg, *J. Org. Chem.*, 1977, **42**, 3567.

[360] T. Matsuura, T. Kunieda, and T. Takizawa, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 239.

bromide in acetic acid gave the 1,6-dibromide (150), which was used to prepare methyl 5-*O*-acetyl-6-bromo-6-deoxy-2,3-*O*-isopropylidene-β-D-mannofuranoside, a 5,6-unsaturated sugar, and some D-rhamnofuranose derivatives (Scheme 30).[361]

References to other halogenated sugar derivatives will be found in Chapters 12, 15, and 19.

[361] K. Bock and C. Pedersen, *Acta Chem. Scand.*, 1977, **B31**, 248.

8
Amino-sugars

Natural Products

2-Amino-2-deoxy-L-altruronic acid, hitherto unknown in Nature, has been identified as a constituent of the *Shigella sonnei* phase I lipopolysaccharide,[362] while an hydrolysate of the cell walls of *Halococcus* sp. strain 24, an extremely halophilic bacterium, has been shown to contain a 2-amino-2,6-dideoxyhexose-6-sulphonic acid.[363] Among the amino-sugars separated and identified from acid hydrolysates of the lipopolysaccharide antigen of *Pseudomonas aeruginosa* immunotype 4 were 2-amino-2-deoxy-D-galactose, 2-amino-2,6-dideoxygalactose as a 2:1-mixture of the D and L enantiomers, and 2-amino-2,6-dideoxy-D-glucose,[364] whereas the constituent amino-sugars of the lipopolysaccharide antigen from *P. aeruginosa* N.C.T.C. 8505 include 2-amino-2-deoxy-D-galactose and -glucose, 2-amino-2-deoxy-L-galacturonic acid, and 2,4-diamino-2,4,6-trideoxy-D-glucose (D-bacillosamine).[365] Muramic acid 6-phosphate and 2-amino-2-deoxyglucose 6-phosphate have been isolated from the lysozyme-resistant cell walls of *Micrococcus lysodeikticus*.[366] 4-*O*-(2-Amino-2-deoxy-α-D-glucopyranosyl)-L-idopyranuronic acid and 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-glucopyranuronic acid have been isolated from an acid hydrolysate of purified porcine heparin.[367] The ratio of the yields of the disaccharides was approximately 3:1, confirming that L-iduronic acid is the principal hexuronic acid in heparin. A simple procedure that involved extraction with hot water, ultrafiltration, and ion-exchange chromatography has been reported for the isolation of gram quantities of *N*-acetylneuraminic acid from edible bird's nests.[368]

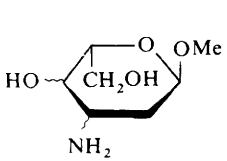
The occurrence of 2-amino-2-deoxy-D-ribose and 2-amino-2-deoxy-D-xylose in a nucleoside antibiotic and seldomycin, respectively, is noted in Chapter 18, while a synthesis of 2-amino-2-deoxy-D-ribose is reported in Chapter 19.

Synthesis

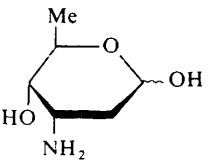
Azide displacements on sugar sulphonates and halides continue to be used extensively in the synthesis of amino-sugars. Such displacements have been used in the synthesis of derivatives of the methyl 3-amino-2,3-dideoxy-α-L-hexopyranosides

[362] T. Kontrohr, *Carbohydrate Res.*, 1977, **58**, 498.
[363] R. Reistad, *Carbohydrate Res.*, 1977, **54**, 308.
[364] D. Horton, G. Rodemeyer, and R. Rodemeyer, *Carbohydrate Res.*, 1977, **56**, 129.
[365] S. G. Wilkinson, *Biochem. J.*, 1977, **161**, 103.
[366] Nasir-ud-Din, M. Tomoda, and R. W. Jeanloz, *Carbohydrate Res.*, 1977, **57**, C1.
[367] M. Kosakai and Z. Yosizawa, *Carbohydrate Res.*, 1977, **58**, 153.
[368] J. E. Martin, S. W. Tanenbaum, and M. Flashner, *Carbohydrate Res.*, 1977, **56**, 423.

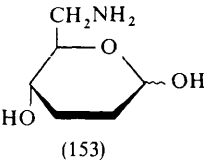
(151) having the *lyxo*, *ribo*, *xylo*, and *arabino* configurations, which are analogues of daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose), the carbohydrate component of daunorubicin;[369] in a new total synthesis of streptolidine (a derivative of 2,3,5-triamino-2,3,5-trideoxy-D-arabinonic acid, see Vol. 9, p. 203) from D-xylose, *via* methyl 2,3,5-tri-*O*-methanesulphonyl-αβ-D-xylofuranoside;[370] in two closely related syntheses of D-ristosamine (3-amino-2,3,6-trideoxy-D-*ribo*-hexose) (152) from methyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-methanesulphonyl-β-D-*arabino*-hexopyranoside;[371,371a] and in syntheses of 6-amino-2,3,6-trideoxy-D-*erythro*-hexose (153) (from methyl 4,6-*O*-benzylidene-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside),[372] the sucrose 6,6′-di- and 1′,6,6′-tri-(3-methyl-3-nitrosoureides) (154),



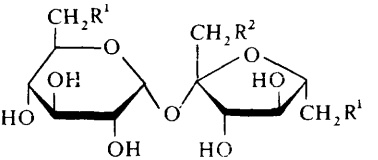
(151)



(152)



(153)

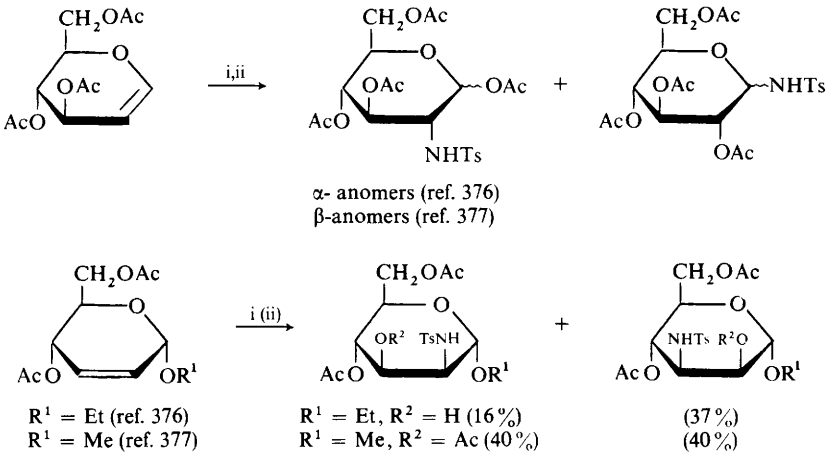


(154) R^1 = NHCONMe, R^2 = R^1 or OH
|
NO

which displayed antitumour activity,[373] and 6-amino-6-deoxy-1,2:3,5-di-*O*-isopropylidene-α-D-glucofuranose, which was converted into 6-bromoacetamido-6-deoxy-, 6-deoxy-6-isothiocyanato-, and 6-(4-azido-2-nitroanilino)-6-deoxy-D-glucose, which are potential substrate-directed alkylating agents for the protein involved in the transport of D-glucose across human-erythrocyte membranes.[374] 2-(4-Azido-2-nitroanilino)-2-deoxy-D-glucose has been synthesized and used as a site-directed probe in labelling adipocyte plasma membranes.[375] The synthesis of aminodeoxy derivatives of lactose is reported[286] in Chapter 6, while those of derivatives of a 4-amino-4-deoxyheptose and 6-amino-3,6-dideoxy-α-D-*arabino*-hexose are mentioned in Chapters 18 and 11, respectively.

Two groups have described the oxyamination of unsaturated sugar derivatives

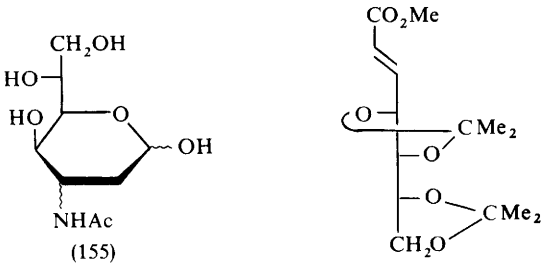
[369] A. Bargiotti, G. Cassinelli, G. Franchi, B. Gioia, E. Lazzari, S. Redaelli, A. Vigevani, F. Arcamone, and S. Hanessian, *Carbohydrate Res.*, 1977, **58**, 353.
[370] S. Kusumoto, S. Tsuji, K. Shima, and T. Shiba, *Bull. Chem. Soc. Japan*, 1976, **49**, 3611.
[371] I. P. Lyvás, F. Sztaricskai, R. Bognár, and G. Bujtás, *Carbohydrate Res.*, 1977, **53**, C17.
[371a] H. H. Baer and F. F. Z. Georges, *Carbohydrate Res.*, 1977, **55**, 253.
[372] A. Canas-Rodriguez, A. M. Tobed, A. G. Sanchez, and C. M. Madero, *Carbohydrate Res.*, 1977, **56**, 289.
[373] R. G. Almquist and E. J. Reist, *J. Medicin. Chem.*, 1977, **20**, 1246.
[374] M. Ramjeesingh and A. Kahlenberg, *Canad. J. Chem.*, 1977, **55**, 3717.
[375] T. Trosper and D. Levy, *J. Biol. Chem.*, 1977, **252**, 181.



Reagents: i, TsN^-ClNa^+ (chloramine T)–OsO_4; ii, Ac_2O–py

Scheme 31

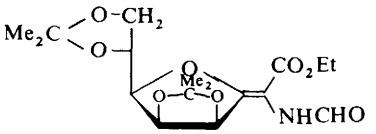
with chloramine T and osmium tetraoxide to give amino-sugar and glycosylamine derivatives (Scheme 31), although there is disagreement over the anomeric configurations of the products finally obtained from 3,4,6-tri-*O*-acetyl-D-glucal.[376,377] This procedure offers a short route to 3-amino-3-deoxy-D-mannose. 3-Acetamido-2,3-dideoxy-D-*gluco*- and -*manno*-heptoses (155) have been prepared by addition of ammonia to the D-*arabino*-hept-2-enonic acid derivative (156) and transformation



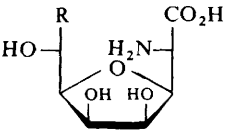
of the resulting 3-amino-2,3-dideoxy-4,5:6,7-di-*O*-isopropylidene-D-heptonamides into the 3-acetamido-2,3-dideoxy-6,7-*O*-isopropylidene-1,4-lactones, prior to reduction with di-isobutylaluminium hydride, *etc.*[378] Formylaminomethylenation of 2,3:5,6-di-*O*-isopropylidene-D-mannono-1,4-lactone with ethyl isocyanoacetate gave mainly the (*E*)-isomer (157), which was transformed by hydrogenation and hydrolysis with acid into L-(2-β-D-mannofuranosyl)glycine (158) or by straight-

[376] I. Dyong, Q. Lam-Chi, G. Schulte, B. Fraser-Reid, and J. L. Primeau, *Angew. Chem. Internat. Edn.*, 1977, **16**, 553.
[377] K. Heyns and J. Feldmann, *Tetrahedron Letters*, 1977, 2789.
[378] I. Dyong and W. Hohenbrink, *Chem. Ber.*, 1977, **110**, 3655.

forward degradative procedures into L-(2-β-D-lyxofuranosyl)glycine (159).[379] Base-catalysed equilibration of the hydrogenation product of (157), namely ethyl 3,6-anhydro-2-deoxy-2-formylamino-4,5:7,8-di-*O*-isopropylidene-D-*erythro*-L-*gluco*-octonate, gave a mixture of the L-*allo*-, L-*altro*-, L-*gluco*-, and L-*manno*-epimers, whose configurations at C-2 and C-3 were established by chemical and physical methods.

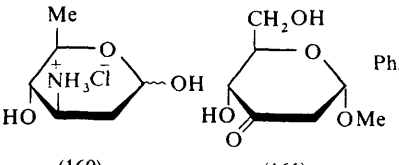


(157)



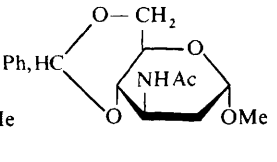
(158) R = CH_2OH
(159) R = H

3-Amino-2,3,6-trideoxy-D-*arabino*-hexose (D-acosamine) hydrochloride (160) and its *N*-trifluoroacetyl derivative have been synthesized by straightforward routes from the keto-sugar (161), *via* methyl 3-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-*arabino*-hexopyranoside (162),[380] and from methyl 2,3,6-trideoxy-3-nitro-α-D-*erythro*-hex-2-enopyranoside (163).[381] *NN*-Dimethylation of the α-glycoside of (160) and hydrolysis of the product with acid was used to obtain D-angolosamine (2,3,6-trideoxy-3-dimethylamino-D-*arabino*-hexose).[381] The addition of nitroalkanols (*e.g.* 2-nitroethanol) to acrylaldehyde(acrolein) in the presence

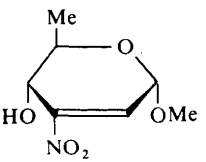


(160)

(161)

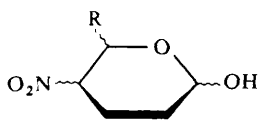


(162)

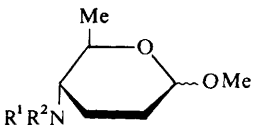


(163)

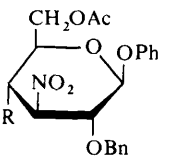
of diethylamine–formic acid afforded mixtures of 2,3,4-trideoxy-4-nitro-DL-aldopyranoses (164), which were converted into the corresponding methyl 2,3,4-trideoxy-4-nitro-DL-aldopyranosides and, with (164; R = Me), subsequently into methyl α- and β-DL-forosaminides (165) and methyl *N*-benzoyl-α- and -β-DL-tolyposaminides (166).[382]



(164) R = H, Me, or Et



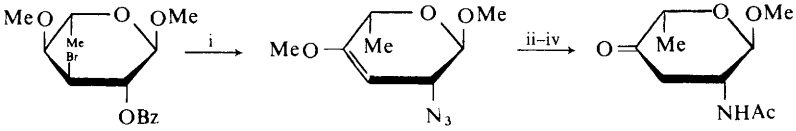
(165) $R^1 = R^2 = Me$
(166) R^1 = H, R^2 = Bz



(167) R = OAc or NHBn

[379] R. H. Hall, K. Bischofberger, S. J. Eitelman, and A. Jordaan, *J.C.S. Perkin I*, 1977, 743.
[380] D. Horton, J. R. Sorenson, and W. Weckerle, *Carbohydrate Res.*, 1977, **58**, 125.
[381] H. H. Baer and F. F. Z. Georges, *Canad. J. Chem.*, 1977, **55**, 1100.
[382] E. Kaji, H. Kohno, and S. Zen, *Bull. Chem. Soc. Japan*, 1977, **50**, 928.

Treatment of the diacetate (167; R = OAc) with benzylamine in THF at 5 °C gave, by elimination–addition, phenyl 6-*O*-acetyl-2-*O*-benzyl-4-benzylamino-3,4-dideoxy-3-nitro-β-D-glucopyranoside (167; R = NHBn) in a yield of 77%.[383] Methyl 2-*O*-benzoyl-3-bromo-3,6-dideoxy-4-*O*-methyl-α-L-altropyranoside (168) gave the unsaturated azido-sugar derivative (169) and its C-2 epimer in the ratio of 5:1 on heating with sodium azide in HMPT at 140 °C (Scheme 32).[384] Catalytic hydrogenation of (169), *N*-acetylation, and mild acidic hydrolysis then gave the potentially useful intermediate methyl 2-acetamido-2,3,6-trideoxy-α-L-*threo*-hexopyranosid-4-ulose (170).

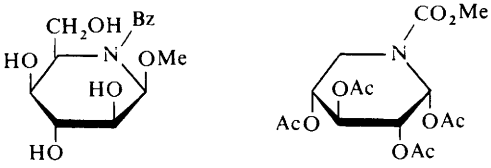


(168) (169) (170)

Reagents: i, NaN_3–HMPT at 140 °C; ii, H_2–Pd; iii, Ac_2O–MeOH; iv, H_3O^+

Scheme 32

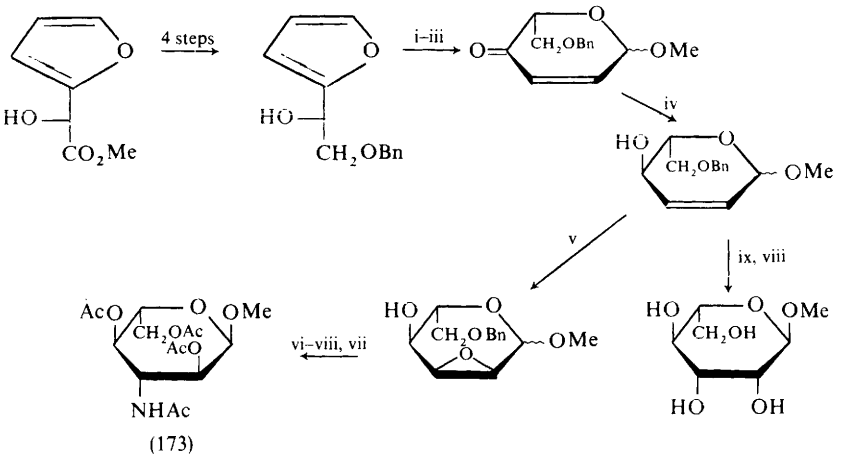
D-Xylose labelled with ^{14}C at all the carbon atoms has been converted into 2-amino-2-deoxy-D-[2—6-^{14}C]gulose hydrochloride either *via* the nitro-olefin route[385] or by using ammonia and hydrogen cyanide, *etc.*, which, after chromatography of the products on cellulose, also gave 2-amino-2-deoxy-D-[2—6-^{14}C]-idose hydrochloride.[385a] D-Galactose, D-mannose, and D-glucose reacted with benzylamine and hydrogen cyanide to give 2-benzylamino-2-deoxyheptononitriles having the D-*glycero*-L-*gluco*, D-*glycero*-D-*galacto*, and D-*glycero*-D-*ido* configurations, respectively.[386] The use of pyridine derivatives in the total synthesis of 5-amino-5-deoxypyranoses, which was illustrated by the preparation of derivatives of 5-amino-5-deoxy-DL-glucopyranose (DL-norjirimycin) in last year's report (Vol. 10, p. 59), has been extended to the synthesis of the derivatives (171) and (172) of 5-amino-5-deoxy-DL-idopyranose[387] and -xylopyranose,[388] respectively. The work



(171) (172)

[383] T. Takamoto, T. Sakakibara, M. Matsumoto, and R. Sudoh, *Carbohydrate Res.*, 1977, **53**, 129.
[384] J.-C. Florent, C. Monneret, and Q. Khuong-Huu, *Carbohydrate Res.*, 1977, **56**, 301.
[385] J. S. Rodia and K. K. De, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 351.
[385a] K. K. De and J. S. Rodia, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 359.
[386] J. A. Galbis Perez, J. I. Fernandez Garcia-Hierro, and P. Areces Bravo, *Anales de Quim.*, 1976, **72**, 820 (*Chem. Abs.*, 1977, **87**, 152 489*a*).
[387] M. Natsume and M. Wada, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 2657.
[388] M. Natsume and M. Wada, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 2651.

of Achmatowicz's group shown in Scheme 1 (p. 6) has also been extended to include the synthesis of the derivatives (173) and (174) of 3-amino-3-deoxy-α-L-glucopyranose and α-L-mannopyranose, respectively (Scheme 33).[17]

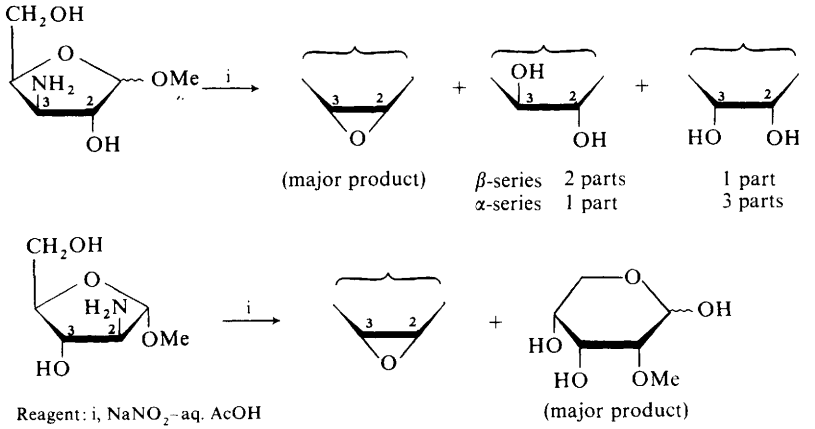


(173)

Reagents: i, Br_2–MeOH; ii, H_3O^+; iii, $HC(OMe)_3$; iv, $NaBH_4$; v, *m*-$ClC_6H_4CO_3H$; vi, NH_3; vii, Ac_2O–py; viii, H_2–Pd; ix, H_2O_2–OsO_4

Scheme 33

The products obtained on deamination of methyl 3-amino-3-deoxy-α- and -β-D-xylofuranoside and methyl 2-amino-2-deoxy-α-D-arabinofuranoside are given in Scheme 34; the formation of these products could be correlated with the expected ground-state conformation of each diazonium ion intermediate.[389]



Reagent: i, $NaNO_2$–aq. AcOH

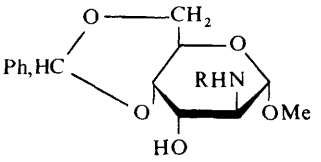
(major product)

Scheme 34

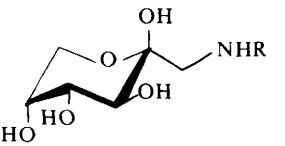
[389] J. G. Buchanan and D. R. Clark, *Carbohydrate Res.*, 1977, **57**, 85.

The principal product from deamination of the aminoarabinoside is 2-*O*-methyl-D-ribopyranose, which arises from 1,2-migration of the glycosidic methoxy-group facilitated by electron-release from the oxygen atom of the furanose ring.

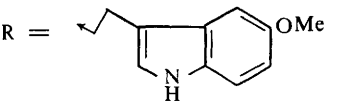
5-Methoxytryptamine reacted with methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside to give the aminoaltroside derivative (175)[390] and with D-glucose to give (176), the product of an Amadori rearrangement.[391,392] Analogues of (176) of biological interest have also been obtained from the reactions of aldoses with catecholamines.[392]



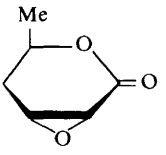
(175)



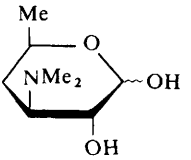
(176)



The racemic anhydro-sugar (177) (obtained by epoxidation of parasorbic acid) has been converted into DL-desosamine (178) by ring-opening with aqueous dimethylamine, lactonization of the resulting 3-dimethylaminohexonic acid, and reduction of the lactone obtained with di-isobutylaluminium hydride.[393]



(177)



(178)

In a study of the reactions of sugars with amino-acids to give 1-amino-1-deoxyketose derivatives, malic acid was found to be the most efficient catalyst and propane-1,2-diol the best solvent.[394]

Modifications to amino-sugars have included the conversion of 2-acetamido-2-deoxy-D-glucopyranose derivatives into 2-amino-2,6-dideoxy-D-glucose[364,395] and of 2-amino-2-deoxy-D(or -L)-glucose into 2-amino-2,3-dideoxy-D(or -L)-*ribo*-hexose by a route involving $O \rightarrow N$ migration of an acetyl group (Scheme 35).[396]

[390] O. V. Lukin, M. M. Vigdorchik, K. F. Turchin, and N. N. Suvorov, *Zhur. org. Khim.*, 1977, **13**, 1091 (*Chem. Abs.*, 1977, **87**, 85 186*j*).

[391] R. P. Turchina, M. M. Vigdorchik, K. F. Turchin, and N. N. Suvorov, *Zhur. org. Khim.*, 1977, **13**, 1316 (*Chem. Abs.*, 1977, **87**, 201 200*j*).

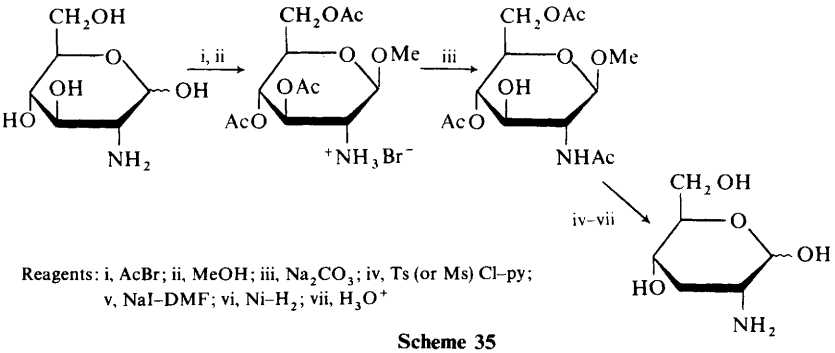
[392] L. Mester and M. Mester, *Amer. Chem. Soc. Symposium Ser.*, 1976, No. 39 (Synthetic Methods Carbohydrates Symposium), 240 (*Chem. Abs.*, 1977, **87**, 68 551*g*).

[393] K. Torssell and M. P. Tyagi, *Acta Chem. Scand.*, 1977, **B31**, 7.

[394] W. Ciusa, G. Barbiroli, and P. Mazzaracchio, *Rass. Chim.*, 1976, **28**, 60 (*Chem. Abs.*, 1977, **86**, 5716*y*).

[395] V. V. Kolesnikov, M. L. Shul'man, and A. Ya. Khorlin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 2331 (*Chem. Abs.*, 1977, **86**, 90 172*k*).

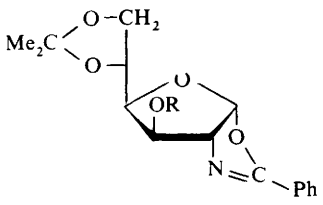
[396] T. Yamasaki, V. Kubota, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1976, **49**, 3190.



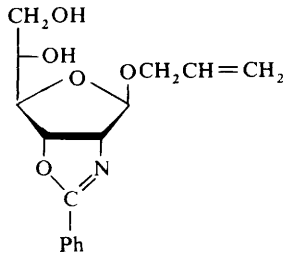
Reagents: i, AcBr; ii, MeOH; iii, Na_2CO_3; iv, Ts (or Ms) Cl–py; v, NaI–DMF; vi, Ni–H_2; vii, H_3O^+

Scheme 35

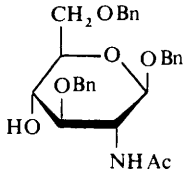
The α-D-glucofuranoso-oxazoline derivative (179; R = H) has been transformed into the L-*talo*-diol (180) and its D-*allo*-isomer, which are potential intermediates for the synthesis of sphingosine, while the benzylated derivative (179; R = Bn) has been used to prepare benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (181), an intermediate required for the synthesis of the immunological



(179) R = H or Bn

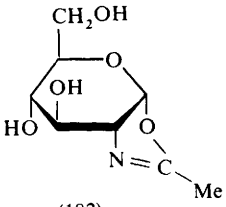


(180)

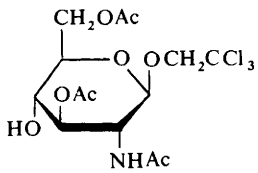


(181)

determinant of the P′-antigen.[397] During the course of this work, the conversion of benzamido- into acetamido-groups was accomplished, without loss of allyl ether and *O*-benzylidene protecting groups, with acetic anhydride–acetic acid containing sodium acetate at reflux. The 3,6- and 4,6-di- and 3,4,6-tri-*O*-benzylated derivatives of the α-D-glucopyranoso-oxazoline (182) have been prepared as intermediates for use in the synthesis of oligosaccharides.[398] The reactivity of these benzylated oxa-



(182)



(183)

[397] R. Gigg and R. Conant, *J.C.S. Perkin I*, 1977, 2006.
[398] P. Rollin and P. Sinaÿ, *J.C.S. Perkin I*, 1977, 2513.

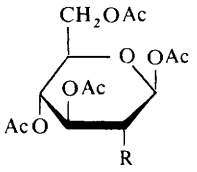
zolines was tested by the preparation of various derivatives of 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactopyranose. The 3,4,6-tri-*O*-acetyl derivative of the oxazoline (182) has been converted into 2,2,2-trichloroethyl 2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranoside (183), a potential intermediate for the synthesis of blood-group antigens.[399] The prop-1-enyl glycosides of 3,4,6-tri-substituted 2-acetamido-2-deoxy-β-D-glucopyranoses can be transformed efficiently into the corresponding oxazoline by treatment with mercury(II) chloride and oxide in refluxing acetonitrile.[400] The synthesis and reactions of a disaccharide oxazoline are reported[157] in Chapter 3.

Syntheses of disaccharides containing amino-sugar residues, of aminoglycoside antibiotics, and of branched-chain amino-sugars are dealt with in Chapters 3, 18, and 13, respectively.

Reactions

Dipeptide and aminoacyl derivatives of 4-nitrophenyl 2-amino-2-deoxy-β-D-glucopyranoside have been prepared, but only the *N*-benzoyl- and *N*-benzyloxycarbonyl-glycyl derivatives were cleaved at a significant rate by the β-D-2-acetamido-2-deoxyhexosidase from the mushroom *Hohenbuechelia serotina*.[401] The synthesis of hexosaminyl derivatives of L-serine is mentioned[121] in Chapter 3.

Several peracetylated 2-deoxy-2-thioureido-β-D-glucopyranoses have been prepared by treating 2-deoxy-2-isothiocyanato-β-D-glucopyranose tetra-acetate with primary or secondary amines.[402] Reaction of 2-amino-2-deoxy-β-D-glucopyranose tetra-acetate (184) with triphenylphosphine and carbon tetrachloride in dichloromethane (the Appel reaction) gave the triphenylphosphonioamino salt (185), which yielded the phosphinimine (186) on treatment with triethylamine and the *N*-methylaminotriphenylphosphonium iodide (187) on further treatment of (186) with



(184) R = NH_2
(185) R = $\overset{+}{N}HPPh_3\ Cl^-$
(186) R = $N{=}PPh_3$
(187) R = $\overset{+}{N}(Me)PPh_3\ I^-$

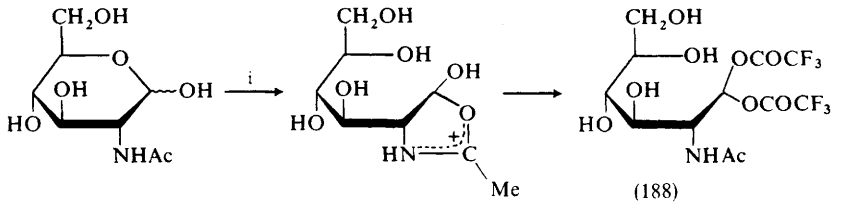
methyl iodide.[403] Analogous triphenylphosphonioamine and phosphinimine derivatives were prepared from 6-amino- and 6-azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose by the Appel or Staudinger reaction, respectively. When 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine was treated under the conditions of the Appel reaction, the principal product was di-(D-glucopyranosyl)amine octa-acetate.[403,404]

[399] J. M. McLaren, R. V. Stick, and S. Webb, *Austral. J. Chem.*, 1977, **30**, 2689.
[400] M. A. Nashed, C. W. Slife, M. Kiso, and L. Anderson, *Carbohydrate Res.*, 1977, **58**, C13.
[401] M. G. Vafina, S. A. Govor, and N. V. Molodtsov, *Carbohydrate Res.*, 1977, **56**, 183.
[402] J. Fuentes Mota and M. Gonzalez Perez, *Anales de Quim.*, 1976, **72**, 996 (*Chem. Abs.*, 1977, **87**, 201 959*q*).
[403] I. Pintér, J. Kovács, and A. Messmer, *Carbohydrate Res.*, 1977, **53**, 117.
[404] I. Pintér, J. Kovács, and A. Messmer, *Magyar Kém. Folyóirat*, 1977, **83**, 231 (*Chem. Abs.*, 1977, **87**, 136 185*t*).

The anomeric methyl 2-amino-2-deoxy-D-glucofuranosides have been isolated from the products of a Fischer glycosidation of 2-amino-2-deoxy-D-glucose.[405] The derived methyl 2-acetamido-2-deoxy-D-glucofuranosides were hydrolysed at closely similar rates, which were also similar to those for the methyl D-glucofuranosides and roughly double those for the methyl D-glucopyranosides, whereas the presence of a free amino-group inhibited the hydrolysis of the methyl 2-amino-2-deoxy-D-glucofuranosides. The rates of hydrolysis of the aminodeoxy- and acetamidodeoxy-glucofuranosides were greater in deuterium oxide than in water and this, together with the markedly negative entropies of activation, suggests that these compounds are hydrolysed by mechanisms similar to those suggested for the hydrolysis of aldofuranosides.

The formation of 2-furaldehyde on acid-catalysed dehydration, *etc.*, of 1-benzylamino-1-deoxy-D-*threo*-pentulose and 1-benzylamino-1-deoxy-D-fructuronic acid has been studied in isotopically labelled water (either acidified deuterium oxide or tritiated water).[406] The pattern of labelling of the 2-furaldehyde derived from the latter compound is consistent with a mechanism involving the decarboxylation of a βγ-unsaturated carboxylic acid intermediate (see also p. 136).

^{1}H N.m.r. spectroscopy has revealed that 2-acetamido-2-deoxyhexoses react with trifluoroacetic acid to give acyclic bis(esters) [*e.g.* (188)], which were unstable and rapidly reverted to the parent 2-acetamido-2-deoxyhexose in water.[407] The reaction sequence proposed to account for the formation of the bis(esters) is shown in Scheme 36. Both ^{13}C and ^{1}H n.m.r. spectroscopy have been used to confirm the



(188)

Reagent: i, CF_3CO_2H

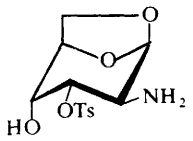
Scheme 36

mechanism suggested (B. Coxon and L. Hough, *J. Chem. Soc.*, 1961, 1577) for the epimerization of 2-acetamido-2-deoxyaldoses in alkali.[408] In agreement with the proposed mechanism, selective deuteriation occurred at C-2 of both 2-acetamido-2-deoxy-D-mannose and -glucose when epimerization of the latter sugar was conducted with sodium deuteroxide in deuterium oxide.[408,409]

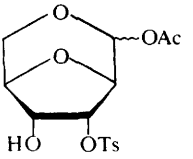
Deamination of the conformationally rigid 2-amino-1,6-anhydro-2-deoxy-3-*O*-toluene-*p*-sulphonyl-β-D-altropyranose (189) with sodium nitrite in acetic acid gave

[405] D. M. L. Morgan and A. Neuberger, *Carbohydrate Res.*, 1977, **53**, 167.
[406] K. B. Hicks and M. S. Feather, *Carbohydrate Res.*, 1977, **54**, 209.
[407] M. Ranganathan, V. S. R. Rao, and P. Balaram, *Carbohydrate Res.*, 1977, **58**, 245.
[408] M. Hanchak and W. Korytnyk, *Carbohydrate Res.*, 1976, **52**, 219.
[409] L. Szilagyi, P. Herczegh, and G. Bujtas, *Z. Naturforsch.*, 1977, **32b**, 296 (*Chem. Abs.*, 1977, **87**, 136 170*j*).

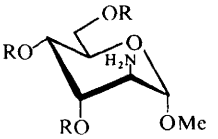
the 2,5-anhydro-D-alloseptanose derivative (190), *via* participation of O-5 in the decomposition of the intermediate diazonium ion, whereas deamination of the conformationally mobile aminoaltrosides (191) yielded only small amounts of ring-contracted products.[410]



(189)



(190)



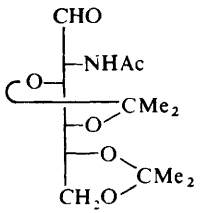
(191) R = H or Bz

Photolysis of 2-(benzyloxycarbonyl)amino-2-deoxy-D-glucopyranose and its α-glycoside in aqueous methanol at 254 nm and 55 °C gave 2-amino-2-deoxy-D-glucose and methyl 2-amino-2-deoxy-α-D-glucopyranoside, respectively.[411] However, photochemical cleavage of more-complex molecules containing three or more (benzyloxycarbonyl)amino-groups was incomplete, leading to mixtures.

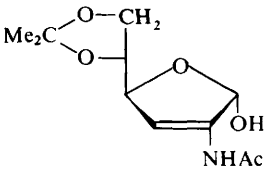
γ-Radiolysis of 2-acetamido-2-deoxy-D-glucose in oxygenated or deoxygenated aqueous solutions yielded acetamidodeoxy derivatives of pent-2-ulose, hexos-4-ulose, hexodialdose, and D-gluconic acid, and scission of carbon–carbon bonds appeared to be more important in oxygenated solution.[412]

Brief oxidation with 0.025 M-sodium metaperiodate at pH 7, followed by estimation with the resorcinol or thiobarbituric acid reagent, has been used in the simultaneous assay of free and ketosidically bonded sialic acids, respectively.[413]

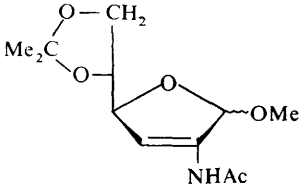
Treatment of 2-acetamido-2-deoxy-3,4:5,6-di-*O*-isopropylidene-*aldehydo*-D-glucose (192) or the analogous D-mannose derivative with alkali gave 2-acetamido-2,3-dideoxy-5,6-*O*-isopropylidene-α-D-*erythro*-hex-2-enofuranose (193), which afforded the methyl 2-enofuranosides (194) with acidified methanol–acetone; (194)



(192)



(193)



(194)

was also obtained from Chromogen I (the principal product of the first phase of the Morgan–Elson reaction) under identical conditions.[414]

The reactions of an epimino derivative of lincosamine with sulphides and the

[410] C.-D. Chang and T. L. Hullar, *Carbohydrate Res.*, 1977, **54**, 217.
[411] S. Hanessian and R. Masse, *Carbohydrate Res.*, 1977, **54**, 142.
[412] A. G. W. Bradbury and C. von Sonntag, *Z. Naturforsch.*, 1976, **31b**, 1274 (*Chem. Abs.*, 1977, **86**, 30020z).
[413] C. F. A. Culling, P. E. Reid, C. W. Ramey, W. L. Dunn, and M. G. Clay, *Canad. J. Biochem.*, 1977, **55**, 778.
[414] J.-M. Beau, P. Rollin, and P. Sinaÿ, *Carbohydrate Res.*, 1977, **53**, 187.

glycosidation of 2-deoxy-2-phthalimido-D-glucopyranosyl halides are referred to in Chapters 10 and 3, respectively.

Physical Measurements

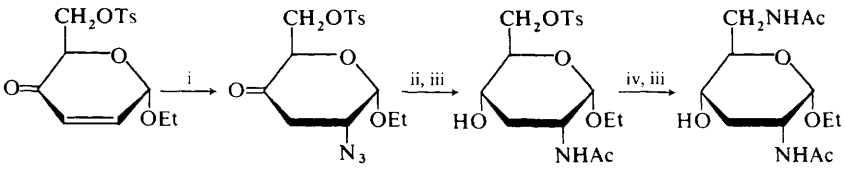
A conformational analysis has been carried out on 2-acetamidopropane-1,3-diol, a model system for the 3-acetamido-3-deoxyhexopyranoses.[415] It was suggested that the acetamido-group may adopt a pyramidal, rather than a planar, conformation, depending on the severity of its interactions with other groups. This gives two possible chiral arrangements that are not equally populated in sugars.

Natural-abundance ^{15}N and ^{13}C n.m.r. spectra and high-resolution ^{1}H n.m.r. spectra of amino-sugar derivatives are discussed in Chapter 20, and the c.d. spectra and analysis of amino-sugar derivatives are referred to in Chapter 21.

Diamino-sugars

The occurrence of 2,3-diamino-2,3-dideoxy-4-*O*-methyl-D-xylopyranose and 2,6-diamino-2,6-dideoxy-D-glucopyranose as constituents of the seldomycin antibiotic complex is noted in Chapter 18.

The derivative (196) of 2,6-diamino-2,3,6-trideoxy-D-*ribo*-hexopyranose, a component of the antibiotic tobramycin, has been prepared by way of addition of the elements of hydrazoic acid to an enone derivative, as shown in Scheme 37.[416]



(195)
+ C-2 isomer

(196)

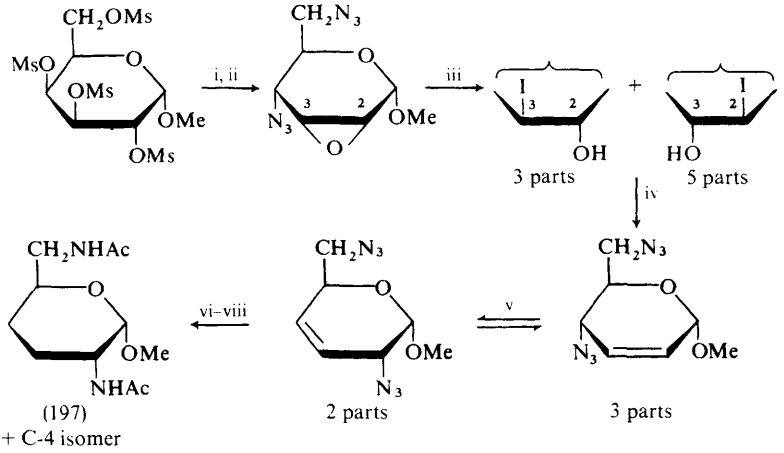
Reagents: i, NaN_3–AcOH; ii, $NaBH_4$; iii, H_2–Pd–C–Ac_2O; iv, NaN_3–DMF

Scheme 37

The D-*erythro*-azide (195) and its C-2 isomer were present in the resulting equilibrium mixture in the ratio of 3:2. Methyl di-*N*-acetyl-α-purpurosaminide C (197) has been synthesized from methyl 2,3,4,6-tetra-*O*-methanesulphonyl-α-D-galactopyranoside by the sequence of reactions outlined in Scheme 38.[417] A related series of reactions was used to obtain the *epi*-purpurosamine C derivative (198) from methyl 2,3,4,6-tetra-*O*-methanesulphonyl-α-D-glucopyranoside.

The ditosylate (199) reacted with ethanolic methylamine at 120°C to yield not only the required bis(methylamino)-sugar but also the azetidine derivative (200), which was also obtained when the 6-methylamino-4-tosyl derivative (201) was heated in ethanol.[263] Cleavage of the azetidine derivative (200) with acetic anhydride at 100°C furnished the (*N*-methylacetamido)galactopyranoside derivative (202).

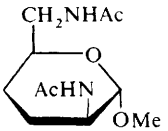
[415] S. Bystrický, I. Tvaroška, and K. Bláha, *Coll. Czech. Chem. Comm.*, 1977, **42**, 1002.
[416] J. Leboul, J. Cleophax, S. D. Gero, A. Rolland, and A. Forchioni, *Tetrahedron*, 1977, **33**, 965.
[417] J. Cleophax, A. Olesker, A. Rolland, S. D. Gero, and A. Forchioni, *Tetrahedron*, 1977, **33**, 1303.



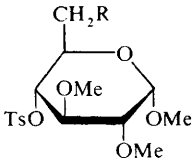
Reagents: i, NaN_3; ii, MeONa–MeOH; iii, NaI–AcONa; iv, $POCl_3$–py; v, heat; vi, Ni–NH_2NH_2; vii, Ac_2O; viii, H_2–Pt

Scheme 38

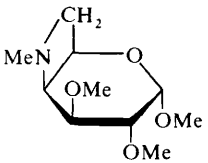
Details have appeared on the synthesis of prumycin (203) and related compounds from benzyl 2-(benzyloxycarbonyl)amino-2-deoxy-5,6-*O*-isopropylidene-β-D-glucofuranoside.[418]



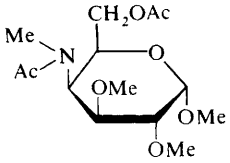
(198)



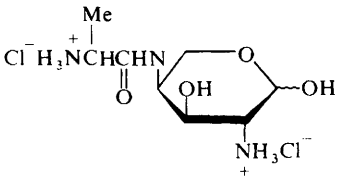
(199) R = OTs
(201) R = NHMe



(200)



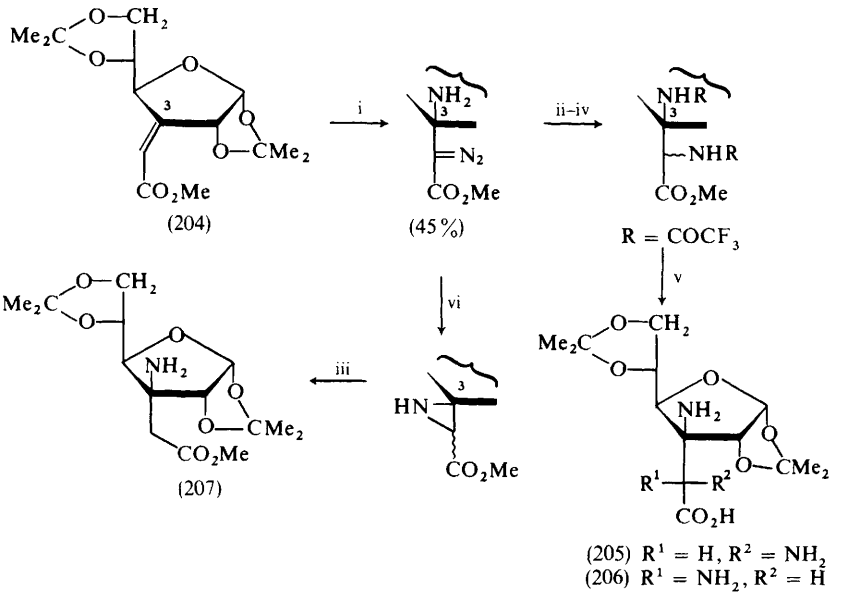
(202)



(203)

The 3-*C*-(methoxycarbonyl)methylene derivative (204) has been converted into the D- and L-2-(3-amino-3-deoxy-α-D-glucofuranos-3-yl)glycine derivatives (205)

[418] A. Hasegawa, N. Aritake, and M. Kiso, *Carbohydrate Res.*, 1976, **52**, 137.

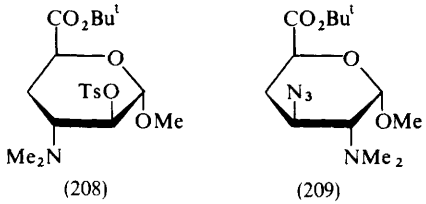


Reagents: i, NaN_3–DMF; ii, H_2–Pd; iii, H_2–Ni; iv, $(CF_3CO)_2O$–py; v, NaOH; vi, $CuSO_4$

Scheme 39

and (206), respectively, and an analogous acetic ester derivative (207) by the reactions shown in Scheme 39.[419] Standard transformations on methyl 2-acetamido-3-*O*-benzyl-2-deoxy-5,6-*O*-isopropylidene-β-D-glucofuranoside have afforded derivatives of 2,5-diamino-2,5-dideoxy-D-gluco- and -L-ido-furanose.[420]

Treatment of the 2-tosylate (208) with sodium azide in DMF gave the 3-azido derivative (209), presumably by ring-opening of an aziridinium ion intermediate.[421] This appears to be the first example of the migration of a dimethylamino-group on a pyranose ring during a displacement reaction.



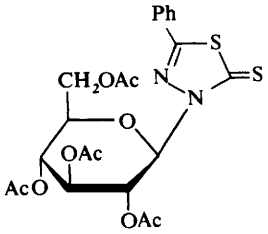
[419] A. Rosenthal and M. Ratcliffe, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 199.
[420] A. Hasegawa, K. Ando, and K. Makoto, *Gifu Daigaku Nogakubu Kenkyu Hokoku*, 1976, **39**, 145 (*Chem. Abs.*, 1977, **87**, 168 311x).
[421] J. Mieczkowski and A. Zamojski, *Roczniki Chem.*, 1976, **50**, 2205.

9

Miscellaneous Nitrogen-containing Compounds

Glycosylamines

Among glycosylamine derivatives prepared by conventional procedures were those derived from hexoses (and pentoses) and 3-aminoacetophenone,[422] D-arabino-furanosylureas,[423] and *N*-acetyl(or benzoyl) derivatives of *N*-methyl-D(and L)-arabino-, -D-galacto-, -D-gluco-, and -D-manno-pyranosylamines,[424] and *N*-β-D-glucosyl derivatives of 3-acylindoles[425] and 5-aryl-2-thioxo-1,3,4-thiadiazoles [*e.g.* (210)], although the corresponding 1-thioglycosides were the principal pro-

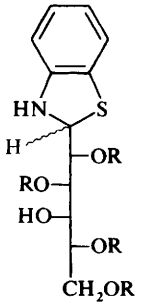


(210)

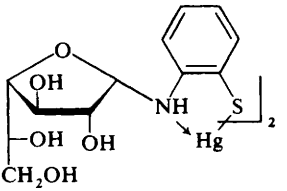
ducts isolated from the reactions of 5-substituted 2-thioxo-1,3,4-thiadiazoles with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of potassium hydroxide.[426] The benzothiazoline (211) formed by the reaction of D-galactose with 2-aminobenzenethiol gave bis-[2-(α-D-galactofuranosylamino)benzenethiol]-mercury(II) (212) on treatment with mercury(II) acetate in refluxing acetic acid.[427] *O*-Acetylation of the chelate (212) and demercuration (hydrogen sulphide) of the product gave 2-(2,3,5,6-tetra-*O*-acetyl-α-D-galactofuranosylamino)benzenethiol, which isomerized to the acetylated benzothiazoline derivative (213) with a catalytic amount of acid or when kept in chloroform solution. The ease with which the benzothiazoline derivative (213) reverted to the corresponding 2-aminobenzene-thiol on treatment with acyl chlorides, *etc.*, limited its use for obtaining D-galacto-syl derivatives specifically modified at C-4.

[422] J. Sykulski, *Acta polon. Pharm.*, 1976, **33**, 295 (*Chem. Abs.*, 1977, **87**, 136 277z).
[423] A. M. Fleischner, J. M. Cross, A. B. Segelman, and T. van Es, *J. Pharm. Sci.*, 1977, **66**, 1206 (*Chem. Abs.*, 1977, **87**, 201 960*h*).
[424] E. Masciorini, I. M. E. Thiel, and J. O. Deferrari, *Anales de Quim.*, 1976, **72**, 946 (*Chem. Abs.*, 1977, **87**, 201 958*p*).
[425] V. M. Tolkachev, M. Z. Kornveits, and M. N. Preobrazhenskaya, *Zhur. org. Khim.*, 1977, **13**, 416 (*Chem. Abs.*, 1977, **87**, 68 564*p*).
[426] F. M. E. Abdel-Megeid, M. A.-F. Elkaschef, and H. M. A. Abdel-Bary, *Carbohydrate Res.*, 1977, **59**, 95.
[427] R. Blattner, R. J. Ferrier, and P. C. Tyler, *Carbohydrate Res.*, 1977, **54**, 199.

Kinetic data for the S_N1 hydrolyses of a number of 1-(β-D-galactopyranosyl)-pyridinium salts between pH 2 and 8 have confirmed that the rate-enhancement of 10^{10}—$10^{12.5}$ during enzymic hydrolysis of the salts with *E. coli* β-D-galactosidase is large enough for meaningful conclusions to be drawn about the enzyme mechanism.[428] Thus, it was concluded that the whole of the catalytic effect of the



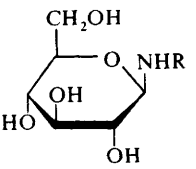
(211) R = H
(213) R = Ac



(212)

enzyme towards these substrates arises from non-covalent interaction with parts of the substrate other than the bond being cleaved (*i.e.* substrate destabilization). It was also argued that such destabilization has comparable importance in the hydrolysis of *O*-glycosides. A synthesis of 1-(β-D-glucopyranosyluronic acid)-pyridinium bromide has been described.[429]

Treatment of β-D-glucopyranosylamine with 2-chloroethyl isocyanate and nitrosation of the product yielded the β-D-glucopyranosylurea derivative (214), which displayed antitumour activity.[430] β-D-Glucopyranosylamine also reacted with dimethyl acetylenedicarboxylate to give a mixture of the *cis*- and *trans*-glycosylaminobutenedioates (215), whereas similar treatment of β-D-xylopyranosylamine gave the *trans*-isomer only.[431] An equimolar mixture of *N*-glycyl-β-D-ribofuranosylamine 5′-phosphate, an intermediate in the biosynthetic pathway

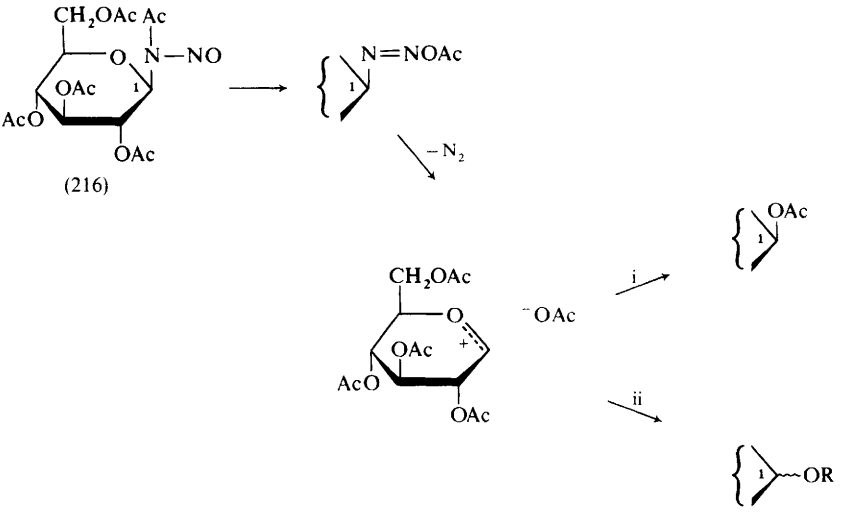


(214) R = $CONCH_2CH_2Cl$ (NO)
(215) R = $C{=}CHCO_2Me$ (CO_2Me)

[428] C. C. Jones, M. L. Sinnott, and I. J. L. Souchard, *J.C.S. Perkin II*, 1977, 1191.
[429] H. Y. Aboul-Enein, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 77.
[430] J. L. Montero, A. Moruzzi, J. Oiry, and J. L. Imbach, *European J. Medicin. Chem.–Chim. Ther.*, 1976, **11**, 183 (*Chem. Abs.*, 1977, **86**, 30 018*e*).
[431] A. Gomez Sanchez and E. Pando Ramos, *Anales de Quim.*, 1976, **72**, 950 (*Chem. Abs.*, 1977, **87**, 184 855*h*).

leading to purines, and its α-anomer has been obtained from 2,3-*O*-isopropylidene-D-ribofuranosylamine toluene-*p*-sulphonate by standard procedures.[432]

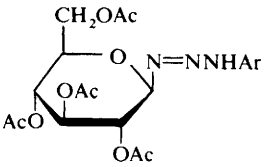
N-Acetyl-2,3,4,6-tetra-*O*-acetyl-*N*-nitroso-β-D-glucopyranosylamine (216) has been synthesized; it decomposed to give principally β-D-glucopyranose penta-acetate in dichloromethane or to give a mixture of anomeric D-glucopyranoside



Reagents: i, CH_2Cl_2; ii, ROH–py

Scheme 40

tetra-acetates with an alcohol in pyridine (Scheme 40).[433] The 1-(β-D-glucopyranosyl)triazene derivative (217) also furnished a mixture of anomeric D-glucopyranosides on reaction with an alcohol in the presence of boron trifluoride.

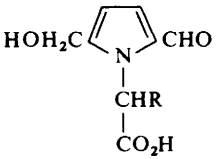


(217) Ar = *p*-ClC_6H_4

There have been a number of reports on the Maillard reaction. Oxidation of the melanoidins produced from D-glucose and glycine with potassium permanganate and peracetic acid yielded, among other products, furan-2,5-dicarboxylic acid, suggesting that 5-hydroxymethyl-2-furaldehyde is a precursor of the melanoidic pigments produced in the Maillard reaction.[434] 2-(2-Formyl-5-hydroxymethyl-

[432] G. Chetter and S. J. Benkovic, *Carbohydrate Res.*, 1977, **56**, 75.
[433] O. Larm, K. Larsson, and M. Wannong, *Acta Chem. Scand.*, 1977, **B31**, 475.
[434] P. Barbetti and I. Chiappini, *Ann. Chim. (Italy)*, 1976, **66**, 485 (*Chem. Abs.*, 1977, **87**, 184 823w).

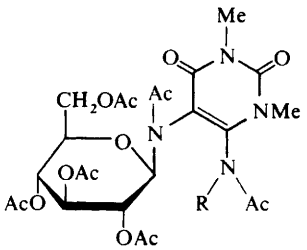
pyrrol-1-yl)acetic acid (218) and the corresponding derivative (219) of 3-methylbutanoic acid have been isolated from the Maillard reaction between D-glucose and glycine or valine,[434a] respectively. The Maillard reaction between D-glucose and methionine (or tryptophan) has also been studied at various temperatures and pH values; the rate of the reaction is highest at pH 11, but the competing formation



(218) R = H
(219) R = $CHMe_2$

of acidic products predominates at pH > 11.[435] The Maillard reaction between lactose and glycine at pH 3—6.2 has been shown to be independent of the hydrolysis of lactose and to proceed by 1,2-enolization followed by allylic elimination of the D-galactopyranosyl residue.[436]

Reduction (sodium borohydride) of the Schiff base formed when D-fructose 1,6-diphosphate reacts with an aminated agarose furnished a matrix suitable for use in the affinity chromatography of yeast aldolase.[327] A convenient, microscale synthesis of *N*-acetylmuramyl-L-[U-^{14}C]alanyl-D-isoglutamine, which is reported to have immunoadjuvant activity, has been described.[437] The *O*- and *N*-β-glycosides of bufotenine (*NN*-dimethyl-5-hydroxytryptamine) have been obtained from the reactions of bufotenine and its 5-*O*-benzyl derivative, respectively, with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide,[438] and D-glucose has been converted into the D-glucopyranosylamine derivatives (220) by acetylation of the Schiff base formed in the reaction with 5,6-diamino-1,3-dimethylpyrimidinedione.[439] A series



(220) R = H or Ac

[434a] H. Kato, H. Sonobe, and M. Fujimaki, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 711.
[435] E. Dworschak and F. Orsi, *Acta Aliment. Acad. Sci. Hung.*, 1977, **6**, 59 (*Chem. Abs.*, 1977, **87**, 147 343*u*).
[436] F. G. Reyes Reyes, F. O. Bobbio, and P. A. Bobbio, *Rev. Bras. Technol.*, 1976, **7**, 93 (*Chem. Abs.*, 1977, **87**, 184 794*n*).
[437] S. Kusumoto, K. Ikenaka, and T. Shiba, *Tetrahedron Letters*, 1977, 4055.
[438] L. S. Krasavina, M. M. Vigdorchik, K. F. Turchin, and N. N. Suvorov, *Zhur. org. Khim.*, 1977, **13**, 1520 (*Chem. Abs.*, 1977, **87**, 202 009*s*).
[439] M. Melgarejo Sampedro and R. Rico Gomez, *Anales de Quim.*, 1976, **72**, 998 (*Chem. Abs.*, 1977, **87**, 201 986*w*).

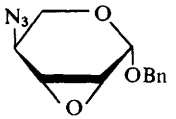
of pyrrole and furan derivatives has been identified among the volatile, ether-soluble products obtained when D-fructose or L-rhamnose react with L-alanine or 3-aminobutanoic acid at pH 3.5.[440]

N-(1-Deoxy-D-fructos-1-yl)-L-valine has been identified as the *N*-terminal unit of the β-chain of haemoglobin A_{1c}, since *N*-(1-deoxy-D-glucitol-1-yl)-L-valyl-L-histidine and the analogous *N*-(1-deoxy-D-mannitol-1-yl)dipeptide were isolated after reduction of the polypeptide chain with sodium borohydride and proteolysis.[441] Model reactions established that D-glucose and D-mannose each reacted with L-valine to give a product that was reduced (sodium borohydride) to a mixture of *N*-(1-deoxy-D-mannitol- and -glucitol-1-yl)valines. A kinetic study of the Amadori rearrangement of *N*-(4,6-*O*-benzylidene-β-D-glucopyranosyl)glycine isopropyl ester has been reported.[442]

The products formed in the reaction of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine with triphenylphosphine and carbon tetrachloride are mentioned [403, 404] in Chapter 8.

Azides

2,3,4,6-Tetra-*O*-acetyl(or benzoyl)-α-D-mannopyranosyl bromide reacted with sodium azide in HMPT to give the β-azide, whose configuration was assigned on the basis of c.d. and ^1H n.m.r. measurements.[443] The assignment of configuration to D-mannopyranosyl azides was discussed and errors in the assignments made by other investigators were corrected. Ring-opening of methyl 2,3-anhydro-α-D-lyxofuranoside and its 5-tosylate with ammonium azide yielded methyl 3-azido-3-deoxy-α-D-arabinofuranoside and the corresponding 3,5-diazide, while similar treatment of methyl 2,3-anhydro-5-*O*-benzoyl(or toluene-*p*-sulphonyl)-α-D-ribofuranoside gave methyl 2-azido-5-*O*-benzoyl-2-deoxy- or 2,5-diazido-2,5-dideoxy-α-D-arabinofuranoside, respectively.[444] Benzyl 2,3-anhydro-4-*O*-toluene-*p*-sulphonyl-α-D-ribopyranoside reacted with sodium azide in DMF at 50 °C to yield benzyl 2,3-anhydro-4-azido-4-deoxy-β-L-lyxopyranoside (221), without ring-opening of the epoxide.[445]



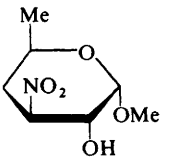
(221)

Nitro-sugars

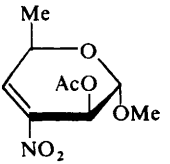
The recent literature on the synthesis and reactions of nitro-sugars has been reviewed.[446]

[440] P. E. Shaw and R. E. Berry, *J. Agric. Food Chem.*, 1977, **25**, 641 (*Chem. Abs.*, 1977, **87**, 6320*x*).
[441] R. J. Koenig, S. H. Blobstein, and A. Cerami, *J. Biol. Chem.*, 1977, **252**, 2992.
[442] J. R. Ramos and O. C. Gonzalez, *Rev. Latinoam. Quim.*, 1977, **8**, 94 (*Chem. Abs.*, 1977, **87**, 68 544*g*).
[443] Z. Györgydeák and H. Paulsen, *Annalen*, 1977, 1987.
[444] F. M. Unger, R. Christian, and P. Waldstätten, *Tetrahedron Letters*, 1977, 4383.
[445] N. Vethaviyasar, R. Kimmich, and W. Voelter, *Chem.-Ztg.*, 1977, **101**, 36 (*Chem. Abs.*, 1977, **86**, 171 745*b*).
[446] A. C. Richardson, M.T.P. International Review of Science, Organic Chemistry, Series Two, 1976, vol. 7, p.131 (*Chem. Abs.*, 1977, **86**, 190 353*p*).

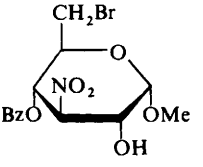
6-Deoxy-3-*O*-methyl-6-nitro-D-altrose, a potential intermediate for the synthesis of new stereoisomers in the deoxynitroinositol series, has been synthesized from methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside by way of 1,2:5,6-di-*O*-isopropylidene-3-*O*-methyl-β-D-altrofuranose.[447] The 4-mono- and 2,4-di-mesylates derived from methyl 3,6-dideoxy-3-nitro-α-D-glucopyranoside are readily converted into the nitro-sugar derivatives (222) and (223), respectively, which are potential intermediates for the synthesis of aminopolydeoxy-sugar components of antibiotics.[448] The 4,6-*O*-benzylidene derivatives of methyl 3-deoxy-3-nitro-α-D-hexopyranosides were found to give good yields of the corresponding 6-bromo-4-benzoates [*e.g.* (224)] with NBS in refluxing carbon tetrachloride.



(222)

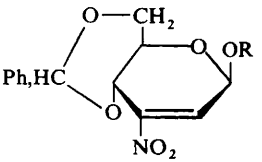


(223)



(224)

Steric and electronic factors governing the addition of nucleophiles to methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranoside (225) have been investigated.[449] The addition of hydrogen cyanide, hydrazoic acid, and



(225) R = Me
(226) R = Ac

toluene-*p*-sulphonic acid gave principally adducts having the D-*gluco* configuration, the reaction with hydrogen cyanide also yielding the 2-cyanohex-2-enopyranoside derivative with loss of the nitro-group. Reaction of 1-*O*-acetyl-4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*-hex-2-enopyranose (226) with hydrazoic acid also gave the thermodynamically more-stable D-glucopyranose derivative, namely 1-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-glucopyranose, whereas the α-anomer of (226) afforded the less-stable D-mannopyranose derivative as the major product with hydrazoic acid and with hydrogen cyanide.[450] Striking solvent effects were observed in the reaction of the α-anomer of (226) with hydrazoic acid, including some epimerization of the D-*manno*-adduct to the D-*gluco*-adduct in DMSO and DMF, but not in such solvents as chloroform, ethyl acetate, and THF.

[447] J. Kovář and H. H. Baer, *Carbohydrate Res.*, 1977, **59**, 103.
[448] H. H. Baer and F. F. Z. Georges, *Canad. J. Chem.*, 1977, **55**, 1348.
[449] T. Sakakibara and R. Sudoh, *J. Org. Chem.*, 1977, **42**, 1746.
[450] T. Sakakibara and R. Sudoh, *Carbohydrate Res.*, 1977, **58**, 31.

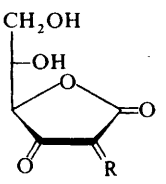
Other references to nitro-sugar derivatives are included in Chapters 8 and 13.

Phenylhydrazones and Osazones

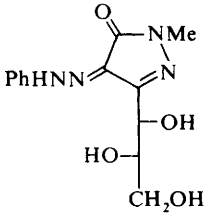
The recent literature on the synthesis and reactions of carbohydrate phenylhydrazones and osazones has been reviewed.[451]

An investigation of the structures of the D-pentose phenylhydrazones in water, methanol, and pyridine by chemical, spectroscopic, and chiroptical methods has indicated that the acyclic form is adopted in these solvents.[452] A related study has shown that D-glucose phenylhydrazone also adopts the acyclic form in DMSO, whereas solutions of the corresponding 4-nitro- and 2,4-dinitro-phenylhydrazones in DMSO contain only 40 and 10%, respectively, of the acyclic form.[453] An n.m.r. study of 2,5-anhydro-D-lyxose and -xylose 4-nitrophenylhydrazones is mentioned in Chapter 20.

El Ashry's group has extended its earlier studies on the chemistry of carbohydrate hydrazones. Reaction of the 2-(phenylhydrazone) (227) derived from L-*threo*-2,3-hexodiulosono-1,4-lactone (228) with methylhydrazine gave a rearranged product, identified as the pyrazoledione derivative (229).[454] Methyl-

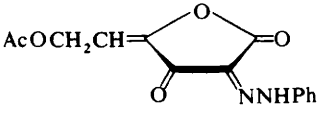


(227) R = NNHPh
(228) R = O

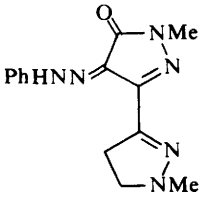


(229)

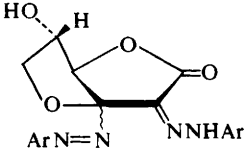
hydrazine reacted with the 2-(phenylhydrazone) (230) of 4-(2-acetoxyethylidene)-4-hydroxy-2,3-dioxobutyro-1,4-lactone to give the heterocyclic derivative (231) by a rearrangement that involves loss of the acetoxy-group and opening of the lactone ring, with further cyclization with one of the hydrazone residues. Oxidation of the 2,3-bis-[(2-chlorophenyl)hydrazone] derived from (228) with copper(II) chloride gave the 3,6-anhydro derivative (232), but without the expected loss of the chlorine



(230)



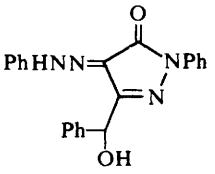
(231)



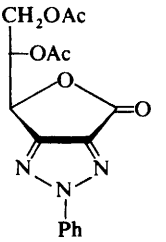
(232) Ar = *o*-ClC_6H_4

[451] H. Simon and A. Kraus, *Amer. Chem. Soc. Symposium Ser.*, 1976, No. 39, Synthetic Methods Carbohydrates Symposium, 188 (*Chem. Abs.*, 1977, **87**, 136 157*k*).
[452] E. Weclawowicz, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1976, **24**, 935 (*Chem. Abs.*, 1977, **86**, 190 369*y*).
[453] S. L. Zdanov and A. A. Potekhin, *Zhur. org. Khim.*, 1977, **13**, 469 (*Chem. Abs.*, 1977, **86**, 190 370*s*).
[454] E. S. H. El Ashry, *Carbohydrate Res.*, 1976, **52**, 69.

atoms.[455] Mixed 2,3-bis(arylhydrazones) of (228) have also been subjected to rearrangement under alkaline conditions to give analogues of the pyrazoledione derivative (229) having varied substituents (*e.g.* 4-tolyl and 4-bromophenyl, *etc.*) on N-1.[456, 456a] Acetylation, benzoylation, and periodate oxidation of these pyrazolediones were examined. 4-Phenylbutano-1,4-lactone 2,3-bis(phenylhydrazone) also rearranged to a pyrazoledione derivative, notably (233), on heating with aqueous sodium hydroxide.[457] The 3-oxime derived from the 2-(phenylhydrazone) (227) was dehydrated and acetylated on boiling with acetic anhydride, furnishing the 1,2,3-triazole derivative (234).[456a]



(233)

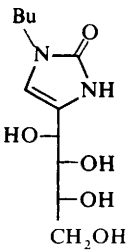


(234)

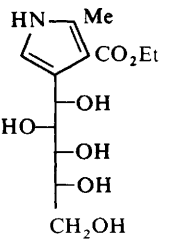
Heterocyclic Derivatives

The synthesis of (polyhydroxy)alkyl-furan, -pyrrole, and -indole derivatives, *etc.*, by condensation of aldoses and ketoses with β-dicarbonyl compounds has been reviewed.[458]

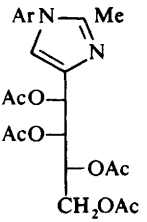
1-Alkylamino-1-deoxy-D-fructoses reacted with potassium isocyanate in the presence of hydrochloric acid to give imidazole derivatives [*e.g.* (235)],[459] while 1-amino-1-deoxy-D-*gluco*-heptulose gave the 4-[(polyhydroxy)alkyl]pyrrole (236)



(235)



(236)



(237) Ar = *p*-MeC_6H_4

[455] E. S. H. El Ashry, G. H. Lahib, and Y. El Kilany, *Carbohydrate Res.*, 1976, **52**, 251.

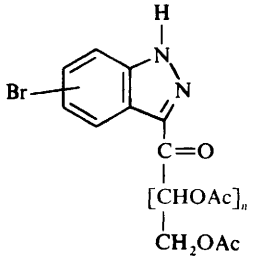
[456] E. S. H. El Ashry, Y. El Kilany, and F. Singab, *Carbohydrate Res.*, 1977, **56**, 93. M. El Sekily, S. Mancy, I. El Kholy, E. S. H. El Ashry, H. S. El Khadem, and D. L. Swartz, *Carbohydrate Res.*, 1977, **59**, 141.

[457] E. S. H. El Ashry, M. Nassr, and F. Singab, *Carbohydrate Res.*, 1977, **56**, 200.

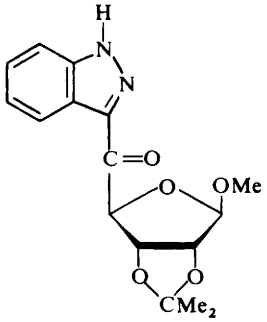
[458] F. Garcia Gonzalez, J. Fernandez Bolanos, and F. J. Lopez Aparicio, *Amer. Chem. Soc. Symposium Ser.*, 1976, No. 39, Synthetic Methods Carbohydrates Symposium, 207 (*Chem. Abs.*, 1977, **86**, 190348*r*).

[459] J. Fernandez Bolanos and J. Viguera Rubio. *Anales de Quim.*, 1976, **72**, 991 (*Chem. Abs.*, 1977, **87**, 184820*t*).

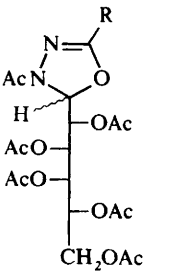
with ethyl acetoacetate.[460] The D-*lyxo*-imidazole derivative (237) was produced as a common product from 2-acetamido-2-deoxy-*N*-(4-tolyl)-β-D-galactosylamine, -D-mannosylamine, and -D-glucosylamine under acetolyzing conditions.[461] Formation of the imidazole is considered to occur by 1,2-enolization and dehydration reactions, and inversion of the configuration at C-4 of the D-mannosyl- and D-glucosyl-amine derivatives presumably involves an intermediate acetoxonium ion. *C*-Glycosylindazoles [*e.g.* (238)] have been prepared from 1-diazo-1-deoxyketoses by 1,3-dipolar addition to the benzynes generated from substituted anthranilic acids.[462] A related approach was also used to obtain (239). Treatment of 2,3,4,5,6-penta-*O*-acetyl-*aldehydo*-D-galactose acetyl(or benzoyl)hydrazone with boiling



(238) $n = 3$ (D-*ribo*)
$n = 4$ (D-*galacto*)



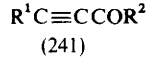
(239)



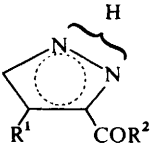
(240) R = Me or Ph

acetic anhydride (or boiling acetyl chloride) gave products that were assigned[463] the 1,3,4-oxadiazoline structure (240), rather than the *NN*-diacylhydrazone structure previously assigned (see Vol. 6, p. 68).

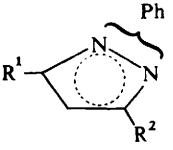
The branched-chain ketols (241) have been prepared by reaction of 3-*C*-ethynyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose with an appropriate aldehyde (*e.g.*



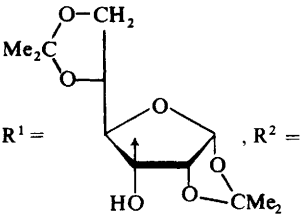
(241)



(242)



(243)

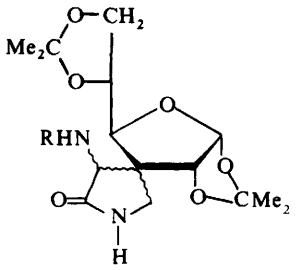


, R^2 = $CHMe_2$, $CHEt_2$, Ph, or *p*-MeC_6H_4, *etc.*

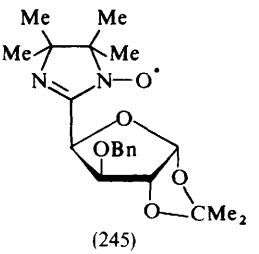
[460] F. Garcia Gonzalez, J. Fernandez Bolanos, and J. Galbis Perez, *Anales de Quim.*, 1976, **72**, 855 (*Chem. Abs.*, 1977, **87**, 168 312*y*).
[461] S. Hirano, R. Yamasaki, and Y. Kondo, *Carbohydrate Res.*, 1977, **59**, 244.
[462] E. García-Abbad, M. T. García-López, G. Garćia-Muñoz, and M. Stud, *J. Heterocyclic Chem.*, 1976, **13**, 1241.
[463] L. Somogyi, *Carbohydrate Res.*, 1977, **54**, C14.

isobutyraldehyde), followed by oxidation of the resulting diol with chromium trioxide.[463a] The substituted pyrazoles (242) and (243) were obtained by condensation of (241) with ethereal diazomethane or ethanolic phenylhydrazine, respectively. Treatment of the 3-*C*-(methoxycarbonyl)methylene derivative (204) with ethereal diazomethane afforded unstable spiro-pyrazoline derivatives, which were converted into a mixture of the spiro-pyrrolidinones (244; R = H) on high-pressure hydrogenation.[464] The structures of the *N*-acetyl derivatives (244; R = Ac) of two of the diastereoisomeric pyrrolidinones were established by *X*-ray crystallography.

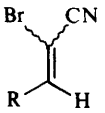
Tronchet's group has reported more examples (see also Vol. 9, p. 75) of stable free radicals derived from heterocyclic derivatives of carbohydrates [*e.g.*(245)], which are potentially interesting for biological studies,[465] and some new reactions of 6-bromo-5,6-dideoxy-3-*O*-methyl-α-D-*xylo*-hept-5-enofuranurononitrile (246)



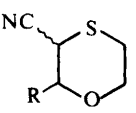
(244) R = H or Ac



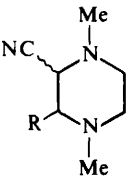
(245)



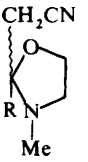
(246)



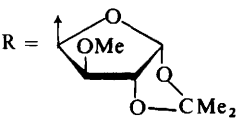
(247)



(248)



(249)



yielding heterocyclic derivatives.[466] For example, (246) reacted with 2-mercaptoethanol or *NN'*-dimethylethylenediamine in the presence of sodium hydroxide to give stereoisomers of the oxathian (247) and of the perhydrodiazine (248), respectively, or with *N*-methylethanolamine–triethylamine and acetic acid, in turn, to give both isomers of the oxazolidine (249).

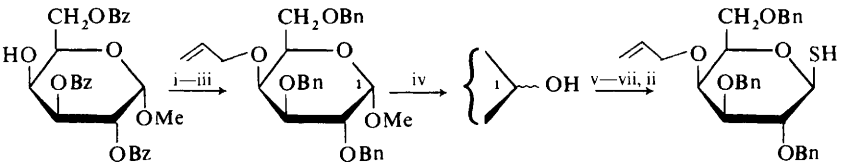
[463a] A. González, A. Llamas, M. A. Pascual, and R. Mestres, *Carbohydrate Res.*, 1977, **59**, 248.
[464] A. Rosenthal and K. Dooley, *Carbohydrate Res.*, 1976, **52**, 79.
[465] J. M. J. Tronchet, J. Ojha-Poncet, E. Winter-Mihaly, B. Kohler, and M. Goeffroy, *Helv. Chim. Acta*, 1977, **60**, 888.
[466] J. M. J. Tronchet and O. R. Martin, *Helv. Chim. Acta*, 1977, **60**, 585.

10

Thio-sugars and Other Sulphur-containing Compounds

Sulphur-containing and other analogues of carbohydrates have been reviewed.[467]

Derivatives of 2,3,6-tri-*O*-benzyl-1-thio-αβ-D-galactopyranose having an acyl group at O-4 have been synthesized for subsequent use in oligosaccharide synthesis by the thioglycoside route on solid supports.[468] For example, the 4-*O*-benzoyl and 4-*O*-(4-nitrobenzoyl) derivatives were obtained in eight steps from allyl 2,6-di-*O*-benzyl-3,4-*O*-dibutylstannylene-α-D-galactopyranoside, *via* selective benzylation at O-3, while a 4-*O*-allyl derivative was prepared by the sequence of reactions outlined in Scheme 41. The boron-trifluoride-catalysed reaction of 1,2-*trans*-related aldose and hexuronic acid peresters with thiols has provided a convenient

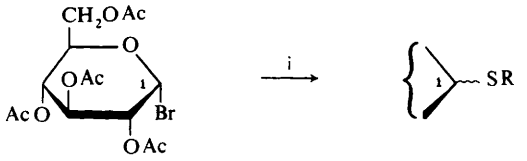


Reagents: i, CH_2=$CHCH_2Br$–Ag_2O; ii, MeONa–MeOH; iii, $PhCH_2Cl$–KOH; iv, HCl–H_2O–*p*-dioxan; v, Ac_2O–py; vi, HCl–CH_2Cl_2; vii, $EtOCS_2K$–EtOH–PhH

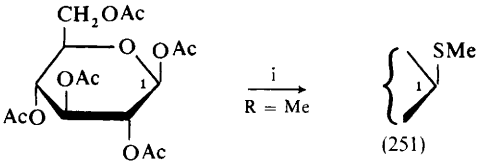
Scheme 41

route to some 1,2-*trans*-1-thioglycosides (*e.g.* phenyl or allyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside).[469] Modification of the nucleophilicity of the thiol group through its transformation into a tributyltin sulphide has been used in a new approach to the synthesis of 1-thioglycosides.[470] Although 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide reacted slowly with methyl tributyltin sulphide in 1,1,2,2-tetrachloroethane at 100 °C to give, after 48 h, methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (251) stereospecifically (45% conversion), it reacted much more readily with ethyl tributyltin sulphide at 15 °C in the presence of tin(IV) chloride to give the α- and β-anomers of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-D-glucopyranoside (250; R = Et) (95%) in a ratio of 1:1, owing to anomerization of the initially formed β-anomer under the reaction conditions

[467] R. L. Whistler and A. K. M. Anisuzzaman, 'A.C.S. Symposium Ser.', 1976, 39, Synthetic Methods Carbohydrates Symposium, 133 (*Chem. Abs.*, 1977, **86**, 190 347*q*).
[468] M. A. Nashed and L. Anderson, *Carbohydrate Res.*, 1977, **56**, 325.
[469] R. J. Ferrier and R. H. Furneaux, *Carbohydrate Res.*, 1976, **52**, 63.
[470] T. Ogawa and M. Matsui, *Carbohydrate Res.*, 1977, **54**, C17.



(250) R = Et (95%; α/β ratio = 1:1), Ph, Bn, or Me_3C, *etc.*

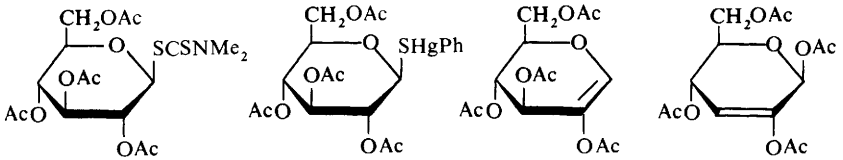


Reagent: i, Bu_3SnSR–$SnCl_4$

Scheme 42

(Scheme 42). By contrast, the reaction of β-D-glucopyranose penta-acetate with methyl tributyltin sulphide in the presence of an equimolar amount of tin(IV) chloride afforded the β-anomer (251) stereospecifically and in high yield. This approach to alkyl 1-thioglycosides was shown to be applicable to furanose derivatives; for example, the α- and β-anomers of ethyl 2,3,5-tri-*O*-benzoyl-1-thio-D-ribofuranoside were obtained in a ratio of 2:3 when 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose reacted with equivalent amounts of ethyl tributyltin sulphide and tin(IV) chloride at room temperature.

Treatment of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl dimethyldithiocarbamate (252) with phenylmercury(II) acetate gave the acetylated 1-*S*-phenylmercury(II)thio-β-D-glucopyranose (253), which could also be prepared in high yield from other dithiocarbamates and from 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose and its *S*-acetyl derivative.[471] Demercuration of (253) with hydrogen sulphide provided a convenient synthesis of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose,



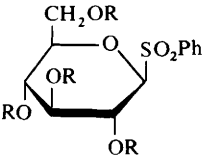
(252) (253) (254) (255)

whereas attempts to extrude mercury(II) sulphide from (253) in refluxing nitrobenzene, to provide a new route to *C*-glycosides, gave a mixture of bis-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl) sulphide and the glycal (254) and the product (255) of its thermal rearrangement. Partial desulphuration of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl phenyl disulphide with tris(diethylamino)phosphine in refluxing benzene afforded roughly equal proportions of phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranoside (40%) and β-D-glucopyranosyl 1-thio-α-D-glucopyranoside octa-acetate, showing that this procedure has limited scope

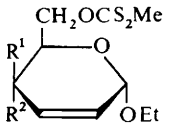
[471] R. J. Ferrier and R. H. Furneaux, *Carbohydrate Res.*, 1977, **57**, 73.

in the efficient synthesis of 1-thio-α-D-glucopyranosides.[472] The phenylsulphonyl group was not readily displaced from β-D-glucopyranosyl phenyl sulphone (256), except intramolecularly, nor could it be displaced from the tetrabenzyl derivative (257), which gave a 1-(phenylsulphonyl)glycal derivative, by β-elimination of benzyl alcohol, when treated with alkoxides or thiolates.

The formation of 1-thioglycosides when 2-thioxo-1,3,4-thiadiazoles react with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide is noted[426] in Chapter 9. Other references to 1-thioglycosides will be found in Chapters 6 and 16.



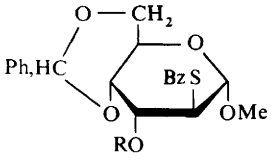
(256) R = H
(257) R = Bn



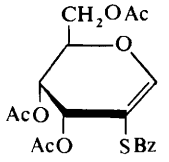
(258) R^1 = H, R^2 = OCS_2Me
(259) R^1 = OCS_2Me, R^2 = H

The 4,6-dixanthates (258) and (259) have been prepared from ethyl 2,3-dideoxy-α-D-*erythro*- and -*threo*-hex-2-enopyranoside, respectively, and on thermolysis they underwent suprafacial [3,3]sigmatropic rearrangements to give the corresponding 3,4-unsaturated 2-(methylthio)carbonylthio compounds.[473]

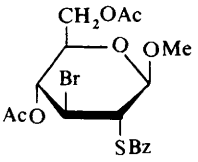
Paulsen's group has synthesized methyl 2-*S*-benzoyl-4,6-*O*-benzylidene-2-thio-α-D-altropyranoside (260; R = H) and the corresponding 2-*S*-benzylthio derivative from methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside.[262] Acetolysis of the 2-*S*-benzoylthiopyranoside (260; R = H) and treatment of the



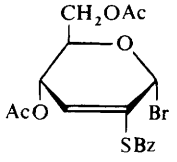
(260) R = H or Me



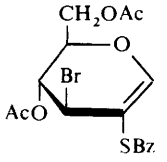
(261)



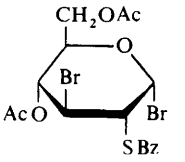
(262)



(263)



(264)

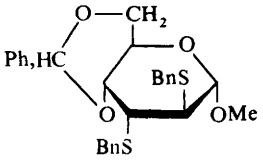


(265)

product (261) with hydrogen bromide in acetic acid and then with methanol in the presence of mercury(II) cyanide gave the 3-bromo-2-thioglucopyranoside (262), which is considered to arise by the pathway (261) → (263) → (264) → (265) → (262). However, the 3-*O*-methyl derivative (260; R = Me) and its *S*-benzylthio analogue, for which an allylic rearrangement is precluded, could be converted into glycosyl bromides (*e.g.*, 4,6-di-*O*-acetyl-2-*S*-benzyl-3-*O*-methyl-2-thio-αβ-D-altropyranosyl

[472] R. J. Ferrier, R. H. Furneaux, and P. C. Tyler, *Carbohydrate Res.*, 1977, **58**, 397.
[473] R. J. Ferrier and N. Vethaviyasar, *Carbohydrate Res.*, 1977, **58**, 481.

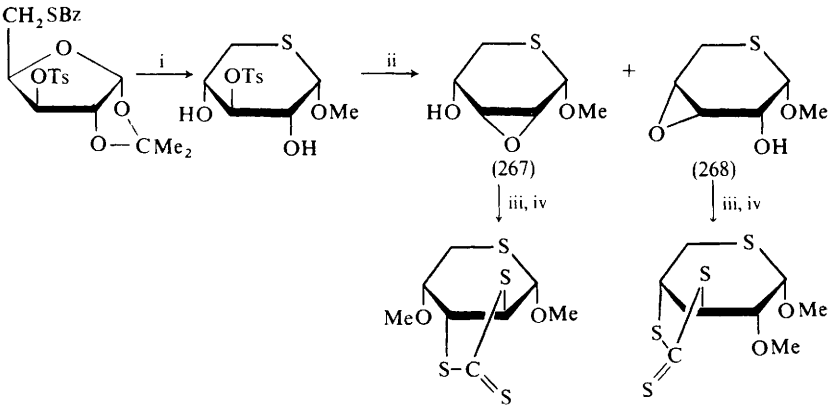
bromide) and afterwards into anomeric mixtures of glycosides (*e.g.* with cholesterol and digitoxigenin) by a related sequence of reactions.[474] The use of this approach to obtain steroidal 2,6-dideoxy-α- and -β-D-*ribo*-hexopyranosides is discussed in the next chapter. 2,3-Dideoxy-2,3-epithio-D-allofuranose and -mannofuranose have been obtained by hydrolysis of the corresponding methyl α-pyranosides with an acid resin,[474a] and derivatives of methyl 2,3-dithio-α-D-altropyranoside have been prepared by thermal isomerization of methyl 3-*S*-benzyl-4,6-*O*-benzylidene-3-thio-α-D-altropyranoside 2-xanthate or of the related 2-*S*-benzylthio 3-xanthate.[475] The pyranoside → furanoside isomerization that occurs on acid-catalysed methanolysis of methyl 2,3-dideoxy-2,3-epithio-α-D-allopyranoside and methyl 2-*S*-benzyl-2-thio-α-D-altropyranoside has been noted[199] in Chapter 3 (see Scheme 11). Related work has shown that methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-epithio-α-D-mannopyranoside and the 2,3-di-*S*-benzylthioaltro-



(266)

pyranoside derivative (266) isomerize to furanosides, with loss of the 4,6-*O*-benzylidene group, on acid-catalysed methanolysis.[476]

The 2,3- and 3,4-anhydro derivatives (267) and (268), respectively, of methyl 5-thio-α-D-ribopyranoside have been prepared by the reactions outlined in Scheme



Reagents: i, MeOH–H^+; ii, MeONa–MeOH; iii, MeI–Ag_2O; iv, $MeOCS_2Na$

Scheme 43

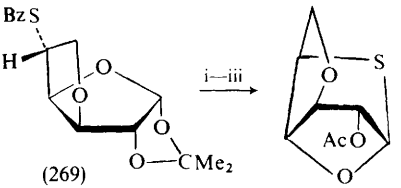
[474] J. Thiem and H.-J. Jürgens, *Chem. Ber.*, 1977, **110**, 2852.

[474a] T. Yamaguchi and M. Kojima, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 1140.

[475] M. Kojima, M. Watanabe, and T. Taguchi, *Yakugaku Zasshi*, 1976, **96**, 905 (*Chem. Abs.*, 1977, **86**, 16 883*b*).

[476] M. Kojima, M. Watanabe, T. Yamaguchi, S. Ishimaru, and T. Taguchi, *Yakugaku Zasshi*, 1976, **96**, 1241 (*Chem. Abs.*, 1977, **86**, 55 661*x*).

43.[477] Reaction of the 4-*O*-methyl derivative of (267) with sodium *O*-methyl dithiocarbonate gave methyl 4-*O*-methyl-2,3,5-trithio-2,3-thiocarbonyl-α-D-arabinopyranoside, and similar treatment of the 2-*O*-methyl derivative of (268) gave the 5-thioxylopyranoside 3,4-trithiocarbonate. 1,6-Anhydro-1(6),5-dithio-β-L-idopyranose was also prepared by hydrolysis of 1,2-*O*-isopropylidene-5,6-dithio-β-L-idofuranose in boiling aqueous acetic acid. The synthesis and reactions of derivatives of 3,6-anhydro-5-thio-D-glucose have been described.[478] Basic solvolysis of 3,6-anhydro-5-*S*-benzoyl-1,2-*O*-isopropylidene-5-thio-α-D-glucofuranose (269), followed by hydrolysis with aqueous trifluoroacetic acid and acetylation, gave 2-*O*-acetyl-1,4:3,6-dianhydro-5-thio-α-D-glucopyranose (270), demonstrating that the thiopyranose ring can be formed in the presence of the 3,6-anhydro bridge, but apparently only when the furanose ring is retained (Scheme 44). An alternative

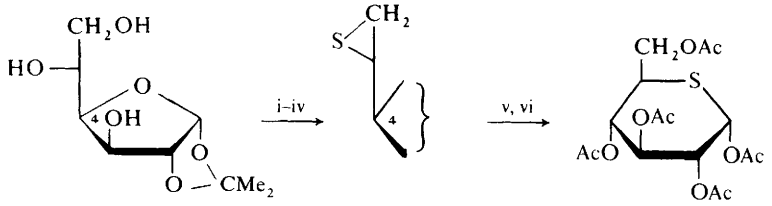


(269) (270)

Reagents: i, MeONa–MeOH; ii, CF_3CO_2H–H_2O; iii, Ac_2O–py

Scheme 44

synthesis of 5-thio-D-glucopyranose derivatives is shown in Scheme 45,[479] while 5-thio-L-rhamnopyranose has been synthesized by routes that use a conventional S_N2 reaction with potassium thioacetate to introduce a sulphur atom at C-5 of methyl 5-chloro-5,6-dideoxy-2,3-*O*-isopropylidene-β-D-gulofuranoside or the corresponding 5-tosylate.[480]

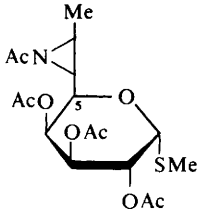


Reagents: i, BzCl(1 mol)–py; ii, TsCl–py; iii, MeONa–MeOH; iv, $(NH_2)_2CS$; v, AcOK–AcOH; vi, Ac_2O–AcOH–H_2SO_4

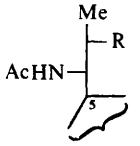
Scheme 45

Ring-opening of the *N*-acetylepimine derivative (271) of methyl α-thiolincosaminide with 2-hydroxyethyl methyl sulphide at 100 °C did not produce the expected derivative (272; R = OCH_2CH_2SMe) but gave instead the sulphides (272; R = SCH_2CH_2OH or SMe), which are formed by nucleophilic attack of the sulphur atom of the reagent at C-7 of the protonated epimine followed by attack

[477] D. M. C. Hull, P. F. Orchard, and L. N. Owen, *J.C.S. Perkin I*, 1977, 1234.
[478] D. M. C. Hull and L. N. Owen, *J.C.S. Perkin I*, 1977, 1777.
[479] M. M. A. Abdel Rahman, *Egypt. J. Chem.*, 1975, **18**, 167 (*Chem. Abs.*, 1977, **86**, 190 364*t*).
[480] A. K. M. Anisuzzaman and R. L. Whistler, *Carbohydrate Res.*, 1977, **55**, 205.



(271)



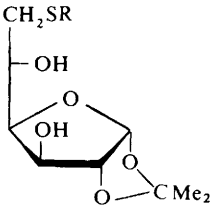
(272) R = OCH_2CH_2SMe, SCH_2CH_2OH, or SMe

of acetate anion at the methyl or α-methylene group of the resulting sulphonium cation.[481] The reactions of other dialkyl sulphides and of dialkyl disulphides with (271) to give α-acetamido-sulphides were examined and discussed.

The yields of 2,5-anhydro-D-xylose and -ribose dibenzyl dithioacetals obtained from the respective pentose dibenzyl dithioacetal increased from *ca.* 35 to 90% when 2 molar equivalents of toluene-*p*-sulphonyl chloride in pyridine were used to effect the cyclization.[482] The observation that 5-*O*-toluene-*p*-sulphonyl-D-xylose dibenzyl dithioacetal rapidly gave the 2,5-anhydride when dissolved in pyridine suggests that cyclizations leading to 2,5-anhydrides generally proceed by displacement of the 5-sulphonate group by O-2. Mixtures of epimeric 2,5-anhydrides were formed when pentose dibenzyl dithioacetals reacted with 1 molar equivalent of mercury(II) chloride in aqueous acetone, probably by an acid-catalysed dehydration between HO-2 and HO-5, whereas the corresponding diethyl dithioacetals and hexose dibenzyl dithioacetals reacted, if only slightly, to give isopropylidene derivatives. The reactions of 1,2:5,6-dianhydro-3,4-*O*-isopropylidene-L-iditol and -D-mannitol with sodium sulphide gave the corresponding 1,6-anhydro-1(6)-thio derivatives, which were isolated as the 2,5-diacetates in yields of 73 and 27%, respectively.[483] The discrepancy in the yields could be explained by destabilizing steric factors in the $_{4,5}TC^{2,3}$ or $^{4,5}TB_{2,3}$ conformation of 1,6-anhydro-3,4-*O*-isopropylidene-1(6)-thio-D-mannitol. 1,5-Anhydro-2-*O*-methyl-5-thio-D-glucitol was formed as a by-product on removal of the acetal group from 1,6-anhydro-3,4-*O*-isopropylidene-2,5-di-*O*-methyl-1(6)-thio-L-iditol with aqueous trifluoroacetic acid, presumably by removal of one of the methyl groups as methanol and formation of a bicyclic sulphonium cation, which was opened at C-1(C-6) by water. Two sulphur-containing products accompanied the expected 6-deoxy compound when a solution of 5,6-anhydro-1,2-*O*-isopropylidene-3-*O*-methanesulphonyl-β-L-idofuranose (86) in THF was reduced with an old sample of lithium aluminium hydride.[258] One of them was the thioether (273), presumably formed by ring-opening of the epoxide with methanethiol produced by reduction at the sulphonate group, while the other, whose mode of formation is obscure, was the dithioacetal (274).

Dethioacetalization of steroidal ethylene and dibenzyl dithioacetals occurred on irradiation of the dithioacetals in hexane under oxygen with a mercury lamp; this method of regenerating a carbonyl group uses neutral conditions and might

[481] B. Bannister, *J.C.S. Perkin I*, 1977, 1057.
[482] T. van Es, *Carbohydrate Res.*, 1977, **58**, 488.
[483] J. Kuszmann and P. Sohár, *Carbohydrate Res.*, 1977, **56**, 105.



(273) R = Me
(274) R = CH_2SMe

be usefully applied to carbohydrates containing acid- or base-sensitive substituents.[484]

Condensation of D-penicillamine with 2,3,4,5,6-penta-*O*-acetyl-*aldehydo*-D-galactose gave 5,5-dimethyl-2(*R*)- and -2(*S*)-(D-*galacto*-1,2,3,4,5-penta-acetoxypentyl)thiazolidine-4(*S*)-carboxylic acid[485] (see Vol. 9, p. 75 for a related reaction with L-cysteine).

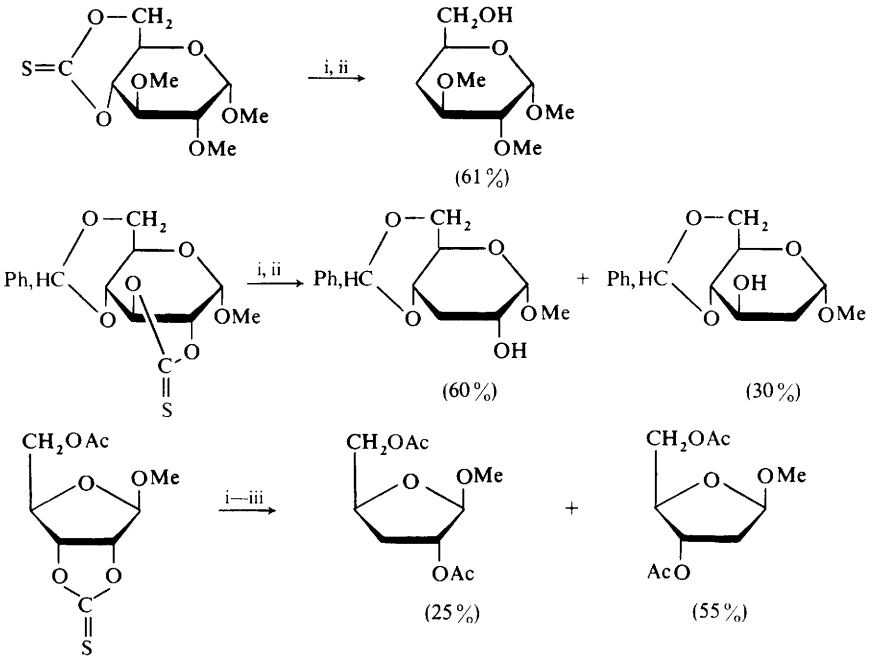
Possible mechanisms for the formation of organic thiocyanates from glucosinolates have been discussed following the observation that extracts of the seed flour of *Thlaspi arvense* converted [1-^{14}C]allylglucosinolate into [3-^{14}C]allyl thiocyanate.[486] The ^{1}H and ^{13}C n.m.r. and mass spectra of glucosinolates (*e.g.* sinigrin) and their derivatives have been recorded to aid in the identification and determination of the vast number of glucosinolates that have been found in plants of the Cruciferae.[487]

5-Thio-D-glucopyranose has been found to reduce significantly the sperm count of male mice and to cause hyperglycemia, while its 6-phosphate is both a competitive inhibitor *in vitro* of *myo*-inositol 1-phosphate synthase from rat testis and a substrate for the reaction catalysed by D-glucose-6-phosphate dehydrogenase from yeast.[488] The effect of 5-thio-D-glucopyranose on the enzymic degradation of glycogen has also been examined.[489]

[484] T. T. Takahashi, C. Y. Nakamura, and J. Y. Satoh, *J.C.S. Chem. Comm.*, 1977, 680.
[485] R. Bognár, Z. Györgydeák, L. Szilágyi, G. Czira, and J. Tamás, *Annalen*, 1977, 1536.
[486] J. Lüthy and M. H. Benn, *Canad. J. Biochem.*, 1977, **55**, 1028.
[487] K. Olsson, O. Theander, and P. Åman, *Carbohydrate Res.*, 1977, **58**, 1.
[488] L. E. Burton and W. W. Wells, *Arch. Biochem. Biophys.*, 1977, **181**, 384.
[489] M. Chen and R. L. Whistler, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 1642.

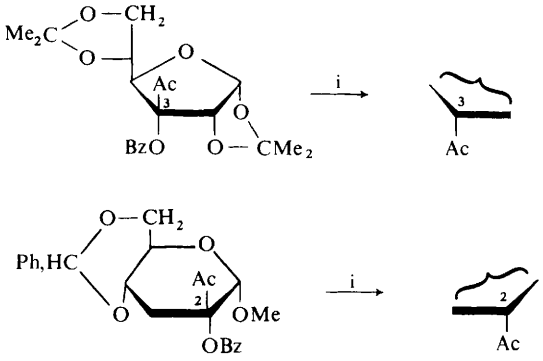
11
Deoxy-sugars

There has been considerable interest in the specific deoxygenation of sugars because the synthesis of many biologically important sugars requires the introduction of one or more deoxy groups. Barton and Subramanian have reported the conversion of thiocarbonates into deoxy-sugars by radical-initiated reduction with tributyltin hydride followed by alkaline hydrolysis.[339] Some examples of the reaction, which complements nicely the ring-opening reactions with methyl iodide (Vol. 9, p. 86), are given in Scheme 46. *C*-Acetyl sugars with a tertiary hydroxy-group at the branch-point can be converted, after benzoylation, into deoxy branched-chain sugars with tributyltin hydride in refluxing toluene in the presence of αα′-azoisobutyronitrile



Reagents: i, Bu_3SnH–refluxing PhMe; ii, NaOH; iii, Ac_2O–py

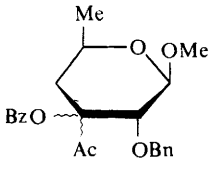
Scheme 46



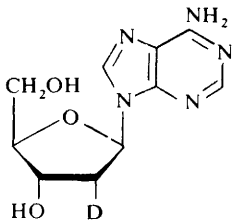
Reagent: i, Bu_3SnH–refluxing PhMe

Scheme 47

(a radical initiator), often with inversion of the configuration at the branch-point (Scheme 47).[490] However, reduction of the isomeric *C*-acetyl benzoates (275) yielded a mixture of methyl 3-*C*-acetyl-2-*O*-benzyl-3,4,6-trideoxy-β-D-*xylo*- and -*ribo*-hexopyranosides, indicating that the reactions proceed by way of a common radical intermediate. Treatment of the TMS derivatives of 9-(2-chloro-2-deoxy-β-D-arabinofuranosyl)adenine or its C-2′ epimer with tributyltin deuteride under free-radical-initiating conditions gave, after deprotection, principally 2′-deoxy-2′-deuterioadenosine (276), with deuteriation of the C-2′ radical intermediate preferably occurring *trans* to the heterocyclic residue at C-1′.[491] A similar stereoselectivity (75%) was also observed on deuterolysis of 1-(3,5-di-*O*-benzoyl-2-chloro-2-deoxy-β-D-ribofuranosyl)uracil.



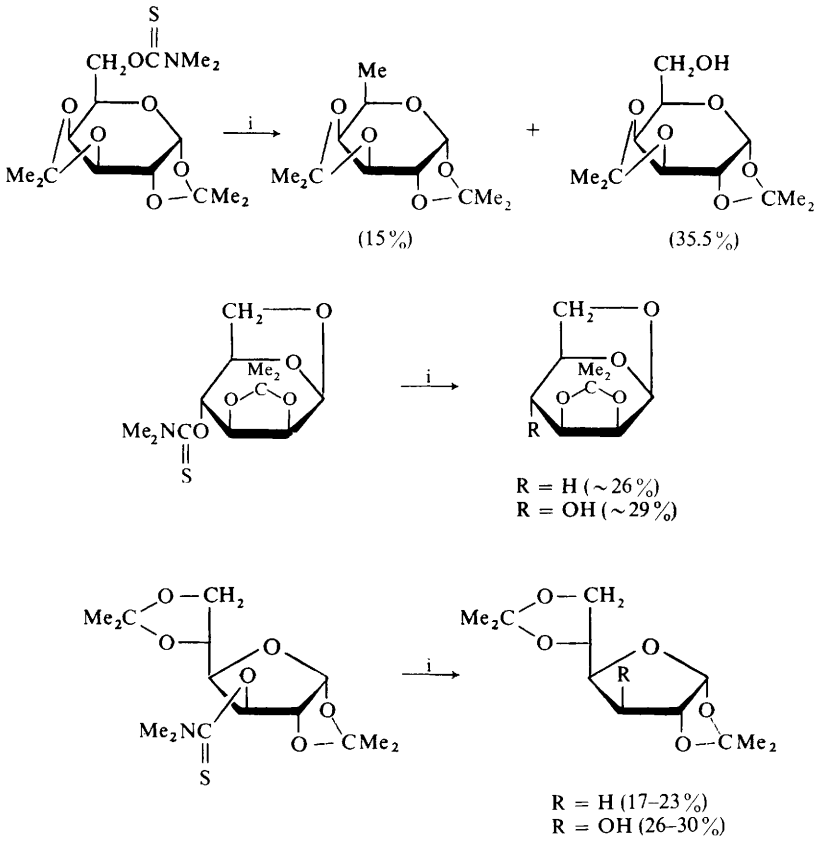
(275)



(276)

Protected sugar derivatives having one free hydroxy-group can be deoxygenated at the alcoholic position by u.v.-irradiation of methanolic solutions of the corresponding dimethylthiocarbamates, although the yields of deoxy-sugars were modest and an accompanying process gave also the original alcohol (Scheme 48).[340]

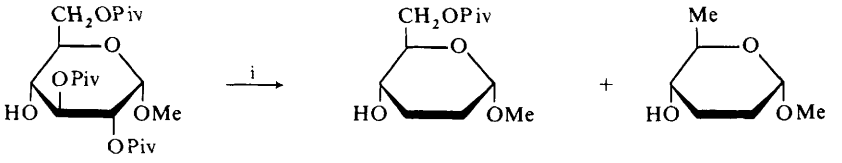
[490] H. Redlich, H.-J. Neumann, and H. Paulsen, *Chem. Ber.*, 1977, **110**, 2911.
[491] M. J. Robins, M. MacCoss, and J. S. Wilson, *J. Amer. Chem. Soc.*, 1977, **99**, 4660.



Reagent: i, *hν*–MeOH

Scheme 48

Photolysis of solutions of acetic and pivalic esters in aqueous HMPT also resulted in deoxygenation; for example, photolysis (λ 254 nm) of methyl 4-*O*-acetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranoside and 1,2:5,6-di-*O*-isopropylidene-3-*O*-pivaloyl-α-D-glucofuranose gave the corresponding 4- and 3-deoxy derivatives, respectively, in yields of 70 and 75%.[304] The method was also extended to hexoses esterified at more than one hydroxy-group; for example, methyl 2,3-dideoxy-4,6-*O*-ethylidene-α-D-*erythro*-hexopyranoside was isolated in 50% yield from the photolysate of methyl 2,3-di-*O*-acetyl-4,6-*O*-ethylidene-α-D-glucopyranoside, while methyl α-D-amicetoside (277) was one of the products obtained on photolysis of methyl 2,3,6-tri-*O*-pivaloyl-α-D-glucopyranoside (Scheme 49). Moreover, photolysis of methyl 2-*O*-acetyl-3,4-*O*-isopropylidene-2-*C*-methyl-β-L-arabinopyranoside (a tertiary alcohol derivative) yielded 2-deoxy-2-*C*-methyl derivatives with the β-L-*ribo* and β-L-*arabino* configurations in a ratio of 85:15.

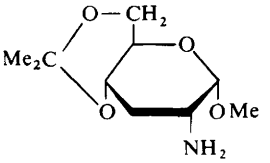


Piv = $COBu^t$ (10%) (277) (20%)

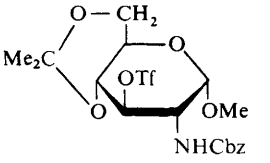
Reagent: i, *hv*–HMPT–H_2O

Scheme 49

Deoxygenation of sugars can also be achieved by way of thio-sugar precursors, which can be desulphurized with Raney nickel or sodium in liquid ammonia or tributyltin hydride. This approach has been used to synthesize methyl 2-amino-2,3-dideoxy-4,6-*O*-isopropylidene-α-D-*ribo*-hexopyranoside (278) from the 3-*S*-phenylthio-α-D-allopyranoside obtained when the 3-triflate (279) reacted with sodium benzenethiolate at 5°C—in this instance, desulphuration was best achieved with sodium in liquid ammonia.[336]



(278)

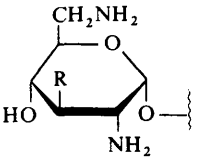


(279) Tf = SO_2CF_3

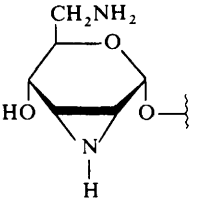
A combination of enzymic and chemical methods has been employed for the selective deoxygenation of aminoglycoside antibiotics.[492] Thus, enzymic phosphorylation of a number of aminoglycoside antibiotics [partial structure (280; R = OH)] gave the corresponding 3′-phosphate (280; R = OPO_3H_2), which was transformed into the 3′-deoxy derivative (*e.g.* 3′-deoxykanamycin B) on treatment with silylating reagents (*e.g.* trimethylchlorosilane and hexamethyldisilazane in pyridine–HMPT at 120°C), hydrolysis to remove the silyl residues, and hydrogenolysis of the resulting 3′-chloro derivative (280; R = Cl). Alternatively, the 3′-phosphates (280; R = OPO_3H_2) could be converted into 3′-deoxy derivatives by hydrogenolysis of the 2′,3′-aziridines (281) formed when they were heated with bis(trimethylsilyl)acetamide and trimethylchlorosilane in pyridine followed by hydrolysis of the products. In another method for synthesizing 3-deoxy-sugars, methyl 5,6-*O*-cyclohexylidene-3-*O*-methanesulphonyl-α- or -β-D-allofuranoside was treated with a large excess of t-butyl magnesium bromide to give methyl 5,6-*O*-cyclohexylidene-3-deoxy-α-D-*arabino*-hexofuranoside or the β-D-*ribo* analogue, respectively.[493] These reactions are thought to proceed *via* the 3-deoxy-2-ulosides formed by a hydride shift.

[492] T. Okutani, T. Asako, K. Yoshioka, K. Hiraga, and M. Kida, *J. Amer. Chem. Soc.*, 1977, **99**, 1278.
[493] M. Kawana and S. Emoto, *Chem. Letters*, 1977, 597.

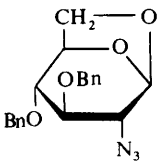
In refluxing hydrazine, the azide (282) gave the monodeoxy and dideoxy compounds (283) and (284), respectively, and, after acetylation, the glycal (285).[494] This procedure can be applied to other azido-sugar derivatives and is suitable for the preparation of 1,5-anhydropentitols from pentopyranosyl azides. The Wolff–Kishner reaction converted 1,5-anhydro-4,6-*O*-benzylidene-2-deoxy-D-*erythro*-hex-3-ulose (286; R = H) into its 3-deoxygenated analogue in high yield, whereas the related α-glycoside (286; R = OMe) afforded a complex mixture of products, mostly arising through elimination processes, or, by a slightly modified procedure, a high yield of the pyrazole derivative (287).[495]



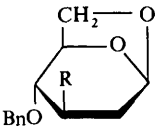
(280) R = OH, Cl, or OPO_3H_2



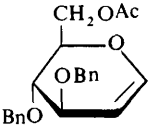
(281)



(282)



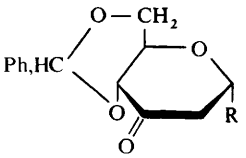
(283) R = OBn
(284) R = H



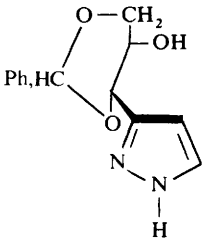
(285)

The 'crossed-formose' reaction between formaldehyde and acetaldehyde has been found to give, in addition to formose sugars, 2-deoxy-DL-pentoses and -glucose.[495a] The sequence of reactions outlined in Scheme 50 has provided a new synthesis of 2-deoxy-D-*erythro*-pentose or its 2-deuteriated analogue (using lithium aluminium deuteride) from 2,3:4,5-di-*O*-isopropylidene-D-arabinose diethyl dithioacetal.[496] Since the 3-*O*-methyl derivative of the keten dithioacetal (288) was not reduced with lithium aluminium hydride, hydride-transfer appears to be directed by an alkoxyaluminium hydride intermediate formed from (288). The key step in a novel synthesis of 2-deoxy-α-glycosides involved the reaction of a glycal (*e.g.* 3,4,6-tri-*O*-acetyl-D-glucal) with an alcohol and NBS in acetonitrile to give mainly the 2-bromo-2-deoxy-α-glycopyranoside.[497] Radical-initiated debromination of the 2-bromo compound with tributyltin hydride then afforded the 2-deoxy-α-glycoside. The *O*-benzylidenehex-3-uloside (286; R = OMe) was converted into 3,5-*O*-

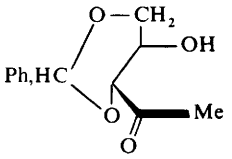
[494] H. Paulsen, D. Schnell, and W. Stenzel, *Chem. Ber.*, 1977, **110**, 3707.
[495] D. Horton, W. Weckerle, L. Odier, and R. J. Sorenson, *J.C.S. Perkin I*, 1977, 1564.
[495a] T. Mizuno, Y. Harada, and Y. Okamura, *Shizuoka Daigaku Nogakubu Kenkyu Hokoku*, 1976, **26**, 45 (*Chem. Abs.*, 1977, **87**, 184 809w).
[496] M. Y. H. Wong and G. R. Gray, *Tetrahedron Letters*, 1977, 1617.
[497] K. Tatsuta, K. Fujimoto, M. Kinoshita, and S. Umezawa, *Carbohydrate Res.*, 1977, **54**, 85.



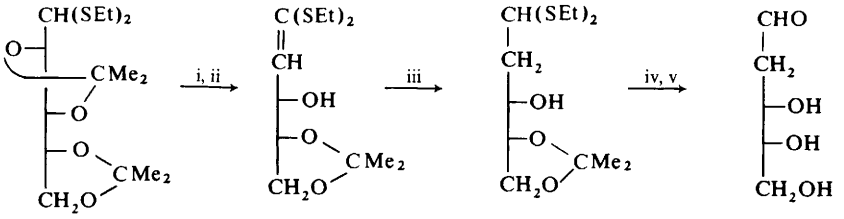
(286) R = H or OMe



(287)



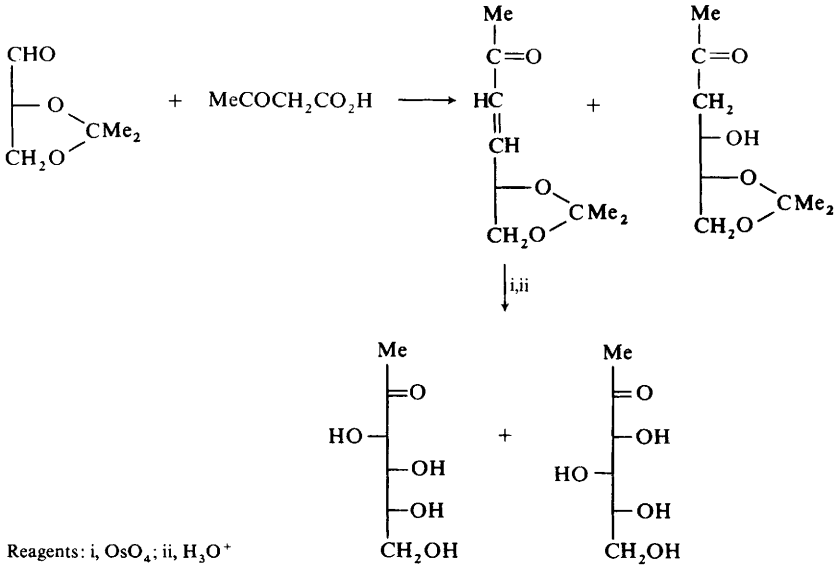
(289)



(288)

Reagents: i, Bu^{t}OK–DMSO–THF; ii, H_2O; iii, $LiAlH_4$–THF; iv, $HgCl_2$–HgO–MeOH; v, H_3O^+

Scheme 50



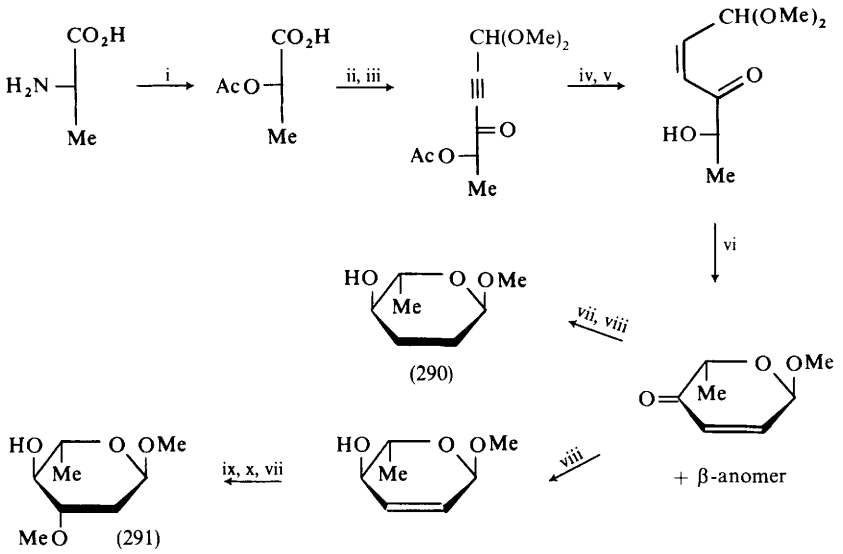
Reagents: i, OsO_4; ii, H_3O^+

Scheme 51

benzylidene-1-deoxy-*keto*-D-*erythro*-2-pentulose (289) by means of lithium hydroxide in a two-phase system.[498] Hydrolysis of (289) gave 1-deoxy-D-*erythro*-2-pentulose, while reduction of (289) with sodium borohydride and hydrolysis of the products gave 1-deoxy-D-ribitol and -arabinitol. A mechanism was suggested for the chain degradation of (286; R = OMe) leading to (289). A route to 1-deoxy- and 1,3-dideoxy-2-hexuloses is available through the reaction of 2,3-*O*-isopropylidene-D-glyceraldehyde with acetoacetic acid (Scheme 51).[499]

A route to derivatives of D-rhamnose is mentioned[361] in Chapter 7 (see Scheme 30), while a new preparation of 5-deoxy-D-lyxose derivatives is based on reductive cleavage of methyl 2,3-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-α-D-lyxofuranoside (prepared from methyl 2,3-*O*-isopropylidene-α-D-mannofuranoside) with sodium borohydride in DMSO.[500] The reductive cleavage of carbohydrate 6-sulphonates with sodium hydrogen bis-(2-methoxyethoxy)aluminate has been discussed[332] in Chapter 6.

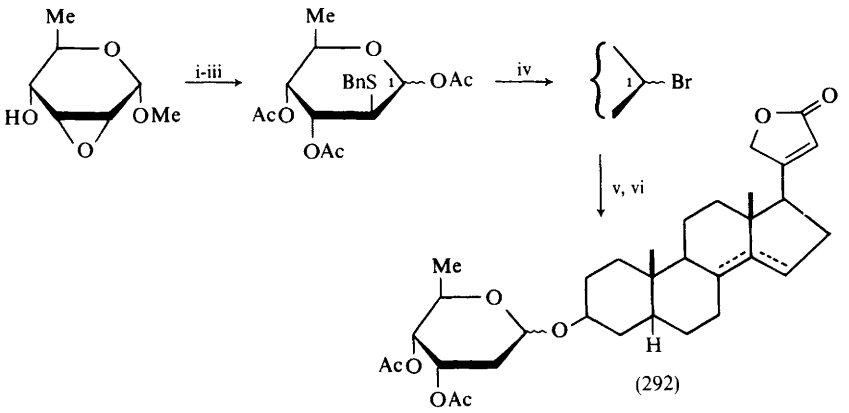
L-Alanine has been used in stereoselective syntheses of methyl α-L-amicetoside (290) and methyl α-L-oleandroside (291), as shown in Scheme 52.[501] Methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside was converted by NBS into methyl 3,4-di-*O*-benzoyl-6-bromo-2,6-dideoxy-α-D-*ribo*-hexopyrano-



Reagents: i, $NaNO_2$–AcOH; ii, SO_2Cl_2; iii, $HC{\equiv}CCH(OMe)_2$–EtMgBr–CuCl; iv, H_2–Pd–$BaSO_4$; v, HO^-; vi, H_3PO_4–CCl_4; vii, H_2–Pd–C; viii, $LiAlH_4$; ix, BnCl–HO^-; x, MeOH–TsOH

Scheme 52

[498] J.-C. Fischer, D. Horton, and W. Weckerle, *Canad. J. Chem.*, 1977, **55**, 4078.
[499] F. J. Lopez Aparicio, M. Gomez Guillen, and I. Izquierdo Cubero, *Anales de Quim.*, 1976, **72**, 938 (*Chem. Abs.*, 1977, **87**, 152 482*t*).
[500] L. M. Lerner, *Carbohydrate Res.*, 1977, **53**, 177.
[501] M. Ohzeki, T. Mizoguchi, K. Koga, and S.-I. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 2676.



Reagents: i, BnSH–MeONa; ii, H_3O^+; iii, Ac_2O–py; iv, HBr–CH_2Cl_2; v, digitoxigenin–$Hg(CN)_2$; vi, Raney Ni

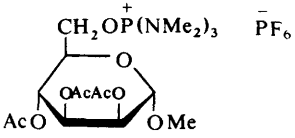
Scheme 53

side, which was the key intermediate in the preparative synthesis of 2,6-dideoxy-D-*ribo*-hexose (digitoxose) and, *via* methyl 3,4-di-*O*-benzoyl-2,6-dideoxy-α-D-*erythro*-hex-5-enopyranoside, of 2,6-dideoxy-L-*lyxo*-hexose (L-oliose).[502] Methyl 3-*O*-benzoyl-2,6-dideoxy-4-*O*-methyl-α-D-*ribo*-hexopyranoside has been synthesized from methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside and shown to differ from benzoylated methyl varioside, which is claimed to have the same structure.[503] 3-(2,6-Dideoxy-D-*ribo*-hexopyranosyl) derivatives of anhydrodigitoxigenin (292) and cholesterol have been synthesized from methyl 2,3-anhydro-6-deoxy-α-D-allopyranoside by a route (Scheme 53) in which the 2-deoxy function was introduced in the final step by desulphuration of the corresponding 2-*S*-benzylthioaltroside.[474]

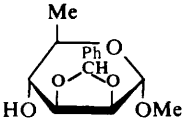
Reduction of the *S*-methyl 3-*O*-dithiocarbonate of 1,2:5,6-di-*O*-isopropylidene-α-D-galacto(or gulo)furanose with tributyltin hydride in refluxing toluene gave 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*xylo*-hexofuranose, which was transformed by conventional reactions into 3,6-dideoxy-D-*xylo*-hexose (abequose).[504] Methyl 3,6-dideoxy-α-D-*arabino*-hexopyranoside (methyl α-D-tyveloside) has been synthesized in seven steps from methyl α-D-mannopyranoside; key steps in the synthesis were the activation of the primary hydroxy-group as the phosphonium salt (293) and the regiospecific cleavage of the *O*-benzylidenerhamnopyranoside (294) with NBS in refluxing carbon tetrachloride.[505] Similar cleavage of the enantiomer of (294), namely methyl 2,3-*O*-benzylidene-α-L-rhamnopyranoside, gave the 3-bromo-2-benzoate (295), which on reduction (lithium aluminium hydride) followed by acid hydrolysis afforded 3,6-dideoxy-L-*arabino*-hexose (ascarylose or

[502] T. M. Cheung, D. Horton, and W. Weckerle, *Carbohydrate Res.*, 1977, **58**, 139.
[503] J. S. Brimacombe, A. S. Mengech, and L. C. N. Tucker, *J.C.S. Perkin I*, 1977, 643.
[504] C. Copeland and R. V. Stick, *Austral. J. Chem.*, 1977, **30**, 1269.
[505] C. Monneret, J.-C. Florent, and Y. Chapleur, *Compt. rend.*, 1977, **285**, *C*, 587.

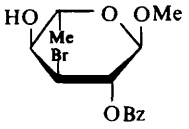
L-tyvelose).[384] Acid-catalysed ring-opening of the racemic epoxide (177) in refluxing methanol and reduction of the product with di-isobutylaluminium hydride gave DL-chalcose (296).[393]



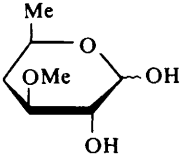
(293)



(294)



(295)



(296)

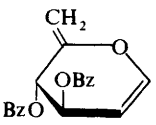
Chapter 19 contains references to dideoxy-sugar derivatives of nucleosides, while Chapters 2, 8, and 13 contain those to 2,3,6-trideoxyhexose derivatives.

12
Unsaturated Derivatives

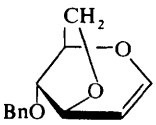
The recent literature on the synthesis and reactions of unsaturated sugar derivatives has been reviewed.[506]

Glycals

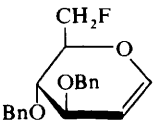
In summarizing their recent work on new derivatives of D-glucal, Guthrie and co-workers have described the preparation of compounds (297)–(300) (see Vol. 10, p. 90 for further details).[507] The dimeric derivative (300) was obtained on methanesulphonylation of 4,6-*O*-isopropylidene-D-glucal, presumably by way of the allylic cation formed on ionization of the initially formed 3-mesylate. The formation of a related dimer (39) as a by-product of an enzyme-catalysed hydration of D-glucal has been noted[141] in Chapter 3. The hydration of D-glucal catalysed by α- or β-D-glucosidase in deuterium oxide is stereospecific, yielding 2-deoxy-D-*arabino*-hexose with a deuterium atom at either the axial or equatorial site, respectively, of the 2-deoxy group.[508] Isotopic labelling has also demonstrated that the hydrogen atom introduced at C-2 is *trans*-related to the aglycone in the addition of glycerol to D-galactal catalysed by the β-D-galactosidase from *E. coli*.[140] 2,6-Anhydro-1-deoxy-D-*galacto*-hept-1-enitol (301) has been prepared by a multistep synthesis from the D-*glycero*-L-*manno*-heptonic acid derivative (302).[509] β-D-Galactosidase catalysed



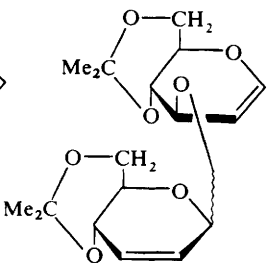
(297)



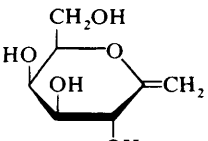
(298)



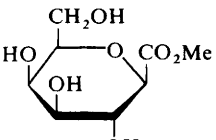
(299)



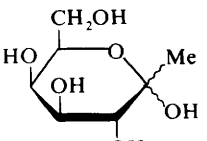
(300)



(301)



(302)



(303)

[506] R. J. Ferrier, *M.T.P. Internat. Rev. Sci., Org. Chem., Series Two*, 1976, **7**, 35 (*Chem. Abs.*, 1977, **86**, 190 351*m*).

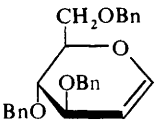
[507] I. D. Blackburne, A. I. R. Burfitt, P. F. Fredericks, and R. D. Guthrie, *A.C.S. Symposium Ser.*, 1976, 39 (Synthetic Methods for Carbohydrates Symposium), 116 (*Chem. Abs.*, 1977, **87**, 68 540*c*).

[508] E. J. Hehre, D. S. Genghof, H. Sternlicht, and C. F. Brewer, *Biochemistry*, 1977, **16**, 1780.

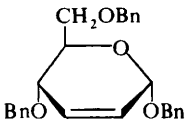
[509] M. Brockhaus and J. Lehmann, *Carbohydrate Res.*, 1977, **53**, 21.

the conversion of (301) into 1-deoxy-D-*galacto*-heptulose (303), and, in the presence of glycerol, into glyceryl 2,6-anhydro-1-deoxy-β-D-*galacto*-heptuloside, which was a substrate for β-D-galactosidase.

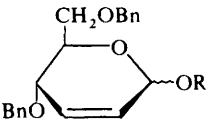
The reaction of glycals with alcohols in the presence of NBS to give the corresponding 2-bromo-2-deoxy-α-glycopyranosides has been noted earlier.[497] Although 3,4,6-tri-*O*-benzyl-D-glucal (304) gave the expected 2-deoxyglycosyl chloride with hydrogen chloride, it yielded the rearranged products (305) and (306) when treated with boron trifluoride etherate or with an alcohol in the presence of boron trifluoride etherate, respectively.[510]



(304)



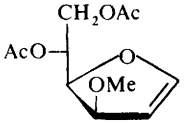
(305)



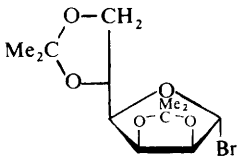
(306) R = Me or Et

1,5-Anhydro-4,6-*O*-benzylidene-2,3-dideoxy-D-*erythro*-hex-1-enitol was among the products formed when methyl 4,6-*O*-benzylidene-3-deoxy-β-D-*erythro*-hexopyranosid-2-ulose reacted with hydrazine hydrate in 2,2'-oxybis(ethanol) (see also p. 103).[495]

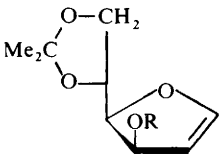
Suppression of acid-catalysed allylic rearrangement and similar reactions in furanoid glycals by the presence of a poor leaving-group at C-3 has permitted the synthesis of (307) in a moderate yield from 1,2,5,6-tetra-*O*-acetyl-3-*O*-methyl-αβ-D-glucofuranose by the Fischer–Zach procedure.[511] Reduction of suitably protected furanosyl bromides [*e.g.* (308)] with sodium in THF ruptured the carbon–bromine bond and, following the elimination of acetone, gave 3-*O*-furanosylfuranoid glycals [*e.g.* (309)] by attack of O-3 (as an anion or a radical) of the initially formed glycal on the unchanged furanosyl halide.[511a]



(307)



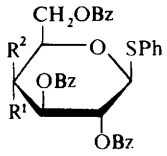
(308)



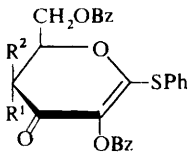
(309) R = 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranosyl

Unusual 1,2-disubstituted glycal derivatives have been encountered during the treatment of phenyl 1-thiohexopyranoside peresters with an excess of NBS in refluxing carbon tetrachloride under bright visible light.[512] For example, phenyl 2,3,4,6-tetra-*O*-benzoyl-1-thio-β-D-glucopyranoside and -galactopyranoside [(310) and (311), respectively] reacted smoothly to give the benzoylated enones (312) and (313), respectively, whereas the corresponding acetates gave enones and small amounts of their 2-bromoacetyl analogues.

[510] G. Descotes and J. C. Martin, *Carbohydrate Res.*, 1977, **56**, 168.
[511] K. Bischofberger and R. H. Hall, *Carbohydrate Res.*, 1976, **52**, 223.
[511a] S. J. Eitelman and A. Jordaan, *J.C.S. Chem. Commun.*, 1977, 552.
[512] R. J. Ferrier and R. H. Furneaux, *J.C.S. Perkin I*, 1977, 1993.

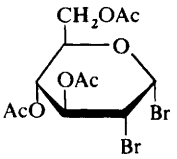


(310) R^1 = OBz, R^2 = H
(311) R^1 = H, R^2 = OBz,

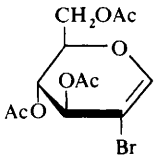


(312) R^1 = OBz, R^2 = H
(313) R^1 = H, R^2 = OBz,

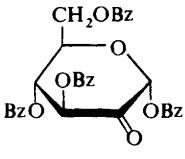
2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide reacted with acetyl bromide–zinc bromide to give the dibromo-compound (314), which readily eliminated hydrogen bromide to give the 2-bromoglycal (315) on treatment with diethylamine.[513] The reaction of the 2-*S*-benzoylthioglycal (261) with hydrogen bromide has been discussed[262] in Chapter 10.



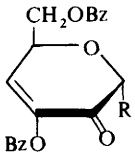
(314)



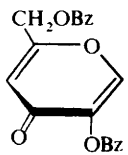
(315)



(316)

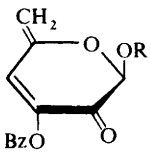


(317) R = OBz, Cl, or Br

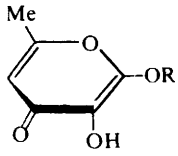


(318)

The chlorination of 2,3,4,6-tetra-*O*-benzoyl-2-hydroxy-D-glucal (138) has been mentioned[347] in Chapter 7. Further work established that the 2-chloro-1,2-benzoxonium salt formed on low-temperature (−30 °C) chlorination of (138) is readily hydrolysed to the glycosulose (316), which readily eliminated benzoic acid to give the enolone (317; R = OBz) on heating with sodium hydrogencarbonate in wet toluene.[514] The enolone (317; R = OBz) afforded α-haloenolones (317; R = Cl



(319) R = Me or Et



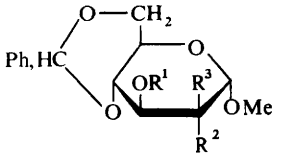
(320) R = Me or Et

[513] A. Fogh, I. Lundt, C. Pedersen, and P. Rasmussen, *Acta Chem. Scand.*, 1977, **B31**, 768.
[514] F. W. Lichtenthaler and U. Kraska, *Carbohydrate Res.*, 1977, **58**, 363.

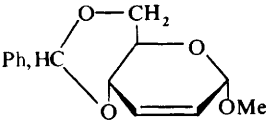
or Br) with hydrogen halides in acetic acid and was transformed into dibenzoylkojic acid (318) on keeping in pyridine at room temperature. Solvolysis of the α-halo-enolones (317; R = Cl or Br) gave β-D-glycenosiduloses, which were converted into (318) on heating with sodium acetate–acetic acid or into the 3,5-dienones (319) on treatment with pyridine at room temperature. The 3,5-dienones (319) were not readily isolated, but were converted into the alkoxyallomaltols (320) upon the addition of water.

Other Unsaturated Derivatives

Reaction of vicinal dixanthates [bis(dithiocarbonates)] with tributyltin hydride in refluxing toluene (or benzene) gave the corresponding olefin in high yield; for example, the dixanthates (321) and (322) were transformed into methyl 4,6-*O*-benzylidene-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (323) in yields of 59 and 79%, respectively, by this procedure, which is conducted at low temperatures under mild, neutral conditions.[341] The elimination of 2- and 3-sulphonyloxy-groups from methyl 4,6-*O*-benzylidene-α- and -β-D-mannopyranosides and -glucopyranosides by the Tipson–Cohen reaction (sodium iodide–zinc in DMF) has been studied.[335] The reactions were found to be strongly influenced by the anomeric configuration of the substrate and by steric and electronic factors affecting the reactivity of the 3-sulphonyloxy-group towards displacement by iodide ion—the order of reactivity found was β-*gluco* > β-*manno* > α-*gluco* > α-*manno*.



(321) $R^1 = CS_2Me$, $R^2 = OCS_2Me$, $R^3 = H$
(322) $R^1 = CS_2Me$, $R^2 = H$, $R^3 = OCS_2Me$



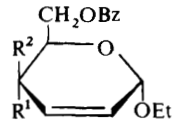
(323)

Iodinolysis of the allylic 4-mesylate (324) in the presence of pyridine gave the 4-iodo derivative (325), which isomerized to (326) when pyridine was omitted.[515] Reduction of (325) with lithium aluminium deuteride gave, after benzoylation, the deuteriated products (327) (with retention of configuration at C-4), (328) (by allylic deuterolysis), and (329) (*via* ring-opening), while dehydroiodination of (325) with 1,5-diazabicyclo[4,3,0]non-5-ene in THF gave the hexadienone (330), presumably by way of an intermediate 2*H*-pyran derivative.

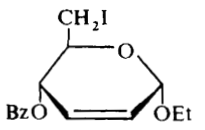
Racemic butyl (α-hex-2-enopyranosid)uronates and the disaccharide derivative (331) have been synthesized by the simple reactions outlined in Scheme 54.[516]

Syntheses of DL-desosamine and DL-chalcose from parasorbic acid (332) have been mentioned[393] in Chapters 8 and 11, respectively. Reduction of (332) with di-isobutylaluminium hydride gave 2-hydroxy-6-methyl-5,6-dihydro-2*H*-pyran, which afforded the 2-alkoxy-6-methyldihydropyrans (333) on treatment with a molar

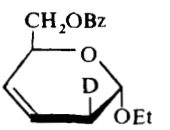
[515] M. B. Yunker and B. Fraser-Reid, *Canad. J. Chem.*, 1976, **54**, 3986.
[516] R. R. Schmidt and R. Angerbauer, *Angew. Chem. Internat. Edn.*, 1977, **16**, 783.



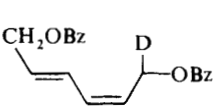
(324) R^1 = OMs, R^2 = H
(325) R^1 = H, R^2 = I
(327) R^1 = H, R^2 = D



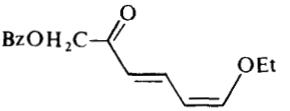
(326)



(328)

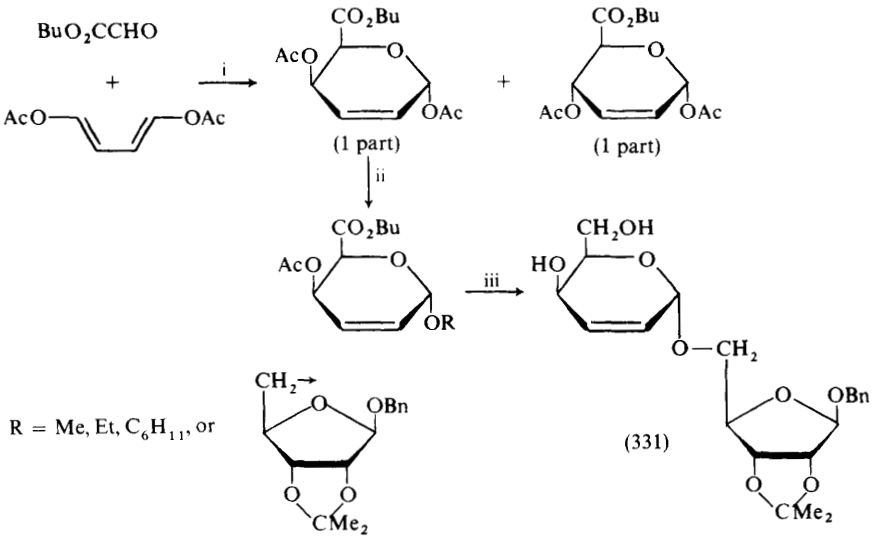


(329)



(330)

equivalent of trimethyl or triethyl orthoformate.[517] On treatment with strong bases (332) gave (2*Z*,4*E*)-hexadienoic acid, which was transformed into osmunda lactone (334) and the isomeric γ-lactone (335) on selective dihydroxylation with 3-chloroperbenzoic acid.



R = Me, Et, C_6H_{11}, or

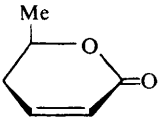
(331)

Reagents: i, heat–PhH, ii, ROH–$BF_3 \cdot Et_2O$; iii, $LiAlH_4$

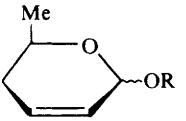
Scheme 54

Oxidation of methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside with DMSO–acetic anhydride yielded mainly the enolone (336; R = OBz), owing to the tendency of the initially formed hexopyranosid-4-ulose to eliminate benzoic acid.[518] The enolone (336; R = OBz) yielded the hydroxymaltol derivative (337) or (338) when treated with acetic acid–sodium acetate or with trifluoroacetic acid, respectively. Mechanisms for the conversions (336) → (337) and (338) were proposed on the

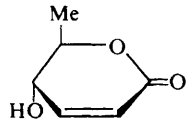
[517] K. Torssell and M. P. Tyagi, *Acta Chem. Scand.*, 1977, **B31**, 297.
[518] F. W. Lichtenthaler, S. Ogawa, and P. Heidel, *Chem. Ber.*, 1977, **110**, 3324.



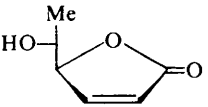
(332)



(333) R = Me or Et

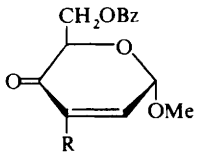


(334)

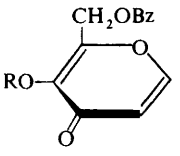


(335)

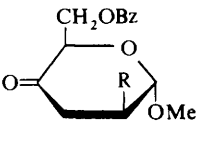
basis of some of the intermediates isolated when the reaction was carried out under mild conditions. Methyl 6-*O*-benzoyl-2,3-dideoxy-α-D-*glycero*-hex-2-enopyranosid-4-ulose (336; R = H) reacted with hydrazoic acid in chloroform to give only the *threo*-adduct (339; R = N_3), and with tributylborane (radical initiation) to give an adduct tentatively identified as the 2-*C*-butyl derivative (339; R = Bu).[519] These and other results show that axial attack (*i.e. trans* to the aglycone) occurs exclusively or predominantly in the nucleophilic and radical reactions of (336; R =



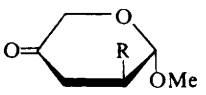
(336) R = OBz or H



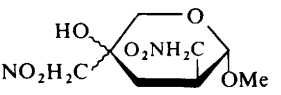
(337) R = Bz
(338) R = H



(339) R = N_3 or Bu

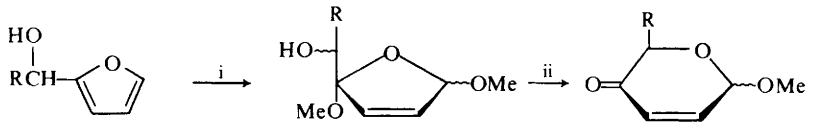


(340) R = CH_2NO_2, $CH(CO_2Et)_2$, or $CH(CN)CO_2Et$



(341)

H) and related α-D-hex-2-enopyranosid-4-ulose derivatives (see also p. 125). Nucleophilic addition of nitroalkanes to 6-(*S*)-methoxy-2*H*-pyran-3(6*H*)-one [*i.e.* a pentose analogue of (336; R = H)] also yielded the 1,4-addition products (340) arising from axial attack, as well as the product (341) of both 1,4- and 1,2-additions with nitromethane.[520] Scheme 55 summarizes a ready preparation of

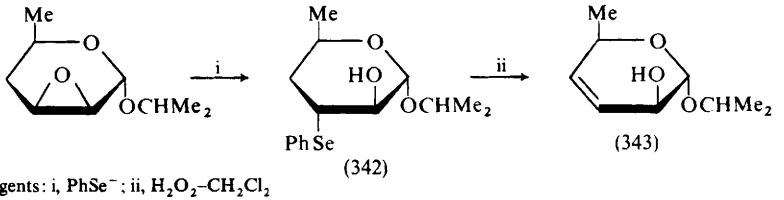


R = H or Me

Reagents: i, $MeOH-Br_2$; ii, HCO_2H–MeOH

Scheme 55

[519] T. Sakakibara, T. Kawahara, and R. Sudoh, *Carbohydrate Res.*, 1977, **58**, 39.
[520] G. Grynkiewicz, O. Achmatowicz, jun., and H. Barton, *Roczniki Chem.*, 1977, **51**, 1663.

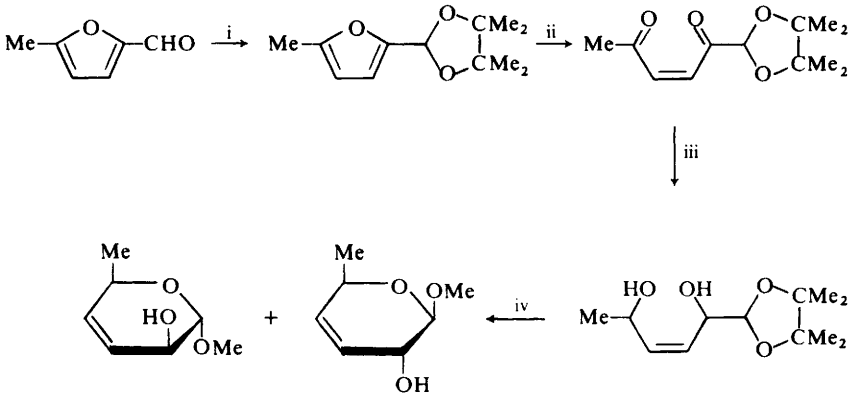


Reagents: i, $PhSe^-$; ii, H_2O_2–CH_2Cl_2

Scheme 56

6-methoxy-2*H*-pyran-3(6*H*)-one and its 2-methyl derivative from furfuryl alcohols.[521]

A new procedure for introducing an olefinic linkage into sugars has involved the treatment of vicinal dimesylates with the sodium–naphthalene reagent.[522] The 3′,4′-dimesylates of butirosin and kanamycin gave the corresponding 3′,4′-unsaturated derivatives with this reagent in yields comparable to those obtained using the Tipson–Cohen procedure. Oxidative elimination of phenyl selenide from the racemic compound (342) gave a modest yield of the 3,4-unsaturated derivative (343) (Scheme 56).[517] Methyl 3,4,6-trideoxy-DL-*threo*- and *-erythro*-hex-3-enopyranosides related to (343) can also be derived from 5-methyl-2-furaldehyde by the reactions shown in Scheme 57.[523]



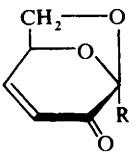
Reagents: i, $Me_2COHCOHMe_2$–H^+; ii, Br_2–H_2O; iii, $NaBH_4$; iv, MeOH–H^+

Scheme 57

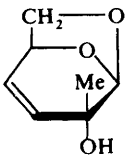
The elimination of hydrogen bromide from methyl 2-*O*-benzoyl-3-bromo-3,6-dideoxy-4-*O*-methyl-α-L-altropyranoside (168) under azidolysis conditions to give the 3,4-unsaturated sugar azide (169), *etc.*, has been referred to[384] in Chapter 8.

[521] P. D. Weeks, D. E. Kuhla, R. P. Allingham, H. A. Watson, jun., and B. Wlodecki, *Carbohydrate Res.*, 1977, **56**, 195.
[522] T. Hayashi, N. Takeda, H. Saeki, and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 2134.
[523] R. Bognár and P. Herczegh, *Carbohydrate Res.*, 1977, **54**, 292.

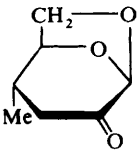
The preparation of levoglucosenone (344) by pyrolysis of waste paper and cellulose, *etc.*, has been discussed.[524] A variety of deoxy-, keto-, and branched-chain sugars was obtained by reduction of (344) and by its reaction with Grignard reagents under different conditions; for example, (344) reacted with methyl magnesium iodide at room temperature to give a 1,2-addition product, which was assigned the structure (345), whereas it gave mainly the 1,4-addition product (346) at −78°C in the presence of tetrakis[iodo(tributylphosphine)copper(I)][$(Bu_3PCuI)_4$].[525] The related enones (347) and (348) have been synthesized from sedoheptulosan using the Corey–Winter procedure to introduce the olefinic linkage, followed by oxidation of the resulting allylic alcohols with manganese dioxide.[526]



(344) R = H
(347) R = CH_2OH
(348) R = Me

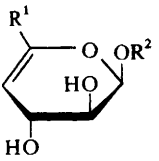


(345)

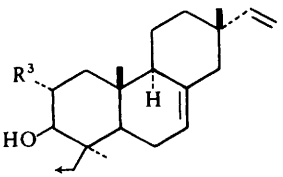


(346)

Two new metabolites, virescenosides D and H, isolated from the fungus *Acremonium luzulae* (Fuckel) Gams have been identified, on the basis of chemical and spectroscopic evidence, as the 4,5-unsaturated glycosides (349) and (350), respectively.[527] Two other metabolites, virescenosides E and L, isolated from this fungus differ from (349) and (350) in the nature of the aglycone (virescenol A instead of virescenol B).[527a] Enantiomeric forms of 9-(5-deoxy-β-*erythro*-pent-4-enofuranosyl)adenine have been synthesized by way of dehydroiodination of appropriately protected 5′-deoxy-5′-iodo-nucleosides with DBU in DMF.[500]

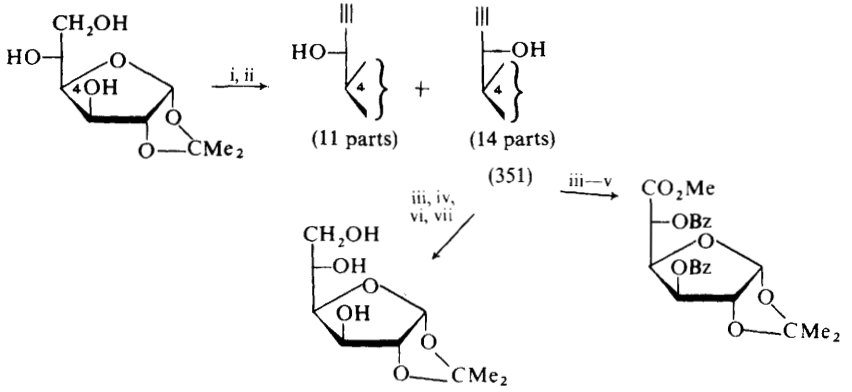


(349) R^1 = CHO, R^2 = virescenol B
(350) R^1 = CH_2OH, R^2 = virescenol B



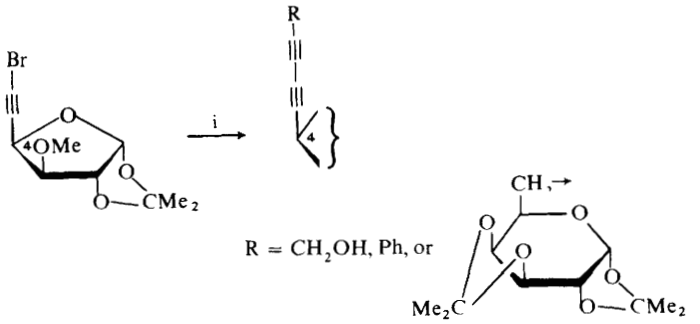
virescenol B R^3 = H
virescenol A R^3 = OH

[524] F. Shafizadeh and P. P. S. Chin, *A.C.S. Symposium Ser.*, 1976, 39 (Synthetic Methods for Carbohydrates Symposium), 179 (*Chem. Abs.*, 1977, **87**, 23 622*q*).
[525] F. Shafizadeh and P. P. S. Chin, *Carbohydrate Res.*, 1977, **58**, 79.
[526] P. Köll and T. Schultek, *Chem. Ber.*, 1977, **110**, 1994.
[527] N. Cagnoli-Bellavita, P. Ceccherelli, M. Ribaldi, J. Polonsky, Z. Baskevitch-Varon, and J. Varenne, *J.C.S. Perkin I*, 1977, 351.
[527a] P. Ceccherelli, N. Cagnoli-Bellavita, J. Polonsky, Z. Baskevitch-Varon, and M. Ribaldi, *Gazz. Chim. Ital.*, 1977, **107**, 51 (*Chem. Abs.*, 1977, **87**, 102 593*j*).



Reagents: i, $NaIO_4$; ii, $HC{\equiv}CMgBr$, iii, BzCl–py; iv, O_3; v, CH_2N_2; vi, B_2H_6; vii, NaOMe

Scheme 58



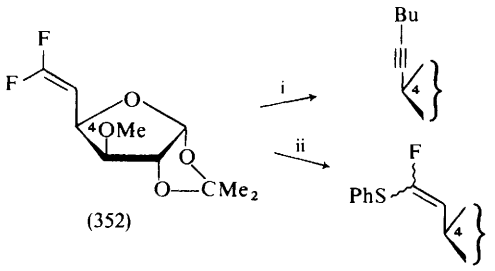
Reagent: i, $RC{\equiv}CH$–CuCl–$EtNH_2$–NH_2OH

Scheme 59

A separable mixture of the 6,7-dideoxy-β-L-*ido*-6-ynofuranose derivative (351) and its α-D-*gluco* analogue was obtained on treatment of the products from the periodate oxidation of 1,2-*O*-isopropylidene-α-D-glucofuranose with ethynyl magnesium bromide.[528] Ozonolysis, *etc.*, of the 3,5-dibenzoate of (351) provided a convenient route to derivatives of L-idose and L-iduronic acid (Scheme 58). Tronchet's group has reported the coupling of a brominated acetylenic sugar with phenylacetylene, prop-2-yn-1-ol, and terminal acetylenic sugars to give novel diynes (Scheme 59) and some reactions of the *gem*-difluoro-olefinic sugar (352) (Scheme 60).[529]

1,2:4,5-Di-*O*-isopropylidene-3-*O*-methyl-β-D-fructopyranose reacted with butyllithium in THF at −30°C to give the 1,2-didehydro-D-*arabino*-hexitol derivative (353).[530] The formation of (2*E*,4*Z*)-2,5-dihydroxy- and (2*E*,4*Z*)-2,5-dialkoxy-6-oxo-2,4-hexadienoic acids by base-catalysed eliminations from the methyl glyco-

[528] D. Horton and J.-H. Tsai, *Carbohydrate Res.*, 1977, **58**, 89.
[529] J. M. J. Tronchet and A. Bonenfant, *Helv. Chim. Acta*, 1977, **60**, 892.
[530] A. Klemer and D. Balkau, *Annalen*, 1977, 181.

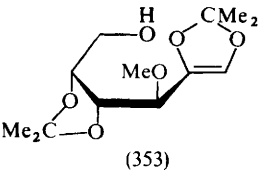


(352)

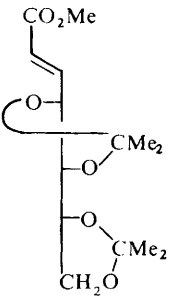
Reagents: i, BuLi; ii, PhS^-

Scheme 60

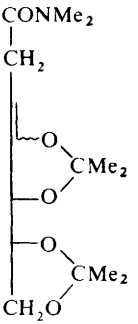
sides of D-glucofuranurono-6,3-lactone and corresponding 2,5-di-*O*-alkyl derivatives has been examined.[531] The cyclization of the 2,3-dideoxyhex-2-enonic ester derivative (84) to the 3,6-anhydro-2-deoxyhexonic acid derivative (85) has already been noted[235] in Chapter 3. The di-*O*-isopropylidene derivative (354) of a related 2,3-dideoxyhept-2-enonic ester rearranged to (355) on treatment with dimethylamine. Removal of the isopropylidene groups from (355) furnished the dimethylamide derivative (356) of 2,3-dideoxy-D-*erythro*-hept-4-ulosonic acid. The value of this and related rearrangements in the synthesis of antibiotic deoxysugars was discussed.



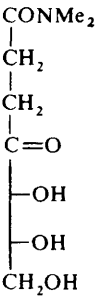
(353)



(354)



(355)



(356)

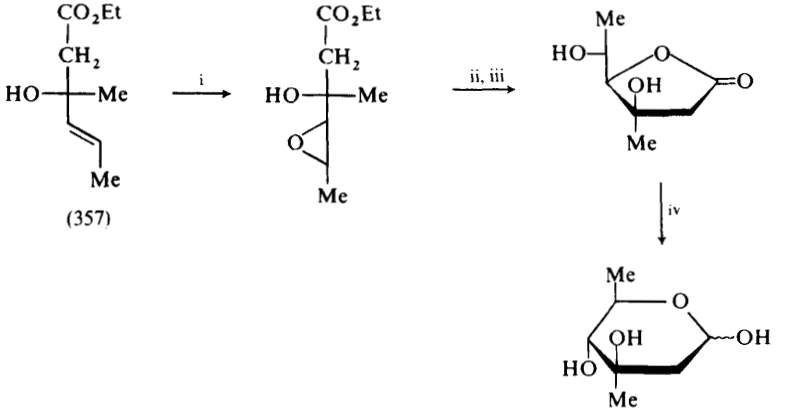
[531] K. Dax, N. A. Gassner, and H. Weidmann, *Annalen*, 1977, 169.

13
Branched-chain Sugars

Compounds with an R^1—C—OR^2 Branch

Details of the synthesis of a derivative of pillarose (2,3,6-trideoxy-4-*C*-glycolyl-L-*threo*-hexose) by Paulsen's group have been published[532] (see Vol. 10, p. 98).

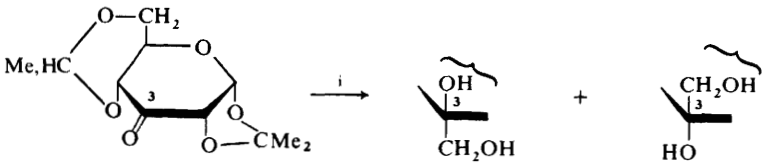
The optically pure acid derivative (357) has been converted into evermicose (2,6-dideoxy-3-*C*-methyl-D-*arabino*-hexose) (358), one of the constituent sugars of everninomicins B and D, by the sequence of reactions shown in Scheme 61.[533]



Reagents: i, $MeCO_3H$; ii, H_3O^+; iii, HO^-; iv, Bu^i_2AlH

Scheme 61

Photochemical cross-pinacolization of acetalated pyranos-3-ulose derivatives with methanol has provided a route to *C*-hydroxymethyl sugar derivatives (Scheme 62), although significant amounts of 4,6-*O*-ethylidene-D-allono-1,5-lactone were

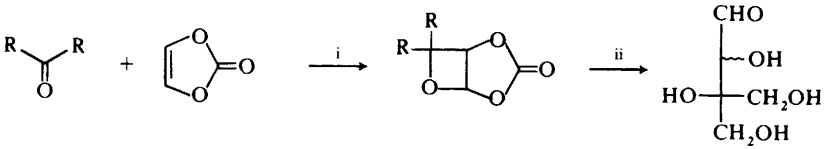


Reagent: i, *hν*–MeOH

Scheme 62

[532] H. Paulsen, K. Roden, V. Sinnwell, and W. Koebernick, *Chem. Ber.*, 1977, **110**, 2146.
[533] I. Dyong and D. Glittenberg, *Chem. Ber.*, 1977, **110**, 2721.

also produced in the reaction illustrated.[534] Photochemical cycloaddition of 1,3-dihydroxypropan-2-one diacetate to 1,3-dioxol-2-one gave an oxetane that furnished DL-apiose on hydrolysis with alkali (Scheme 63).[535] Photoamidation of

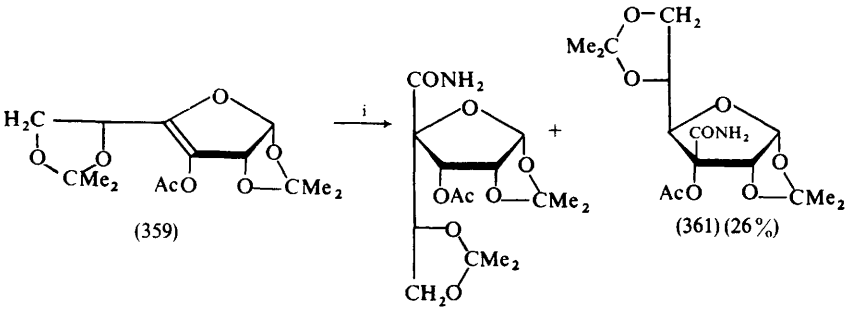


R = CH_2OAc

Reagents: i, *hv*–PhH; ii, HO^-–H_2O

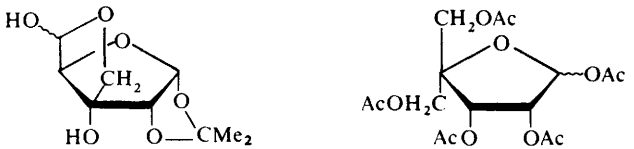
Scheme 63

the enol acetate (359) afforded the 4- and 3-*C*-carbamoyl derivatives (360) and (361) (Scheme 64).[536] The structure of (361) was determined by correlation with the known 5,3^1-hemiacetal (362), while standard transformations on (360) gave 4,4-di-*C*-acetoxymethyl-1,2,3-tri-*O*-acetyl-αβ-D-erythrofuranose (363), whose conversion into isomers of psicofuranine is mentioned in Chapter 19.



Reagent: i, $HCONH_2$–Me_2CO–*hv*

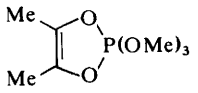
Scheme 64



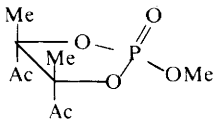
Partial hydrolysis of the *meso*-oxyphosphorane obtained when butanedione reacts with the 1,3,2-dioxaphospholene (364) gave two cyclic phosphotriesters [e.g. (365)] that differ in the configuration at the phosphorus atom.[537] Further

[534] P. M. Collins, V. R. N. Munasinghe, and N. N. Oparaeche, *J.C.S. Perkin I*, 1977, 2423.
[535] Y. Araki, J.-I. Nagasawa, and Y. Ishido, *Carbohydrate Res.*, 1977, **58**, C4.
[536] A. Rosenthal and M. Ratcliffe, *Carbohydrate Res.*, 1977, **54**, 61.
[537] F. Ramirez, J. S. Ricci, O. P. Madan, J. F. Marecek, and H. Tsuboi, *J. Amer. Chem. Soc.*, 1977, **99**, 5135.

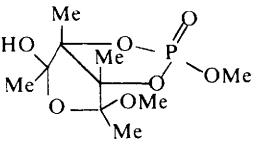
reaction of (365) or its isomer with methanol yielded diastereoisomeric cyclic derivatives [*e.g.* (366)], which rearranged to the furanoic cyclic phosphate (367) on keeping in acetonitrile.



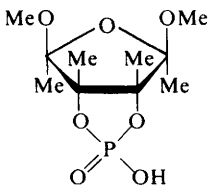
(364)



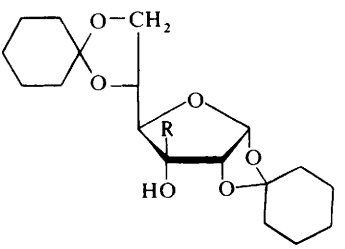
(365)



(366)



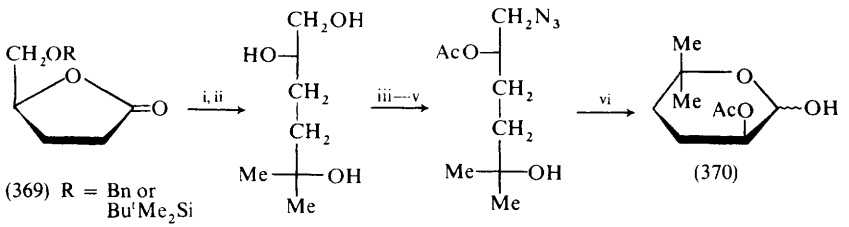
(367)



(368) R = CN or $CONH_2$

The cyanohydrin (368; R = CN) has been prepared in high yield by reaction of 1,2:5,6-di-*O*-cyclohexylidene-α-D-*ribo*-hexofuranos-3-ulose with potassium hexacyanoferrate(III) and potassium carbonate.[537a] Mild hydrolysis of (368; R = CN) gave the 3-*C*-carboxamide (368; R = $CONH_2$).

The lactone derivatives (369) prepared from L-glutamic acid (2-amino-2,3,4-trideoxy-L-*glycero*-pentaric acid) can be converted into the 5,5-di-*C*-methylpentose derivative (370) by the reactions outlined in Scheme 65.[538] A salient feature of the synthesis is that the chirality of L-glutamic acid is preserved at C-2 of (370). The enone (371) [prepared from 2-(2-furyl)propan-2-ol] has been converted into methyl 2,3-*O*-isopropylidene-5,5-di-*C*-methyl-β-DL-ribopyranoside (372) by *cis*-dihydroxylation (silver chlorate–osmium tetroxide) and stereoselective reduction (lithium aluminium hydride) of the 2,3-*O*-isopropylidene derivative of the resulting diol.[15]



(369) R = Bn or Bu^tMe_2Si

(370)

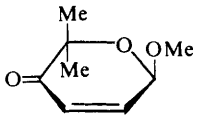
Reagents: i, MeMgI; ii, NH_4F–THF (or H_2–Pd); iii, TsCl–py; iv, NaN_3–DMF; v, Ac_2O–py; vi, *hν*–PhH

Scheme 65

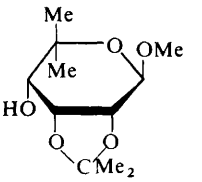
[537a] Yu. A. Zhdanov, Yu. E. Alekseev, G. V. Zinchenko, and S. S. Doroshenko, *Doklady Akad. Nauk S.S.S.R.*, 1976, **231**, 868 (*Chem. Abs.*, 1977, **86**, 121 645*y*).

[538] W. A. Szarek, D. M. Vyas, and L. Chen, *Carbohydrate Res.*, 1977, **53**, C1.

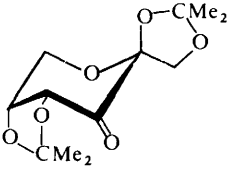
The value of the 1,3-dithian route to branched-chain sugar derivatives has been illustrated further by stereospecific syntheses of 3-*C*-formyl, 3-*C*-hydroxymethyl, and 3-*C*-methyl derivatives of D-psicose from the ketone (373) *via* the dithianyl adduct (374).[539] Addition of nitromethane to (373) gave both 3-*C*-nitromethyl-D-psicose and -fructose derivatives, which, after separation by chromatography, were reduced to the corresponding 3-*C*-aminomethyl derivatives. The use of dithianyl derivatives in the synthesis of streptomycin components is dealt with in Chapter 18.



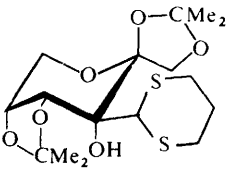
(371)



(372)

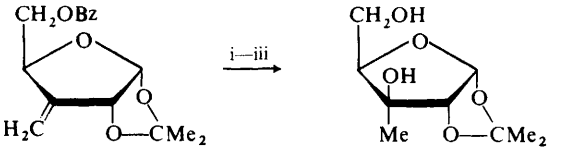


(373)



(374)

Derivatives of 3-*C*-methyl-D-xylofuranose have been obtained by the route shown in Scheme 66.[540] A key intermediate (363) in the synthesis of 4′-*C*-hydroxymethyl-nucleosides (Chapter 19) was obtained by way of aldol coupling of the furanose 5-aldehyde (375) with formaldehyde and subsequent Cannizzaro reduction of the condensation product by formaldehyde.[541]



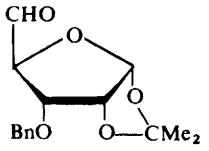
Reagents: i, OsO_4; ii, TsCl–py; iii, $LiAlH_4$

Scheme 66

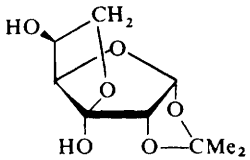
Treatment of methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-*arabino*-hexopyranosid-2-ulose with an excess of methyl magnesium iodide gave, as the main product, methyl 4,6-*O*-benzylidene-2-*C*-methyl-α-D-glucopyranoside.[27]

The hemiacetal (376) formed by 1,2-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose has been converted into the 5,6-diacetate (377) by protection of the carbonyl group as the oxime, acetylation of the oxime, and deoximation with chro-

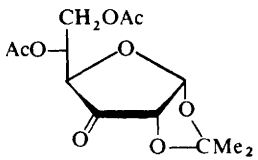
[539] G. Vass, A. M. Sepulchre, and S. D. Gero, *Tetrahedron*, 1977, **33**, 321.
[540] J. M. J. Tronchet and J. Tronchet, *Helv. Chim. Acta*, 1977, **60**, 1984.
[541] R. Youssefyeh, D. Tegg, J. P. H. Verheyden, G. H. Jones, and J. G. Moffatt, *Tetrahedron Letters*, 1977, 435.



(375)



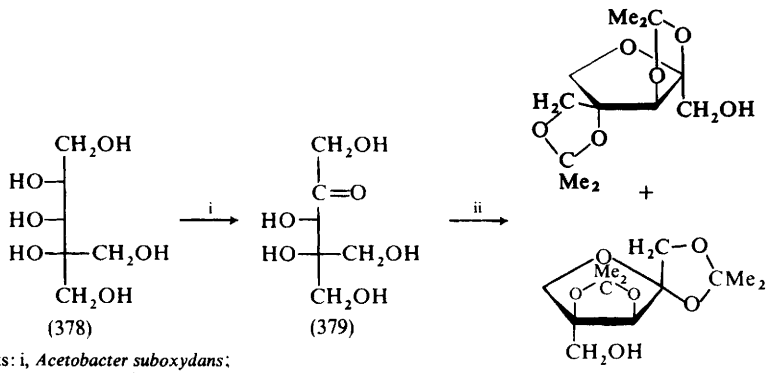
(376)



(377)

mium(II) acetate.[542] Condensation of nitromethane with (376) and (377) gave 3-*C*-nitromethyl derivatives having the D-*gluco* and D-*allo* configurations, respectively. The difference in the stereoselectivity was attributed to isomerization of the D-*allo* to the D-*gluco* compound when (376) condenses with nitromethane. The products obtained from analogous condensations with 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose were shown to depend on the reaction conditions, with low temperatures favouring the formation of the D-*allo* adduct.

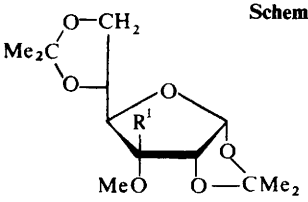
A synthesis of L-dendroketose (379) has been achieved by microbiological oxidation of 2-*C*-hydroxymethyl-D-*erythro*-pentitol (378) with *Acetobacter suboxydans* (Scheme 67).[543] Acetonation of L-dendroketose (379) gave 1,2:3,4- and 2,3:4,4[1]-di-*O*-isopropylidene derivatives, whose configurations at C-4 and the anomeric centre were assigned with the aid of ^{13}C n.m.r. spectroscopy.



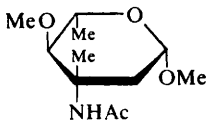
(378) (379)

Reagents: i, *Acetobacter suboxydans*;
ii, Me_2CO–H^+ or Me_2CO–$ZnCl_2$–$CuSO_4$

Scheme 67



(380) R^1 = C≡CH
(381) R^1 = C≡CCHOHR^2, R^2 = Pr^i or C_9H_{17}
(382) R^1 = CH=CHC(O)$C_6H_4R^2$-*p*, R^2 = H, Me, or Cl



(383)

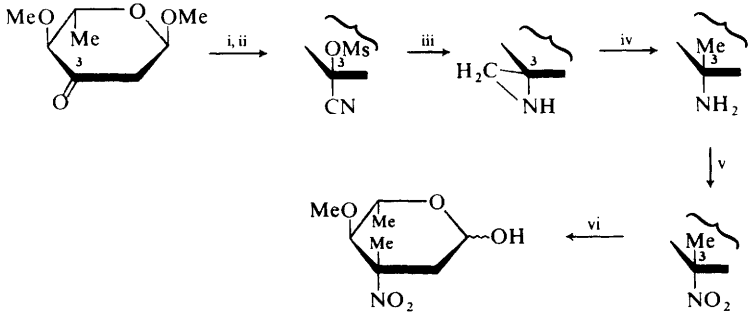
[542] K. Sato, J. Yoshimura, and C. Shin, *Bull. Chem. Soc. Japan*, 1977, **50**, 1191.
[543] W. A. Szarek, G. W. Schnarr, H. C. Jarrell, and J. K. N. Jones, *Carbohydrate Res.*, 1977, **53**, 101.

The branched-chain acetylenic sugar derivative (380) reacted with aliphatic and aromatic aldehydes in the presence of a strong base to give the alkynols (381) and the enones (382), respectively.[544] The synthesis of analogues of (381) and the conversion of the analogues into substituted pyrazole derivatives are referred to[463a] in Chapter 9.

The structure of nogalamycin, an antibiotic containing the branched-chain sugar nogalose, and that of D-hamamelose in solution are mentioned in Chapters 18 and 20, respectively. The preparation of an unusual trihalogenated, branched-chain heptulose (147) from xylaric acid has been discussed[359] in Chapter 7.

Compounds with an R—C—N Branch

X-Ray crystallography has established the stereochemistry at the branching point of methyl 3-acetamido-2,3,6-trideoxy-3*C*,4*O*-dimethyl-β-L-*arabino*-hexopyranoside (383), a derivative of evernitrose (384) (the nitro-sugar component of everninomicins B, C, and D).[545] Evernitrose (384) has also been synthesized from methyl 2,6-dideoxy-4-*O*-methyl-α-L-*erythro*-hexopyranosid-3-ulose (Scheme 68).[546]



(384)

Reagents: i, HCN–py; ii, MsCl; iii, $LiAlH_4$; iv, H_2–Ni; v, *m*-$ClC_6H_4CO_3H$; vi, H_3O^+

Scheme 68

Compounds with an R—C—H Branch

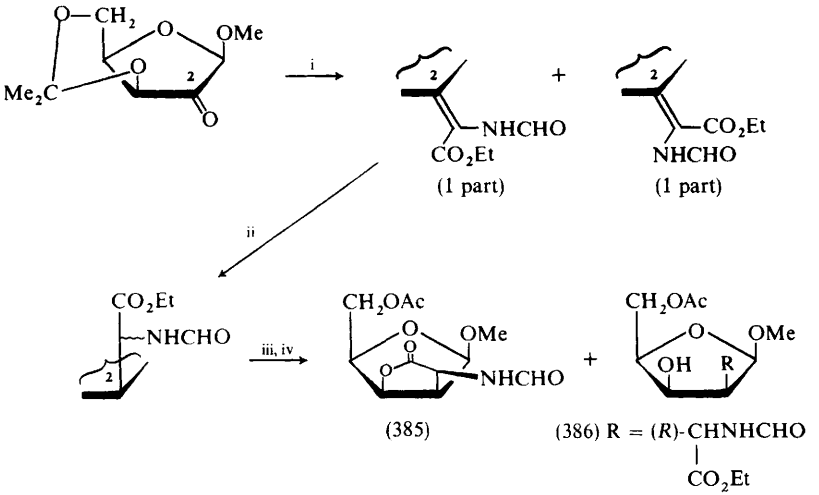
The deoxygenation of branched-chain sugars containing a tertiary hydroxy-group has been referred to[490] in an earlier chapter. Hydrogenation of the products obtained on formylaminomethylenation (see also p. 70) of methyl 3,5-*O*-isopropylidene-β-D-*threo*-pentofuranosid-2-ulose has provided a route to branched-chain compounds bearing a protected amino-acid residue at C-2 (Scheme 69).[547] Hydrolysis, *etc.*, to (385) or (386) established the *S*- or *R*-configuration of the branched-chain precursors, since the formation of a lactone was seriously impeded by the methoxy-group at C-1 when the amino-acid residue had the *R*-configuration. Formation of the 2^1,3-lactone (387), as well as c.d. measurements, was simi-

[544] A. Gonzalez, M. Orzaex, and R. Mestres, *Anales de Quim.*, 1976, **72**, 954 (*Chem. Abs.*, 1977, **87**, 168 300t).

[545] A. K. Ganguly, O. Z. Sarre, A. T. McPhail, and K. D. Onan, *J.C.S. Chem. Comm.*, 1977, 313.

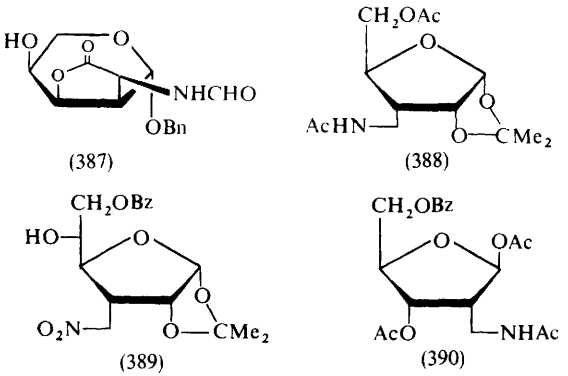
[546] J. Yoshimura, M. Matsuzawa, K.-I. Sato, and M. Funabashi, *Chem. Letters*, 1977, 1403.

[547] K. Bischofberger, A. J. Brink, O. G. de Villiers, R. H. Hall, and A. Jordaan, *J.C.S. Perkin I*, 1977, 1472.



Reagents: i, EtO_2CCH_2NC–KH–THF; ii, H_2–Ni–EtOH; iii, H_3O^+; iv, Ac_2O–NaOAc

Scheme 69

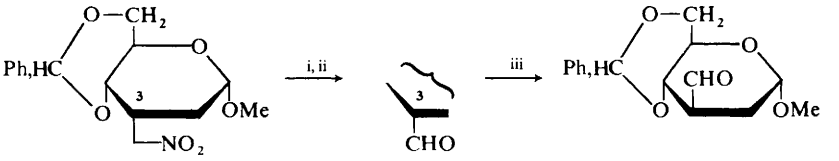


larly used to assign the *S*-configuration to related branched-chain compounds isolated from the reaction of benzyl 3,4-*O*-isopropylidene-β-L-*erythro*-pentopyranosid-2-ulose with ethyl isocyanoacetate–potassium hydride in THF.

The facile conversion of *C*-nitromethyl- into aldehydo-groups with aqueous titanium(III)chloride has provided a convenient route to several *C*-formyl sugars, which can be isomerized to the thermodynamically favoured isomers with methanolic sodium methoxide (Scheme 70).[548] Conventional reactions have been used to obtain the 3-*C*-acetamidomethyl-3-deoxy-D-ribofuranose derivative (388) from 1,2-*O*-isopropylidene-5-*O*-trityl-α-D-*erythro*-pentofuranos-3-ulose,[549] and the 6-benzoate (389) of 3-deoxy-1,2-*O*-isopropylidene-3-*C*-nitromethyl-α-D-allofuranose has been converted, by a conventional chain-shortening procedure, into

[548] J. J. Nieuwenhuis and J. H. Jordaan, *Tetrahedron Letters*, 1977, 369.
[549] M. M. A. Abdel-Rahman, *Bull. Chem. Soc. Japan*, 1977, **50**, 1619.

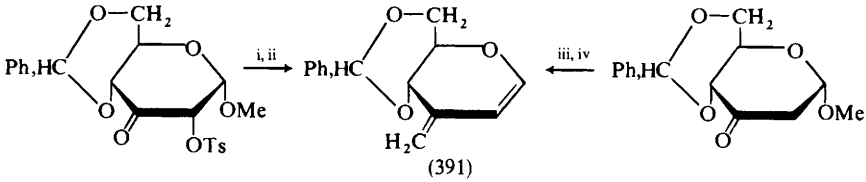
the 2-*C*-acetamidomethyl-2-deoxy-β-D-ribofuranose triester (390).[550] The preparation of 2′-*C*-(nitromethyl)uridines from (389) is reported in Chapter 19.



Reagents: i, NaH–DMF; ii, $TiCl_3$–HCl–NH_4^+ AcO^-; iii, MeONa

Scheme 70

Several multistage syntheses of the conjugated diene (391) have been developed.[551] The later stages of two recommended routes, which furnished (391) from methyl α-D-glucopyranoside in yields of 31 and 24%, are shown in Scheme 71.



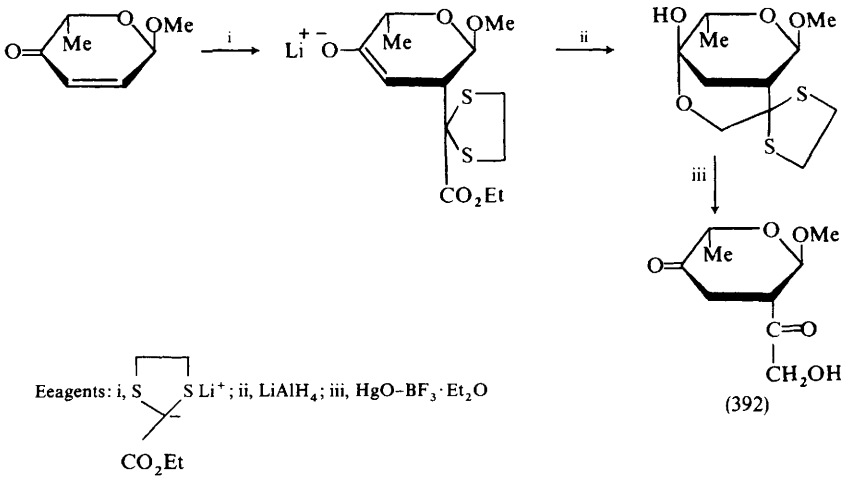
(391)

Reagents: i, CH_2I_2–Mg(Hg); ii, MeLi; iii, $Ph_3P{=}CH_2$; iv, Bu^{t}OK

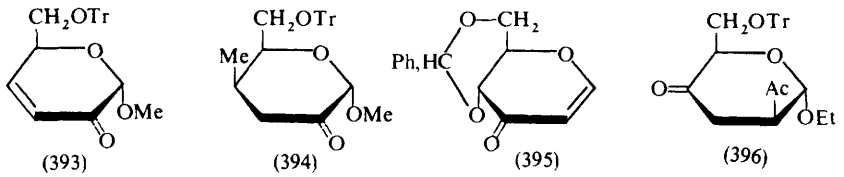
Scheme 71

Paulsen's group has examined the conjugate addition of lithiocopper organyles and other carbanions to methyl(or ethyl) 2,3,6-trideoxy-α- and -β-L-*glycero*-hex-2-enopyranosid-4-ulose and 6-(*R*)-methoxy-2*H*-pyran-3(6*H*)-one.[552,553] All the 1,4-additions yielded only the product in which the substituent introduced at C-2 is *trans*-related to the aglycone. The reactions with 2-ethoxycarbonyl-2-lithio-1,3-dithiolan can be used to prepare branched-chain sugars (392) having a functionalized side-chain (Scheme 72). Similar stereoselectivities have been reported by Fraser-Reid's group for the conjugate addition of lithium dimethylcuprate to hex-2-enopyranosid-4-uloses and hex-3-enopyranosid-2-uloses [*e.g.* (393) → (394)], whereas the hex-1-enopyranos-3-ulose (395) gave a complex mixture of products containing both 1,4-adducts, namely 2,6-anhydro-5,7-*O*-benzylidene-1,3-dideoxy-D-*arabino*-hept-4-ulose and its D-*ribo* analogue.[554] This group has also published details of the photochemical addition of simple alcohols,[555] dioxolans, aldehydes, and polyfunctional species[556] to ethyl 6-*O*-trityl-α-D-*glycero*-hex-2-enopyranosid-4-ulose and related enones (see Vol. 9, p. 104). For example, the photochemically induced addition of acetaldehyde to this enone gave the 2-*C*-acetyl derivative (396) having the D-*threo* configuration. The photochemical reactions are regiospecific and, in most cases, stereoselective. The reactions of

[550] A. J. Brink, O. G. de Villiers, and A. Jordaan, *J.C.S. Perkin I*, 1977, 1608.
[551] D. E. Iley, S. Y.-K. Tam, and B. Fraser-Reid, *Carbohydrate Res.*, 1977, **55**, 193.
[552] H. Paulsen and W. Koebernick, *Carbohydrate Res.*, 1977, **56**, 53.
[553] H. Paulsen and W. Koebernick, *Chem. Ber.*, 1977, **110**, 2127.
[554] M. B. Yunker, D. E. Plaumann, and B. Fraser-Reid, *Canad. J. Chem.*, 1977, **55**, 4002.
[555] B. Fraser-Reid, N. L. Holder, D. R. Hicks, and D. L. Walker, *Canad. J. Chem.*, 1977, **55**, 3978.
[556] B. Fraser-Reid, R. C. Anderson, D. R. Hicks, and D. L. Walker, *Canad. J. Chem.*, 1977, **55**, 3986.

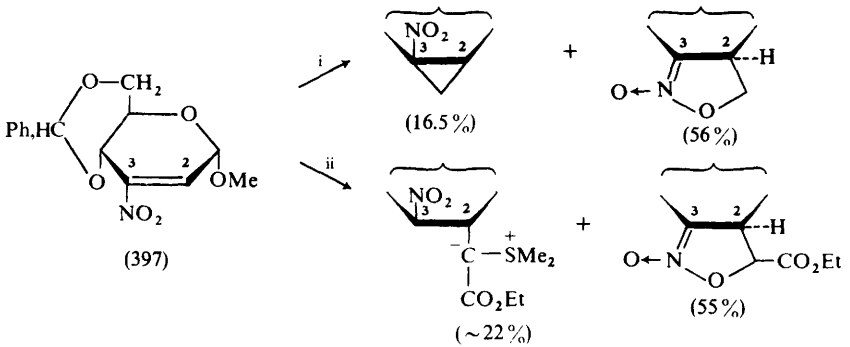


Scheme 72



(336; R = H) with tributylborane,[519] of 6-(*S*)-methoxy-2*H*-pyran-3(6*H*)-one with nitroalkanes,[520] and of levoglucosenone with Grignard reagents[525] are discussed in Chapter 12.

The nitro-olefin (397) underwent predominantly axial attack by *S*-ylides to give isoxazoline *N*-oxides, whereas equatorial attack afforded 1,2-adducts (Scheme 73).[557] The structures of the isoxazoline *N*-oxides were revealed by their reduction



Reagents: i, $Me_2SO\bar{C}H_2$–DMSO; ii, $Me_2\overset{+}{S}-\bar{C}HCO_2Et$

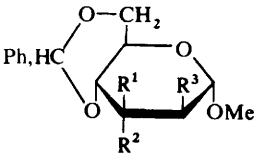
Scheme 73

[557] T. Sakakibara and R. Sudoh, *J.C.S. Chem. Comm.*, 1977, 7.

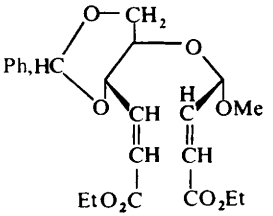
with triphenylphosphine to the corresponding isoxazoline and with Raney nickel to methyl 2-*C*-alkyl-3-amino-2,3-dideoxy-α-D-altropyranoside derivatives. The base-catalysed reactions of diethyl malonate and pentane-2,4-dione with the nitro-olefin (397) gave principally 2-*C*-alkyl derivatives (398) having the D-*manno* configuration (*i.e.* the thermodynamically more-stable isomer), although the D-*altro* compound (399) could be obtained in high yield after brief reaction of (397) with pentane-2,4-dione.[558]

The 'dialdehydes' from periodate oxidation of methyl 4,6-*O*-benzylidene-α- and -β-D-glucopyranoside reacted with appropriate Wittig reagents to give such dienes as (400) that afforded cyclized products [*e.g.* (401)] with nitromethane by a double Michael condensation, whereas only one of the olefinic linkages was attacked, without cyclization, with ethyl cyanoacetate and malononitrile.[559] Details of the reactions of these 'dialdehydes' with nitroalkanes (see Vol. 8, p. 108), ethyl cyanoacetate (see Vol. 8, p. 110), and other carbanions to give various branched-chain heptoseptanosides have also been reported.[560]

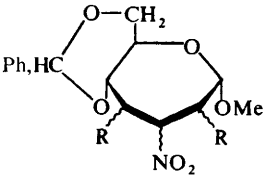
Methyl 2,3-anhydro-4-*O*-benzyl-β-L-ribopyranoside reacted with dimethylmagnesium in ethanol to give methyl 4-*O*-benzyl-3-deoxy-3-*C*-methyl-β-L-xylopyranoside (402).[561]



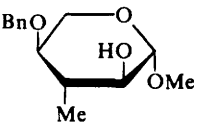
(398) $R^1 = NO_2$, $R^2 = H$, $R^3 = CH(CO_2Et)_2$ or $CH(COMe)_2$
(399) $R^1 = H$, $R^2 = NO_2$, $R^3 = CH(COMe)_2$



(400)



(401) R = CH_2CO_2Et



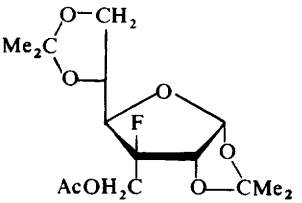
(402)

Compounds with an R—C—F Branch

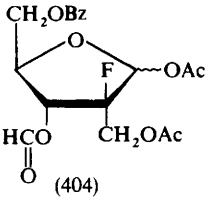
The use of trifluoro(fluoroxy)methane for the stereospecific introduction of a fluorine atom at the branching point of branch-chain sugars has been reported previously (see Vol. 9, p. 56). The fluorinated branched-chain derivative (403) has been converted into 3-*C*-acetoxymethyl-6-*O*-benzoyl-3-deoxy-3-fluoro-D-glucose,

[558] T. Sakakibara, A. Seta, Y. Tachimori, T. Takamoto, and R. Sudoh, *Chem. Letters*, 1977, 1365.
[559] C. L. Bhardwaj, J. C. Ireson, J. B. Lee, and M. J. Tyler, *Tetrahedron*, 1977, **33**, 3279.
[560] M. E. Butcher, J. C. Ireson, J. B. Lee, and M. J. Tyler, *Tetrahedron*, 1977, **33**, 1501.
[561] A. Ya. Shmyrina, A. F. Sviridov, O. S. Chizhov, A. S. Shashkov, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1977, 461 (*Chem. Abs.*, 1977, **87**, 6296u).

which on oxidation with sodium metaperiodate and acetylation gave the 2-*C*-acetoxymethyl-2-deoxy-2-fluoro-D-arabinofuranose derivative (404).[562] The synthesis of nucleosides from fluorinated branched-chain sugars is mentioned in Chapter 19.



(403)

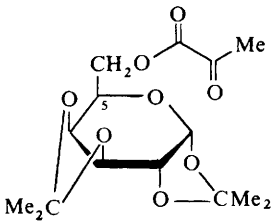


(404)

[562] A. J. Brink, O. G. de Villiers, and A. Jordaan, *Carbohydrate Res.*, 1977, **54**, 285.

14
Aldosuloses, Dialdoses, and Diuloses

U.v.-irradiation of the pyruvic esters [*e.g.* (405)] of suitably protected monosaccharides in benzene gave the corresponding dialdose [*e.g.* (406)] or aldosulose, avoiding the undesirable competing reactions sometimes encountered in DMSO-based oxidations.[563] This photochemical process permitted the oxidation of of 1,2,3,4- and 1,3,4,6-tetra-*O*-acetyl-D-glucopyranoses without further reaction of the labile oxidation products, which may, however, eliminate acetic acid during purification. Excellent yields of glycopyranosiduloses have been obtained on oxidation of protected glycopyranosides with DMSO–trifluoroacetic anhydride in dichloromethane at −65°C.[564] Oxidation of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside with DMSO–acetic anhydride gave methyl 2-*O*-acetyl-4,6-*O*-benzylidene-β-D-*ribo*-hexopyranosid-3-ulose (72%) and methyl 3-*O*-acetyl-4,6-*O*-benzylidene-β-D-*arabino*-hexopyranosid-2-ulose (12%).[565]



(405)



(406)

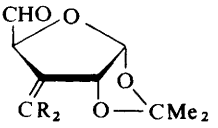
Further studies on the chelation of methyl α-D-*arabino*-hexopyranosid-2-ulose and methyl α-D-*ribo*-hexopyranosid-3-ulose by magnesium, barium, and calcium salts have been reported[566] (see Vol. 8, p. 113).

Microbiological oxidation of methyl α- and β-D-xylopyranosides with *Acetobacter suboxydans* gave the corresponding glycosid-4-uloses; all pentopyranosides tested showed some oxidation, whereas hexopyranosides showed very limited or no oxidation.[567] The substrate specificity of D-galactose oxidase has been examined.[568] The primary alcoholic groups of D-glyceraldehyde, glycerol, D-threitol,

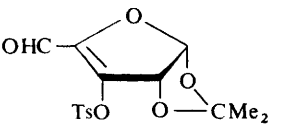
[563] R. W. Binkley, *J. Org. Chem.*, 1977, **42**, 1216.
[564] J. Yoshimura, K.-I. Sato, and H. Hashimoto, *Chem. Letters*, 1977, 1327.
[565] J. Defaye and A. Gadelle, *Carbohydrate Res.*, 1977, **56**, 411.
[566] J. Defaye, H. Driguez, and A. Gadelle, *Appl. Polymer Symposium*, 1976, 28 (8th Proc. Cellulose Conf., 1975, Vol. 3), 955 (*Chem. Abs.*, 1977, **86**, 190 404*f*).
[567] W. A. Szarek and G. W. Schnarr, *Carbohydrate Res.*, 1977, **55**, C5.
[568] J. H. Pazur, H. R. Knull, and G. E. Chevalier, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 129.

and 1,5-anhydro-D-galactitol (but not galactitol) and of D-galactopyranosyl or 2-acetamido-2-deoxy-D-galactopyranosyl residues occurring in nucleotides were oxidized to the corresponding aldehyde by the enzyme, although prolonged treatment gave the carboxylic acid.

1,2-*O*-Isopropylidenepentodialdofuranoses having the α-D-*ribo*-, β-L-*lyxo*-, or β-L-*arabino* configuration and analogues (407) and (408) having one(C-3) or two(C-3 and -4) hybrid sp^2 carbon atoms, respectively, have been synthesized.[569] The orientation of these compounds about the C-4—C-5 bond is virtually independent of modifications at C-3 and seems to be directed by orbital factors, which favour a conformer having the carbonyl group and the O—C-4 bond nearly eclipsed.

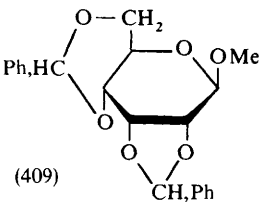


(407) R = H or F

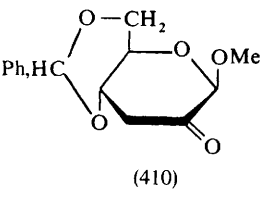


(408)

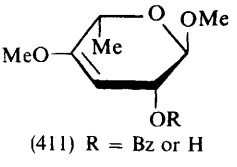
Aspects of the cleavage of benzylidene derivatives [*e.g.* (409)] with butyl-lithium to give deoxyketones [*e.g.* (410)] have been described by Horton's group; with such systems as (409) and its α-D-*manno* analogue, the axial hydrogen atom at C-2 or C-3, respectively, is abstracted and replaced, after the expulsion of benzaldehyde, with a carbonyl group.[570] The enol ether (411; R = Bz), which is obtained from methyl 2-*O*-benzoyl-3-bromo-3,6-dideoxy-4-*O*-methyl-α-L-altropyranoside under azidolysis conditions, yielded methyl 2,3,6-trideoxy-α-L-*glycero*-hex-2-enopyranosid-4-ulose on debenzoylation and hydrolysis of the product (411; R = H) with aqueous acetic acid at room temperature.[384]



(409)



(410)



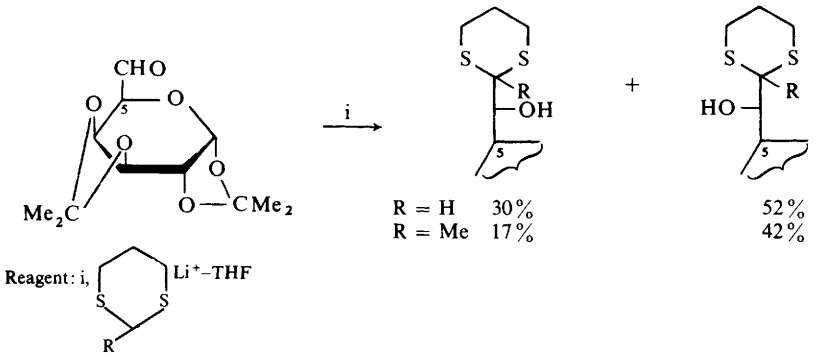
(411) R = Bz or H

Heptose and octose derivatives have been synthesized by way of nucleophilic *C*-alkylation of 1,2:3,4-di-*O*-isopropylidene-α-D-*galacto*-1,5-hexodialdopyranose with the carbanions derived from 1,3-dithian and 2-methyl-1,3-dithian (Scheme 74).[330] The diastereoisomeric products could be formed in different proportions, depending on the solvent used.

The principal products formed on γ-radiolysis of D-glucose in oxygenated solu-

[569] J. M. J. Tronchet, B. Gentile, J. Ojha-Poncet, G. Moret, D. Schwarzenbach, F. Barbalat-Rey, and J. Tronchet, *Carbohydrate Res.*, 1977, **59**, 87.

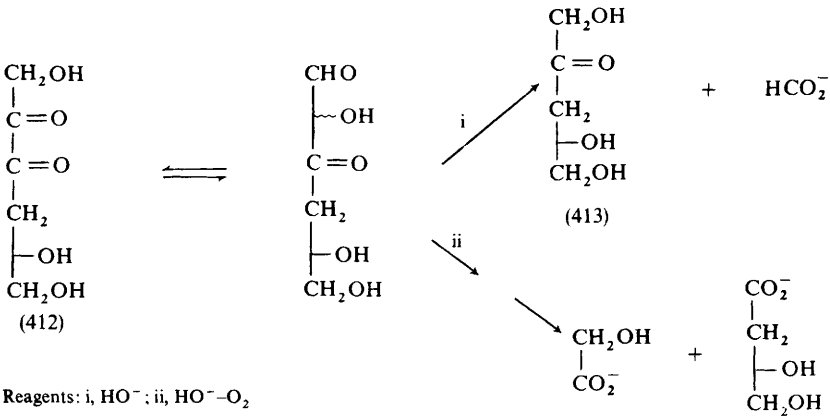
[570] D. Horton and W. Weckerle, *Amer. Chem. Soc. Symposium Ser.*, 1976, 39 (Synthetic Methods for Carbohydrates Symposium), 22 (*Chem. Abs.*, 1977, **87**, 68 539j).



Scheme 74

tion have been identified as D-gluconic acid, D-*arabino*-hexosulose, D-*ribo*-hexos-3-ulose, D-*xylo*-hexos-4-ulose, D-*xylo*-hexos-5-ulose, D-*gluco*-hexodialdose, and L-*threo*-tetrodialdose.[570a] These products are thought to be formed by the attack of hydroxy-radicals at C-1—C-6, followed by the addition of oxygen and either elimination of $HO_2^{\bullet}$ or fragmentation of carbon–carbon bonds. Dose-rate studies showed that the probability of abstraction by hydroxy-radicals is *ca.* 20% for H-1 and H-2, *ca.* 10% for H-3 and H-4, *ca.* 15% for H-5, and *ca.* 30% for H-6, and that the rates for elimination of $HO_2^{\bullet}$ are in the order C-1 ≫ C-2, -3, -4 > C-6 ≫ C-5.

The 4-deoxy-2,3-hexodiulose (412) decomposed to the 3-deoxypentulose (413) and formic acid in oxygen-free, alkaline solutions, whereas it was cleaved to give 3,4-dihydroxybutanoic and glycolic acids in oxygenated, alkaline solutions (Scheme 75).[571]



Scheme 75

[570a] M. N. Schuchmann and C. von Sonntag, *J.C.S. Perkin II*, 1977, 1958.
[571] L.-Å. Lindström and O. Samuelson, *Acta Chem. Scand.*, 1977, **31B**, 479.

Deuteriated Raney nickel in refluxing deuterium oxide caused deuterium exchange, presumably by a dehydrogenation–deuteriogenation process, with carbon-bonded hydrogen atoms geminal to primary and secondary alcoholic groups in mono-, di-, and tri-saccharide derivatives.[572] No loss of absolute configuration was observed, although excessive reaction times can sometimes lead to isomerization. The exchange reaction is particularly useful for the deuteriation of methyl glycosides (for example, it was used to prepare methyl α-D-[2,3,4,6,6′-^{2}H]mannopyranoside from methyl α-D-mannopyranoside) and failed only when large neighbouring groups were present (for example, neither 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose nor 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose incorporated deuterium).

Other aspects of the chemistry of glycosiduloses are dealt with in Chapters 3, 7, 11, and 21.

[572] H. J. Koch and R. S. Stuart, *Carbohydrate Res.*, 1977, **59**, C1.

15
Sugar Acids and Lactones

Aldonic Acids

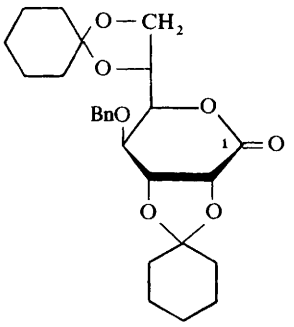
The oxidative decarboxylation of D-glucono-1,5-lactone with cerium(IV) sulphate has been discussed[103] in Chapter 2. The yield of potassium D-arabinonate obtained by oxidation, *etc.*, of D-glucose with gaseous oxygen in aqueous potassium hydroxide at *ca.* 40 C was improved in the presence of Methylene Blue.[104] The kinetics of oxidation of D-glucose to D-gluconic and D-glucuronic acids with persulphate anions have been correlated with a free-radical mechanism,[92] and the amounts of D-gluconic, 2-deoxy-D-*arabino*-hexonic, and other acids formed on γ-radiolysis of aqueous solutions of D-glucose saturated with nitrous oxide have been measured.[88] D-Gluconic acid was oxidized with gaseous oxygen (using platinum on carbon as the catalyst) to D-glucaric acid *via* L-guluronic acid.[573] Yields of 50–55% of D-glucaric acid could be obtained under optimal conditions.

Specific rate constants for the hydrolysis of D-mannono-1,4-lactone have been determined,[574] and the rate of acid-catalysed hydrolysis of the 4,6-dideoxy-L-hexono-1,5-lactones at 35°C in water, aqueous *p*-dioxan, and aqueous acetone was shown to decrease along the series *xylo*, *lyxo*, *ribo*, and *arabino*.[575] The effects of the composition of mixed-solvent systems on the rate of hydrolysis were also investigated.

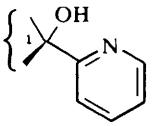
The D-*glycero*-D-*gulo*-heptono-1,5-lactone derivative (414) gave the heterocyclic heptopyranose derivative (415) on successive treatments with butyl- or phenyl-lithium and 2-bromopyridine.[575a] Heterocyclic derivatives related to (415) were also obtained when (414) reacted with 2-methylpyridine and benzothiazole, whereas it reacted with furan, thiophene, and 1-methylindole to give acyclic derivatives [*e.g.* (416)]. Chelation of the nitrogen atom of the heterocyclic ring with the intermediate alkoxide anion appears to deactivate C-1 towards further reaction with the former series of compounds.

The principal volatile products formed on pyrolytic decomposition of D-xylan, xylotriose, xylobiose, and methyl β-D-xylopyranoside were 3-hydroxy-2-penteno-1,5-lactone and 2-furaldehyde.[576] These products appear to be formed in independent reactions, the lactone being derived from non-reducing D-xylopyranosyl residues. Oxidation of ethyl 4,6-di-*O*-acetyl-2,3-dideoxy-α-D-*erythro*-hex-2-eno-

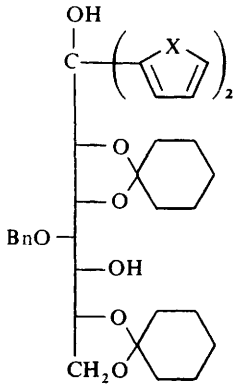
[573] J. M. H. Dirkx, H. S. van der Baan, and J. M. A. J. J. van der Broek, *Carbohydrate Res.*, 1977, **59**, 63.
[574] S. K. Dutta and S. B. Roy, *Indian J. Chem.*, 1976, **14A**, 698 (*Chem. Abs.*, 1977, **86**, 121 662b).
[575] L. Helešic, K. Kefurt, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1977, **42**, 2193.
[575a] Yu. A. Zhdanov, V. G. Alekseeva, E. L. Korol, and V. A. Polenov, *Doklady Akad. Nauk S.S.S.R.*, 1976, **230**, 856 (*Chem. Abs.*, 1977, **86**, 90 157j).
[576] A. Ohnishi, K. Katō, and E. Takagi, *Carbohydrate Res.*, 1977, **58**, 387.



(414)

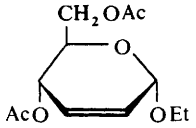


(415)

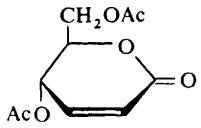


(416) X = O or S

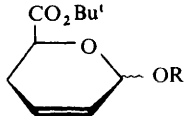
pyranoside (417) with hydrogen peroxide in the presence of molybdenum trioxide gave the 1,5-lactone (418) in a yield of 41%, following dehydration of the resulting hydroperoxide with acetic anhydride–pyridine.[577] The intermediate hydroperoxide (419; R = OH) was isolated following oxidation of butyl 5,6-dihydro-2-methoxy-2*H*-pyran-6-carboxylate (419; R = Me).



(417)



(418)



(419) R = Me or OH

Elemental analysis and i.r. spectroscopy have indicated that pangamic acid (vitamin B_{15}) is 6-*O*-(dimethylglycyl)-D-gluconic acid.[578]

Aspects of the chemistry of unsaturated aldonic acid derivatives are also referred to in Chapters 8 and 12.

Aldaric Acids

The formation of D-glucaric acid by platinum-catalysed oxidation of D-gluconic acid has been noted[573] in the previous section. Mono- and per-allyl ether derivatives of xylaric and galactaric acids have been prepared by treating the aldaric acid with allyl alcohol in the presence of an appropriate acid catalyst.[579] Diallyl 3-*O*-allyl-2,4-*O*-methylenexylarate was obtained in good yield when 2,4-*O*-methylenexylaric acid reacted with allyl bromide in the presence of alkali. The reaction of 2,3,4-tri-*O*-acetylxylaryl dichloride with diazomethane has been mentioned[359] in Chapter 7.

[577] J. Mieczkowski, J. Jurczak, M. Chmielewski, and A. Zamojski, *Carbohydrate Res.*, 1977, **56**, 180.

[578] L. Telegdy Kovats, E. Berndorfer-Kraszner, A. Juhasz, and T. Gabor, *Acta Ailment, Acad. Sci. Hung.*, 1976. **5**. 281 (*Chem. Abs.*, 1977, **87**, 49 193a).

[579] V. L. Lapenko, B. I. Mikhant'ev, and A. I. Slivkin, *Izvest. Vyssh. Uchebn. Zaved., Khim. i khim. Tekhnol.*, 1977, **20**, 659 (*Chem. Abs.*, 1977, **87**, 168 313z).

Ulosonic Acids

A route to hept-2-ulosonic acid derivatives is available by way of the reaction of 2,3:4,5-di-*O*-isopropylidene-L-arabinose and 2,4:3,5-di-*O*-ethylidene-L-xylose with ethyl nitrosoacetate.[580] Isomerization of the intermediate nitroso derivatives with alcoholic sodium nitrite yielded the corresponding oximes, which can be deoximated with lead tetra-acetate in acetic acid. 3-Deoxy-D-*manno*-oct-2-ulosonic acid 5-(dihydrogen phosphate) and its D-*gluco* analogue have been obtained by base-catalysed condensation of D-arabinose 2-phosphate (obtained by way of phosphorylation of benzyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside) with oxalacetic and separation of the isomers by ion-exchange chromatography.[581]

The efficiency of the oxidation of 2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose to 2,3:4,6-di-*O*-isopropylidene-α-L-*arabino*-hexulopyranosonic acid (a stage in the manufacture of L-ascorbic acid) with sodium hypochlorite and nickel sulphate (as a catalyst) has been shown to depend on the characteristics of the catalyst, the temperature of the reaction, and the concentration of the reactants.[582] The catalytic activities of a number of metal cations (*e.g.* Cu^{2+}, Ag^{+}, Co^{2+}, Fe^{3+}, and Ce^{4+}) in the electrochemical oxidation of 2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose have also been examined, but none were as effective as Ni^{2+} cations.[583]

Uronic Acids

The synthesis of derivatives of L-iduronic acid from 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose by way of an acetylenic intermediate is mentioned[528] in Chapter 12 (see Scheme 58). A related sequence of reactions with methyl 2,3-*O*-isopropylidene-β-D-*ribo*-pentodialdo-1,4-furanoside has furnished methyl (methyl 2,3-*O*-isopropylidene-α-L-talofuranosid)uronate and its β-D-*allo* analogue from acetylenic precursors.[584] Malonic acid reacted with methyl 2,3-*O*-isopropylidene-β-D-*ribo*-pentodialdo-1,4-furanoside to give the unsaturated uronic acid derivative (420).

Oxidation of 1,2-*O*-isopropylidene-α-D-gluco-, -allo-, and -galacto-furanoses with silver carbonate–Celite in boiling methanol gave the corresponding methyl 1,2-*O*-isopropylidene-α-D-pentofuranuronates as the principal products.[585] 1,2-*O*-Isopropylidenepentodialdo-1,4-furanoses are intermediates in the oxidation reaction, and various amounts of hexofuranuronates are also formed as by-products. Differences in the ratios of hexuronic and penturonic esters obtained were explained by the way in which the substrate is absorbed onto the surface of the oxidant prior to oxidation. Derivatives of stereoisomeric hexuronic acids having the *altro*, *manno*, *gluco*, *gulo*, *galacto*, and *talo* configurations have been prepared from *cis,trans*-t-butyl 2-methoxy-5,6-dihydro-2*H*-pyran-6-carboxylate

[580] Yu. A. Zhdanov, V. I. Kornilov, and B. B. Paidak, *Zhur. obshchei Khim.*, 1977, **47**, 936 (*Chem. Abs.*, 1977, **87**, 102 546w).

[581] R. S. Sarfati, M. Mondange, and L. Szabo, *J. C. S. Perkin I*, 1977, 2074.

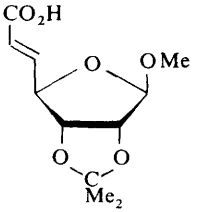
[582] K. B. Pal'chik, V. V. Terent'ev, A. E. Maslov, G. B. Shnaiderman, V. G. Sidorov, N. S. Zolotarev, Z. G. Tkhorevskaya, A. D. Khafizova, L. F. Beshevli, and O. V. Gorbunov, *Khim.-farm. Zhur.*, 1977, **11**, 92 (*Chem. Abs.*, 1977, **87**, 44 195t).

[583] L. G. Seleznev, F. I. Luknitskii, L. M. Sukhmaneva, M. E. Meller, T. A. Kargopolova, and T. V. Sazonova, *Khim.-farm. Zhur.*, 1976, **10**, 125 (*Chem. Abs.*, 1977, **86**, 90 161f).

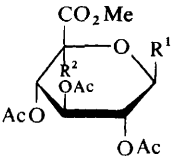
[584] N. Berg and O. Kjolberg, *Carbohydrate Res.*, 1977, **57**, 65,

[585] S. Morgenlie, *Carbohydrate Res.*, 1977, **59**, 73.

(419; R = Me).[586] The syntheses involved epoxidation of (419; R = Me), opening of the epoxides with dimethylamine, Cope degradation of the dimethylamino derivatives, and dihydroxylation of the double bond in the resulting t-butyl hex-3-enuronates.



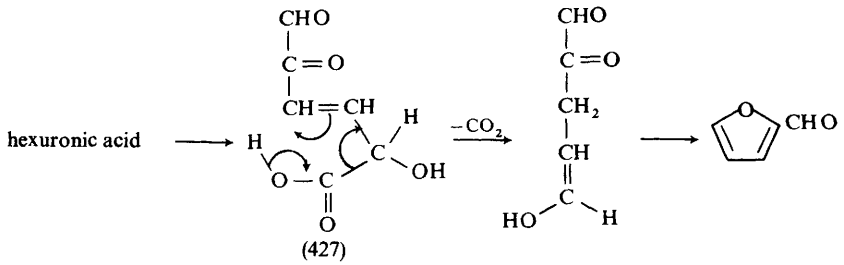
(420)



(421) R^1 = SPh, R^2 = H
(422) R^1 = SPh, R^2 = Br
(423) R^1 = R^2 = H
(424) R^1 = OAc, R^2 = H
(425) R^1 = H, R^2 = Br
(426) R^1 = OAc, R^2 = Br

The radical-induced reaction of methyl (phenyl 2,3,4-tri-*O*-acetyl-1-thio-β-D-glucopyranosid)uronate (421) with NBS in refluxing carbon tetrachloride gave, as well as the expected hex-1-enopyranos-3-ulose (see p. 109), the 5-bromo derivative (422).[587] The D-glucopyranuronic acid derivatives (423) and (424) were similarly converted into the brominated derivatives (425) and (426). Methylation of methyl (methyl α-D-galactopyranosid)uronate with methyl iodide–silver oxide —methyl sulphide has been found to give a mixture of methyl (methyl 2,3,4-tri-*O*-methyl-α-D-galactopyranosid)uronate and, by epimerization at C-5, the analogous β-L-altropyranosiduronate.[588] Methyl (methyl α-L-altropyranosid)uronate was obtained following hydrogenolysis of one of the products formed on treatment of methyl (methyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranosid)uronate with these reagents.[588a]

The mechanism shown in Scheme 76 has been proposed for decarboxylation of the β,γ-unsaturated acid (427) formed during the acid-catalysed conversion of hexuronic acids into 2-furaldehyde.[406] This mechanism is qualitatively consistent with the labelling pattern observed following degradation of D-glucuronic acid to 2-furaldehyde in acidified tritiated water or deuterium oxide.



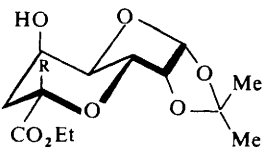
(427)

Scheme 76

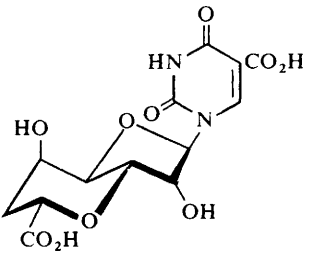
[586] J. Mieczkowski and A. Zamojski, *Carbohydrate Res.*, 1977, **55**, 177.
[587] R. J. Ferrier and R. H. Furneaux, *J.C.S. Perkin I*, 1977, 1996.
[588] P. Kováč, J. Hirsch, R. Palovčík, I. Tvaroška, and S. Bystrický, *Coll. Czech. Chem. Comm.*, 1976, **41**, 3119.
[588a] P. Kováč, J. Hirsch, I. Tvaroška, R. Palovčík, V. Kováčik, and T. Sticzay, *Coll. Czech. Chem. Comm.*, 1976, **41**, 3804.

The acidity constants of D-galacturonic and D-glucuronic acids in water have been determined by conductance and e.m.f. measurements.[589] The structures of the isomeric products obtained on trimethylsilylation of naturally occurring hexuronic acids and their sodium salts and lactones have been established using n.m.r. spectroscopy (for the anomeric configuration) and mass spectrometry (for the ring-size).[590] The equilibria of some of the hexuronic acids in DMSO involved a larger proportion of furanoid forms than those in water or pyridine.

Details have appeared on the synthesis of 3,7-anhydro-6-deoxyoctofuranuronic acid derivatives [*e.g.* (428)] related to the carbohydrate components of the octosyl acids isolated from the fermentation broth of *Streptomyces cacaoi* var. *asoensis*[591]



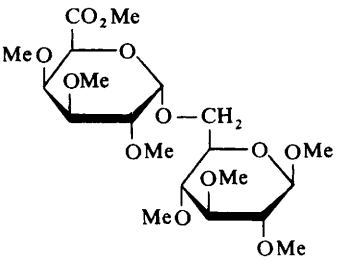
(428) R = CO_2Et



(429)

(see Vol. 10, p. 117). This work has also been extended to the synthesis of nucleosides related to octosyl acid A (429), *etc.*[591a]

Details of the selective cleavage of D-glucuronidic linkages in saponins have been published[592] (see Vol. 10, p. 117). A modified procedure for determining the sites of attachment of hexuronic acid residues in methylated acidic polysaccharides by base-catalysed degradation has been modelled on experiments with the methylated aldobiouronic acid derivative (430), which eliminated methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranoside (89%) on treatment with sodium methylsulphinylmethanide in DMSO at room temperature.[593] When this procedure is applied to



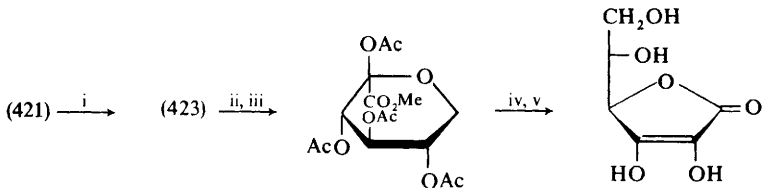
(430)

[589] H. Holvik and H. Hoeiland, *J. Chem. Thermodynamics*, 1977, **9**, 345.
[590] J. F. Kennedy, S. M. Robertson, and M. Stacey, *Carbohydrate Res.*, 1977, **57**, 205.
[591] K. Anzai and T. Saita, *Bull. Chem. Soc. Japan*, 1977, **50**, 169.
[591a] K. Anzai and T. Saita, *Nucleic Acids Res., Special Publ.*, 1976, 2 (4th Symposium Nucleic Acids Chem., 1976), 87 (*Chem. Abs.*, 1977, **86**, 190 390y).
[592] I Kitagawa, M. Yoshikawa, K. Sik Im, and Y. Ikenishi, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 657.
[593] G. O. Aspinall and K.-G. Rosell, *Carbohydrate Res.*, 1977, **57**, C23.

acidic polysaccharides, re-alkylation with trideuteriomethyl or ethyl iodide after the elimination reaction can be used to label the site(s) to which the uronic acid residues were attached. 2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylitol was rapidly epimerized to 2-*O*-(4-*O*-methyl-β-L-idopyranosyluronic acid)-D-xylitol in hot alkali, and both epimers were degraded to give 2-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-D-xylitol and xylitol.[594] Treatment of 4-*O*-methyl-D-glucuronic acid with an aqueous solution containing chlorine gave 4-*O*-methyl-D-glucaric acid, which was degraded to *O*-methyl-pentaric and -tetraric acids and small amounts of demethylated aldaric acids.[595] Similar treatment of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose resulted in its rapid oxidation to 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylonic acid, which was slowly hydrolysed to 4-*O*-methyl-D-glucuronic and D-xylonic acids; these acids gave the expected products on oxidation and further degradation.

L-Ascorbic Acid

Specific photochemical bromination of (423) has been used in a new synthesis of L-ascorbic acid (Scheme 77).[596]



Reagents: i, H_2–Ni; ii, NBS–*hv*; iii, $Hg(OAc)_2$–AcOH; iv, MeONa; v, H_3O^+

Scheme 77

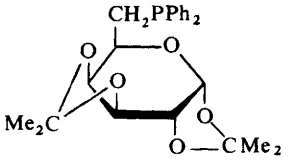
Other reports have discussed the reactions of L-ascorbic acid with fatty acids in strong sulphuric acid,[597] 4-toluidine,[598] and various oxidizing agents.[599–606]

[594] M. H. Johansson and O. Samuelson, *Carbohydrate Res.*, 1977, **54**, 295.
[595] T. Ericsson and O. Samuelson, *Acta Chem. Scand.*, 1977, **31B**, 209.
[596] R. J. Ferrier and R. H. Furneaux, *J. C. S. Chem. Comm.*, 1977, 332.
[597] R. C. Cousins, P. A. Seib, R. C. Hoseney, C. W. Deyoe, Y. T. Liang, and D. W. Lillard, jun., *J. Amer. Oil Chem. Soc.*, 1977, **54**, 308 (*Chem. Abs.*, 1977, **87**, 168 315b).
[598] T. Ozawa, Y. Nakamura, and N. Kinae, *Yakugaku Zasshi*, 1976, **96**, 932 (*Chem. Abs.*, 1977, **86**, 5700p).
[599] J. J. Ruiz, A. Aldaz, and M. Dominguez, *Canad. J. Chem.*, 1977, **55**, 2799.
[600] R. H. Schuler, *Radiation Res.*, 1977, **69**, 417 (*Chem. Abs.*, 1977, **87**, 6288t).
[601] A. D. Nadezhdin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1977, 1016 (*Chem. Abs.*, 1977, **87**, 67608u).
[602] B. S. Rawat and M. C. Agrawal, *Indian J. Chem.*, 1976, **14A**, 482 (*Chem. Abs.*, 1977, **86**, 30 008b).
[603] D. N. Samios, M. I. Karayannis, and T. P. Hadjiioannou, *Z. phys. Chem. (Wiesbaden)*, 1977, **104**, 189 (*Chem. Abs.*, 1977, **87**, 201 963m).
[604] Y.-H. Tsai, *T'ai-wan K'o Hsueh*, 1976, **30**, 153 (*Chem. Abs.*, 1977, **86**, 140 376c).
[605] R. Matusch, *Z. Naturforsch.*, 1977, **32B**, 562 (*Chem. Abs.*, 1977, **87**, 201 961j).
[606] C. M. Paleos and P. Dais, *J.C.S. Chem. Comm.*, 1977, 345.

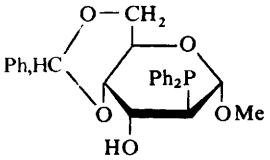
16
Inorganic Derivatives

Carbon-bonded Compounds

The sugar phosphines (431) and (432) have been prepared and used as ligands for Wilkinson's catalysts in the asymmetric hydrogenation of prochiral olefins.[607]

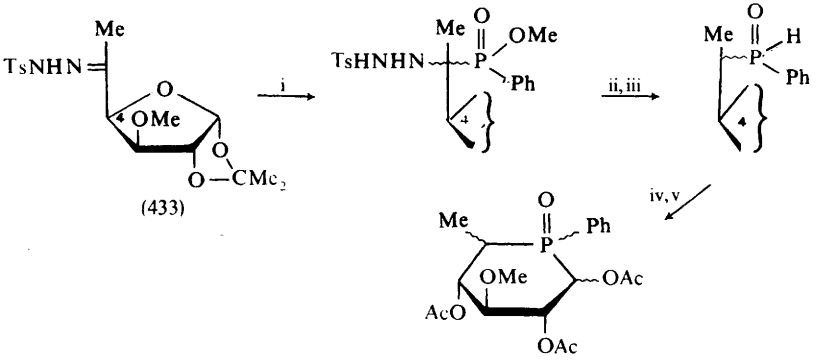


(431)



(432)

The optical purities obtained were generally low (≤40%). The acid-catalysed reaction of 6-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*xylo*-hexofuranos-5-ulose 5-(toluene-*p*-sulphonylhydrazone) (433) with methyl phenylphosphonite has been used in a novel method for the synthesis of D-gluco- and L-ido-pyranose derivatives containing a phosphorus atom in the hemiacetal ring (Scheme 78).[608]



(433)

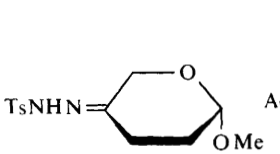
Reagents: i, PhP(O)(OMe)H–TsOH; ii, $NaBH_4$; iii, $NaAlH_2(OCH_2CH_2OMe)_2$; iv, H_3O^+; v, Ac_2O–py

Scheme 78

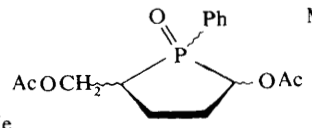
[607] T. Iwami, T. Yoshida, and M. Sato, *Nippon Kagaku Kaishi*, 1976, 1652 (*Chem. Abs.*, 1977, **86**, 90 155g).

[608] M. Yamashita, Y. Nakatsukasa, M. Yoshikane, H. Yoshida, T. Ogata, and S. Inokawa, *Carbohydrate Res.*, 1977, **59**, C12.

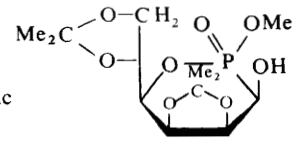
An analogous sequence of reactions with methyl 2,3-dideoxy-(1*S*)-pentopyranosid-4-ulose 4-(toluene-*p*-sulphonylhydrazone) (434) afforded 1,5-di-*O*-acetyl-2,3,4-trideoxy-4-*C*-(phenylphosphinyl)-DL-*glycero*-pentofuranose (435). The Abramov reaction of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose with dimethyl phosphite yielded the oxaphosphorinane (436) by intramolecular transesterification.[609]



(434)

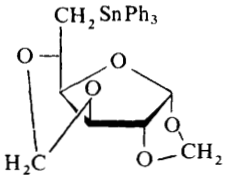


(435)

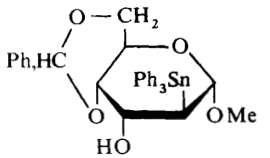


(436)

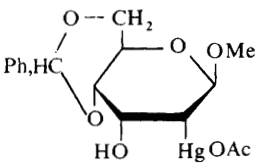
C-Stannyl derivatives of sugars [*e.g.* (437) and (438)] have been prepared by either the displacement of primary tosylates or the reaction of suitably protected epoxides with triphenyltin lithium in anhydrous THF.[610]



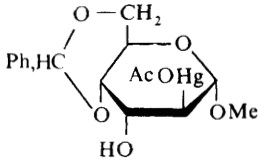
(437)



(438)

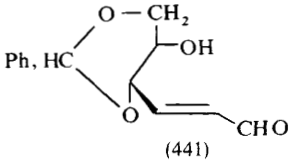


(439)

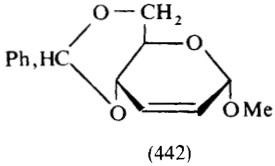


(440)

The methoxymercuration of 4,6-*O*-benzylidene-D-allal yielded isomeric acetoxymercurial adducts having the β-D-*allo* (439) and α-D-*altro* (440) configurations.[611] Demercuration of (439) with sodium borohydride gave methyl 4,6-*O*-benzylidene-2-deoxy-β-D-*ribo*-hexopyranoside (72%), the α,β-unsaturated aldehyde (441) (8.3%), and a trace of the original glycal, while demercuration of (440) gave methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside (40%), (442) (23.5%), and traces of other products.



(441)



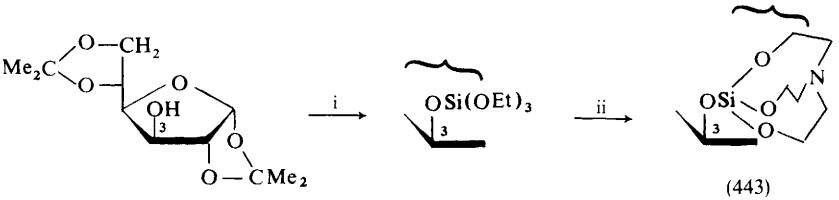
(442)

[609] J. Thiem, M. Günther, H. Paulsen, and J. Kopf, *Chem. Ber.*, 1977, **110**, 3190.
[610] L. D. Hall, D. C. Miller, and P. R. Steiner, *Carbohydrate Res.*, 1976, **52**, C1.
[611] T. M. Cheung, D. Horton, and W. Weckerle, *Carbohydrate Res.*, 1977, **59**, 276.

Sulphur- or Oxygen-bonded Compounds

Several stable metal–thio-sugar derivatives have been prepared by the reaction of either 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose with such reagents as Bu_3SnOH, Ph_3PbOH, Ph_3GeOH, and Bu_2SnO, *etc.*, or of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with R_3SnSLi (R = Bu or Ph).[612]

The silylated derivative (443) has been obtained from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose by the reactions shown in Scheme 79.[613]



(443)

Reagents: i, $(EtO)_3SiH$; ii, $(HOCH_2CH_2)_3N$

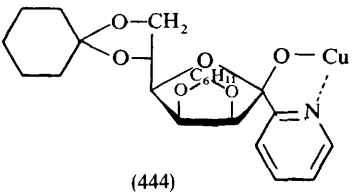
Scheme 79

The Mössbauer spectra of several *O*-stannyl derivatives of mono-, di-, and oligosaccharides, for example, 1,4,6-tri-*O*-tributylstannyl-β-D-glucopyranose, have been examined in connection with the impregnation of cellulosic materials with dilute solutions of the fungicide $(Bu_3Sn)_2O$.[614] The results indicated that *O*-tributylstannyl derivatives are not formed by cellulosic materials, but that the fungicide is converted into polymeric organotin carbonate by reaction with carbon dioxide in the atmosphere. Regiospecific benzoylation, methylation, and allylation of allyl 2,6-di-*O*-benzyl-α-D-galactopyranoside at O-3 has been achieved *via* its 3,4-*O*-dibutylstannylene derivative[249] (see Vol. 10, p. 122).

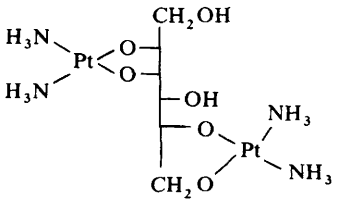
The work of Angyal's group on the Fischer glycosidation of sugars in the presence of Ca^{2+} cations has been discussed[110] in Chapter 3, and details have appeared on the formation of complexes between sugars (*e.g.* L-sorbose, D-ribose, and methyl β-D-ribopyranoside) and Na^+ cations in such non-aqueous solvents as pyridine and isopropylamine[615] (see Vol. 10, p. 124). D-Glucose and D-xylose have been shown to form 1:1 complexes with Al^{3+}, In^{3+}, Ga^{3+},[616] and Ti^{3+} cations in acid solution;[616a] they also formed 1:1 complexes with Cu^{2+}, Fe^{3+}, and Ce^{4+} cations at pH 1, although complexes containing 2, 3, and 4 monosaccharide residues, respectively, were formed with these transition-metal cations at pH 5.[617,618]

[612] A. F. Husain and R. C. Poller, *J. Organometallic Chem.*, 1976, **118**, C11.
[613] B. N. Stepanenko, V. I. Kopkov, and A. P. Luzin, *Doklady Akad. Nauk S.S.S.R.*, 1977, **235**, 969 (*Chem. Abs.*, 1977, **87**, 201 926h).
[614] P. J. Smith, A. J. Crowe, D. W. Allen, J. S. Brooks, and R. Formstone, *Chem. and Ind.*, 1977, 874.
[615] J. Grandjean and P. Laszlo, *Helv. Chim. Acta*, 1977, **60**, 259.
[616] V. I. Krupenskii, I. I. Korol'kov, and T. V. Dolgaya, *Zhur. priklad. Khim.*, 1977, **50**, 1334 (*Chem. Abs.*, 1977, **87**, 184 803q).
[616a] V. I. Krupenskii and N. P. Mikush, *Khim. Drev.*, 1977, 110 (*Chem. Abs.*, 1977, **87**, 201 930y).
[617] V. I. Krupenskii, I. I. Korol'kov, and T. V. Dolgaya, *Khim. Drev.*, 1977, 92 (*Chem. Abs.*, 1977, **86**, 190 366v).
[618] V. I. Krupenskii, I. I. Korol'kov, and T. V. Dolgaya, *Izvest. Vyssh. Uchebn. Zaved., Lesn. Zhur.*, 1977, **20**, 102 (*Chem. Abs.*, 1977, **87**, 136 191s).

Such sugar–metal complexes as (444) were formed in high yield by electrolysis of the appropriate ligand (see p. 133) in methanolic solution using anodes of the metal (Cu or Ni or Co) and a platinum cathode.[619] The interaction between Cu^{2+} cations and adenosine, guanosine, cytidine, and uridine in aqueous DMSO at various pH values has been studied by e.s.r. and n.m.r. spectroscopy.[620] Since Cu^{2+} cations were shown to complex specifically with O-2′ and O-3′ of these nucleosides at pH 8.5–10, complex formation can be used to distinguish 5′-ribonucleotides from 2′- and 3′-ribonucleotides and 2′-deoxyribonucleosides. Under certain conditions, Cu^{2+} cations can form complexes having as many as four bases bound to the metal.



(444)



(445)

C.d. and ^1H and ^{13}C n.m.r. spectroscopy have been used to assign the structure (445) to one of two complexes formed by ligand-exchange between D-mannitol and *cis*-$Pt(NH_3)_2(OH)_2$ at pH 7.[621] The formation of complexes between reduced technetium-99 and polyhydroxy compounds (*e.g.* D-*glycero*-D-*gulo*-heptonic acid, D-gluconic acid, and perseitol) at various pH values has been examined.[622] Xylitol interacted with lanthanide cations to form 1:1 and 2:1 xylitol–lanthanide complexes, the stability of which increased along the series Pr^{3+}, Nd^{3+}, and Eu^{3+}.[623] An interpretation of the induced shifts in these and related systems indicated that nuclei close to the site of lanthanide complexation experience large contact shifts.

Colorimetric and pH measurements have suggested that two successive equilibria are involved when D-mannitol interacts with borate anions; the formation constants determined for the complexes differed from those obtained previously.[624] During paper electrophoresis in the presence of periodate anions at pH 11, oxidation was very slow for raffinose, sucrose, and methyl α-D-glucopyranoside, slightly faster for *myo*-inositol, and more so for D-mannitol.[625] Since the sugars did not migrate unless previously treated with periodate, their electrophoretic mobilities provide evidence for anionic periodate–sugar complexes, which polarimetric and conductivity measurements indicated to have a 1:1 stoicheiometry for raffinose, methyl α-D-glucopyranoside, sucrose, D-mannitol, lactose, maltose, and D-ribose, and a 2:1 stoicheiometry for *myo*-inositol.

[619] Yu. A. Zhdanov, O. A. Osipov, V. P. Grigor'ev, A. D. Garnovskii, Yu. E. Alekseev, N. M. Gontmakher, V. G. Alekseeva, T. A. Yusman, and V. G. Zaletov, *Koord. Khim.*, 1977, **3**, 3 (*Chem. Abs.*, 1977, **86**, 140 356w).

[620] Y.-Y. H. Chao and D. R. Kearns, *J. Amer. Chem. Soc.*, 1977, **99**, 6425.

[621] M. Eshaque, M. J. McKay, and T. Theophanides, *J. Clin. Hematol. Oncol.*, 1977, **7**, 338.

[622] J. Steigman, L. Hwang, and S. Srivastava, *J. Labelled Compounds, Radiopharm.*, 1977, **13**, 160 (*Chem. Abs.*, 1977, **87**, 77 676d).

[623] J. Reuben, *J. Amer. Chem. Soc.*, 1977, **99**, 1765.

[624] W. J. Evans, V. L. Frampton, and A. D. French, *J. Phys. Chem.*, 1977, **81**, 1810.

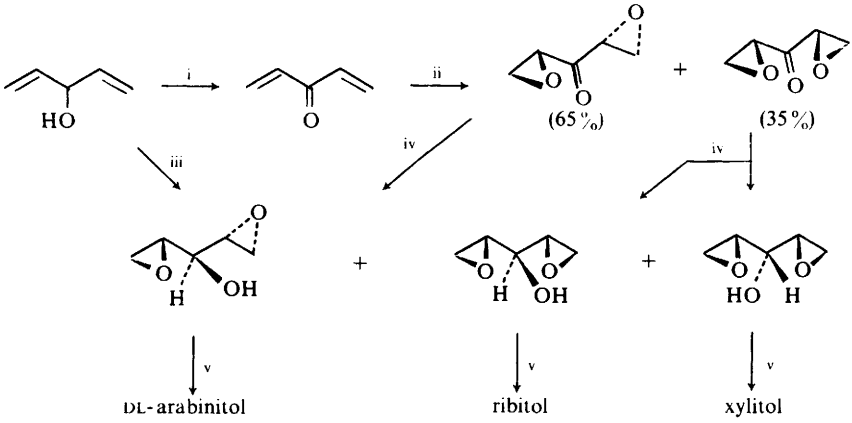
[625] W. J. Popiel and M. S. Rustom, *Carbohydrate Res.*, 1977, **56**, 407.

17
Alditols and Cyclitols

Alditols

A monograph (in Russian) has appeared on catalytic hydrogenation and hydrogenolysis of carbohydrates.[626] The influence of the composition of nickel–alumina and other catalysts on the hydrogenolysis of D-glucose has been investigated.[627, 628]

DL-Arabinitol, ribitol, and xylitol have been synthesized from 3-hydroxypenta-1,4-diene (divinyl carbinol) by the routes outlined in Scheme 80.[629]



Reagents: i, MnO_2–$CHCl_3$; ii, H_2O_2–HO^-; iii, *p*-$NO_2C_6H_4CO_3H$; iv, $NaBH_4$; v, NaOH

Scheme 80

Radical-initiated reduction of peracetylated glycopyranosyl bromides with tributyltin hydride has provided a route to the corresponding 1,5-anhydropentitols and -hexitols, including 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-iditol.[630]

[626] N. K. Nadirov and R. L. Slutskin, 'Catalytic Hydrogenation and Hydrogenolysis of Carbohydrates', Khimiya, Moscow, 1976 (*Chem. Abs.*, 1977, **87**, 202 015r).

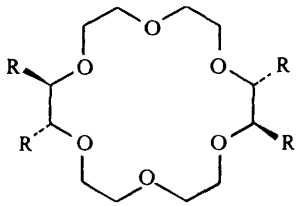
[627] T. I. Poletaeva, T. V. Ipatova, E. P. Leikin, I. A. Mamaeva, G. F. Epishko, E. Z. Golosman, and V. I. Yakerson, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 2592 (*Chem. Abs.*, 1977, **86**, 90 160e).

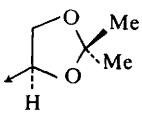
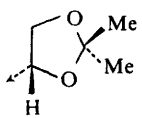
[628] N. K. Nadirov, D. V. Sokol'skii, T. A. Isaev, and A. F. Savel'ev, *Kinetika i Kataliz*, 1977, **18**, 812 (*Chem. Abs.*, 1977, **87**, 168 296w).

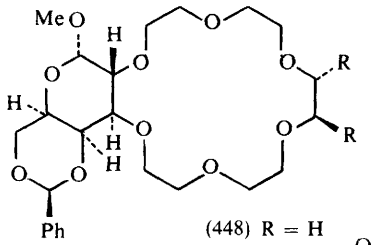
[629] P. Chautemps, *Compt. rend.*, 1977, **284**, *C*, 807.

[630] J. Augé and S. David, *Carbohydrate Res.*, 1977, **59**, 255.

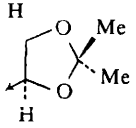
Interest continues in the incorporation of carbohydrate residues into the 18-crown-6 framework. Optically pure chiral cryptands have been synthesized from L-tartaric acid, D-mannitol [*e.g.* (446)],[631] and L-iditol [*e.g.* (447)][632] by Stoddart's group by procedures similar to those previously described (see Vol. 9, p. 198). The DD-crown (446) formed strong complexes with alkali-metal cations in methanol and with primary alkylammonium salts in dichloromethane, and, in contrast to the LL-crown (447), exhibited enantiomeric differentiation in complexation equilibria towards (*RS*)-α-phenylethylammonium hexafluorophosphate. The LL-crown (447), however, showed chiral recognition in favour of the (*R*)-isomer towards the perchlorate salt of (*RS*)-phenylglycine methyl ester and formed slightly stronger complexes with t-butylammonium thiocyanate than did the DD-crown (446). Space-filling molecular models indicated that chiral recognition of the LL-crown (447) towards the perchlorate salt of (*RS*)-phenylglycine methyl ester may be accounted for by the effect of steric compression in the diastereoisomeric complexes. The chiral, asymmetric 18-crown-6 macrocycles (448)–(451) incorporating D-galactose and D-glucose residues have also been synthesized.[633] They formed weaker complexes than 18-crown-6 itself with t-butylammonium thiocyanate, although the trend in complexing ability, *viz.* (448) > (449) > (450) > (451), was

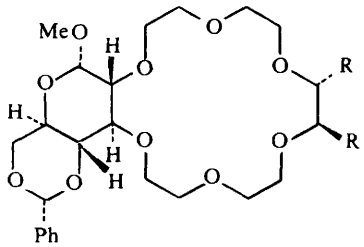


(446) R =  (447) R = 

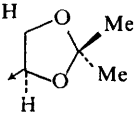


(448) R = H

(449) R = 



(450) R = H

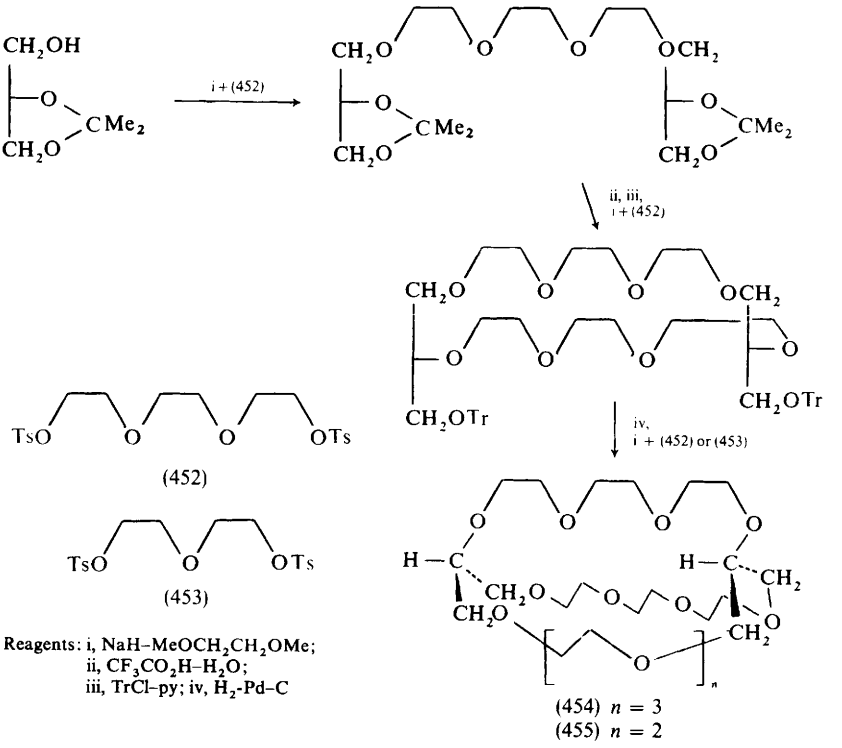
(451) R = 

[631] W. D. Curtis, D. A. Laidler, J. F. Stoddart, and G. H. Jones, *J.C.S. Perkin I*, 1977, 1756.

[632] W. D. Curtis, D. A. Laidler, J. F. Stoddart, J. B. Wolstenholme, and G. H. Jones, *Carbohydrate Res.*, 1977, **57**, C17.

[633] D. A. Laidler and J. F. Stoddart, *Carbohydrate Res.*, 1977, **55**, C1.

unexpected on steric grounds, but could be rationalized if O-4 of the D-*galacto*-18-crown-6 derivatives (448) and (449) is available to participate, together with the oxygen atoms of the polyether, in hydrogen-bonding with the ammonium hydrogen atoms of the cation in a distorted face-to-face complex. The reactions shown in Scheme 81 have yielded two macrobicyclic polyethers (454) and (455) from 2,3-*O*-isopropylidene-D-glycerol.[634] $^{13}C\{^1H\}$ N.m.r. spectroscopy showed that the cryptands (454) and (455) undergo *in, out–out, in* isomerization by passage of any one chain through the ring formed by the other chains and the bridgehead carbon atoms (homeomorphic isomerization); in the case of the cryptand (455), the interconversion may be slowed sufficiently by cooling for the bridgehead carbon atoms to be distinguished spectroscopically.

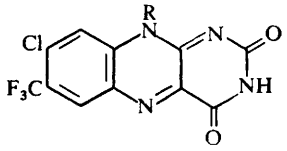


Scheme 81

Several 8-chloro-7-trifluoromethylisoalloxazines (456) have been synthesized by the reaction of 1-amino-1-deoxy-hexitols and -pentitols with 2,4-dichloro-5-nitrotrifluoromethylbenzene, followed by reduction of the nitro-group of the product with Raney nickel and cyclization with alloxan.[634a]

[634] B. J. Gregory, A. H. Haines, and P. Karntiang, *J.C.S. Chem. Comm.*, 1977, 918.

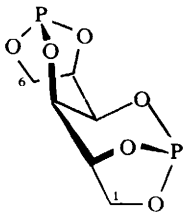
[634a] N. A. Plashkina, V. I. Troitskaya, Z. V. Pushkareva, and T. P. Ryapasova, *Khim. geterotsikl. Soedinenii*, 1976, 1136 (*Chem. Abs.*, 1977, **86**, 16 857w).



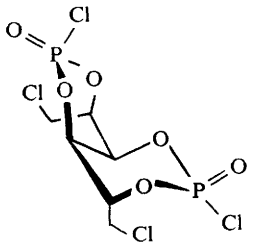
(456) R = 1-deoxyalditol-1-yl

Complexes of lithium aluminium hydride with 1,4:3,6-dianhydro- and 1,3:4,6-di-*O*-benzylidene-D-mannitol, each of which contains a two-fold axis of symmetry, have been used to achieve asymmetric reductions of some alkyl aryl and dialkyl ketones (*e.g.* methyl or ethyl phenyl ketone and 3,3-dimethylbutan-2-one).[635] All reductions with lithium 1,4:3,6-dianhydro-D-mannitolatodihydridoaluminate(III), using a 2:1 molecular ratio of ketone to reducing agent, gave the *S*-enantiomer of the secondary alcohol preferentially (selectivity 1.1—5.3%), whereas those of alkyl aryl ketones and dialkyl ketones gave more of the *S*- and *R*-alcohols, respectively, when lithium 1,3:4,6-di-*O*-benzylidene-D-mannitolatodihydridoaluminate(III) was used.

D-Mannitol reacted with phosphorus trichloride in *p*-dioxan to give the 1,2:3,4:5,6-tris(phosphorochloridate) and the bis(cyclic phosphite) (457), which was converted into the 1,6-dichloro-1,6-dideoxy-D-mannitol derivative (458) on treatment with chlorine.[328]



(457)



(458)

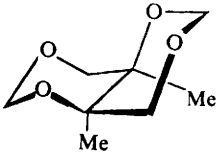
The acid-catalysed reaction of *meso*-2,3-dimethylbutanetetraol with paraformaldehyde yielded the 1,2:3,4- and 1,4:2,3-di-*O*-methylene derivatives, whereas that of DL-2,3-dimethylbutanetetraol afforded principally the 1,2:3,4- and 1,3:2,4-di-*O*-methylene derivatives.[636] The latter derivative (459) was shown to favour the 'O-inside' conformation, despite the introduction of two methyl groups.

Oxidation of allitol with one molecular equivalent of periodate yielded ribose (17%) and unreacted allitol (23%) among the products, indicating that ribose was the first product of the oxidation.[637] This work extends earlier studies (J. C. Schwarz, *J. Chem. Soc.*, 1957, 276) on the partial oxidation of alditols and implies that, in the absence of *threo*-glycol groups, cleavage between neighbouring primary and secondary hydroxy-groups is preferred to that of an *erythro*-glycol group. Kinetic

[635] N. Baggett and P. Stribblehill, *J.C.S. Perkin I*, 1977, 1123.

[636] N. Frederiksen, R. B. Jensen, S. E. Jørgensen, J. U. R. Nielsen, L. Norskov, and G. Schroll, *Acta Chem. Scand.* (*B*), 1977, **31**, 694.

[637] L. Hough, P. N. Shankar Iyer, and B. E. Stacey, *Carbohydrate Res.*, 1976, **52**, 228.



(459)

studies of the degradation of 1,5-anhydro-ribitol and -xylitol by gaseous oxygen in aqueous sodium hydroxide solution at 120 °C have indicated that the reactions proceed by a free-radical mechanism involving intermediate α-hydroxyhydroperoxidic species.[638] In contrast to those in the reactions of 1,5-anhydroribitol, the α-hydroxyhydroperoxidic species formed in the reactions of 1,5-anhydroxylitol appear to be stabilized by intramolecular hydrogen-bonding, and because of their augmented stability increase the importance of peroxy radical–peroxy radical termination reactions producing non-radical products. Possible pathways to account for formic, acetic, lactic, glycolic, glyceric, and 3-*O*-(carboxymethyl)-glyceric acids, and 1,4-anhydro-2-*C*-carboxytetritols among the acidic degradation products were discussed.

A micro method for determining the structures of substituted 2-acetamido-2-deoxy-D-galactitol and -glucitol in glycoproteins, *etc.*, has been reported.[639] Aminodeoxy[^{3}H]alditols resulting from periodate oxidation, reduction with sodium borotritide, and hydrolysis of nmole amounts of substituted 2-acetamido-2-deoxyalditols were separated by t.l.c. and detected by autoradiography.

Volumetric specific heats and densities have been measured for several alditols in water and in salt solutions.[640] The lower apparent molal heat capacities of D-glucitol and xylitol suggest that alditols which adopt a bent conformation in solution are relatively more disruptive of the solvent structure than those which adopt a planar zig-zag conformation.

Cellulose chromatography has been used in isolating erythritol, arabinitol, and mannitol from extracts of several species of lichen.[641]

Other references to alditols can be found in Chapters 3, 16, and 18.

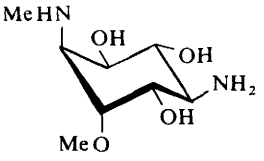
Cyclitols

Fortamine (460), a new aminocyclitol isolated from the aminoglycoside antibiotics fortimicin A and B (see also Chapter 18), has been identified as a derivative of 1,4-diamino-1,4-dideoxy-*chiro*-inositol.[642]

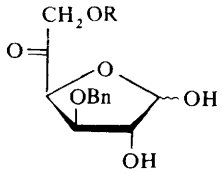
An epimerase from bovine brain has been shown to catalyse the interconversion of *myo*- and *scyllo*-inositol, also producing *neo*-inositol.[643]

3-*O*-Benzyl-D-*xylo*-hexofuranos-5-ulose (461) and the corresponding 3,6-di-*O*-benzyl derivative (463) have been converted into the *myo*-inositol derivatives (462) and (464) by base-catalysed intramolecular cyclization and reduction (sodium boro-

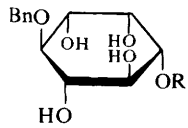
[638] E. C. Millard, L. R. Schroeder, and N. S. Thompson, *Carbohydrate Res.*, 1977, **56**, 259
[639] D. H. van den Eijnden, J. F. Codington, and R. W. Jeanloz, *Carbohydrate Res.*, 1976, **52**, 209.
[640] G. Dipaola and B. Belleau, *Canad. J. Chem.*, 1977, **55**, 3825.
[641] G. Pueyo, *Ann. Falsif. Expt. Chim.*, 1977, **70**, 27 (*Chem. Abs.*, 1977, **87**, 2372t).
[642] R. S. Egan, R. S. Stanaszek, M. Cirovic, S. L. Mueller, J. Tadanier, J. R. Martin, P. Collum, A. W. Goldstein, R. L. de Vault, A. C. Sinclair, E. E. Fager, and L. A. Mitscher, *J. Antibiotics*, 1977, **30**, 552.
[643] P. P. Hipps, W. H. Holland, and W. R. Sherman, *Biochem. Biophys. Res. Comm.*, 1977, **77**, 340.



(460)

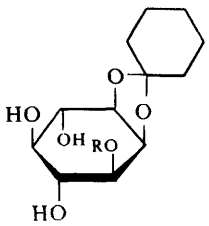


(461) R = H
(463) R = Bn

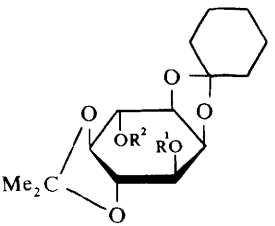


(462) R = H
(464) R = Bn

hydride) of the resulting inososes[644] (see also Vol. 9, p. 124). Acid-catalysed acetonation of 1,2-*O*-cyclohexylidene-*myo*-inositol (465; R = H) and its 3-*O*-toluene-*p*-sulphonyl and 3-*O*-benzoyl derivatives (465; R = Ts and Bz, respectively) with 2,2-dimethoxypropane in DMF gave the 4,5-*O*-isopropylidene derivatives (466; R^1 = H, Bz, or Ts, R^2 = H) and, after chromatography, the corresponding 5,6-*O*-isopropylidene derivatives.[645] *chiro*-Inositol derivatives were obtained by nucleophilic displacement of the 3-toluene-*p*-sulphonyloxy group of (466; R^1 = Ts, R^2 = H) with the benzoate anion, whereas the 3,6-ditosylate (466; R^1 = R^2 = Ts) afforded 2,5-di-*O*-benzoyl-3,4-*O*-cyclohexylidene-1,6-*O*-isopropylidene-*allo*-inositol in 10% yield on prolonged treatment with sodium benzoate in refluxing DMF.



(465) R = H, Bz, or Ts

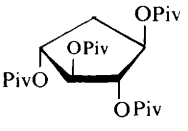


(466) R^1 = H, Bz, or Ts
R^2 = H or Ts

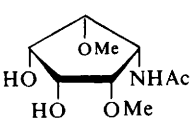
1,2,3,4-Tetra-*O*-pivaloyl-1,3/2,4-cyclopentanetetraol (467) reacted with antimony(V) chloride in dichloromethane to give a mixture of pivaloxonium ions that equilibrated by 1,2- and 1,3-rearrangements.[646] Base-catalysed condensation of the product of periodate oxidation of the *cis*-tetrahydroxycyclopentylamine derivative (468) with nitromethane, *etc.*, furnished a mixture of the *myo*-2,5-, *epi*-1,4-, and *cis*-1,4-inosadiamine derivatives (469)—(471).[647]

The synthesis of a carbocyclic analogue of 9-β-D-arabinofuranosyladenine is mentioned in Chapter 19; the hydroxycyclopentylamine (472) required for this synthesis was obtained by the route shown in Scheme 82.[648]

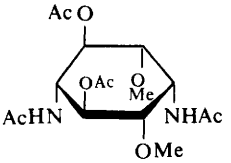
[644] A. P. Kaplun, V. A. Basharuli, V. E. Kalugin, V. K. Shvets, and R. P. Evstigneeva, *Trudy Khim. i Khim. Tekhnol.*, 1975, **5**, 82 (*Chem. Abs.*, 1977, **86**, 90 168p).
[645] S. Ogawa, S. Oki, H. Kunitomo, and T. Suami, *Bull. Chem. Soc. Japan*, 1977, **50**, 1867.
[646] H. Paulsen and O. Brauer, *Chem. Ber.*, 1977, **110**, 342.
[647] K. Tadano, T. Shiratori, and T. Suami, *Bull. Chem. Soc. Japan*, 1976, **49**, 3193.
[648] R. Vince and S. Daluge, *J. Medicin. Chem.*, 1977, **20**, 612.



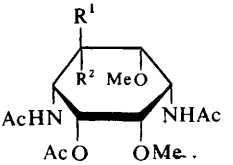
(467) Piv = COBu^t



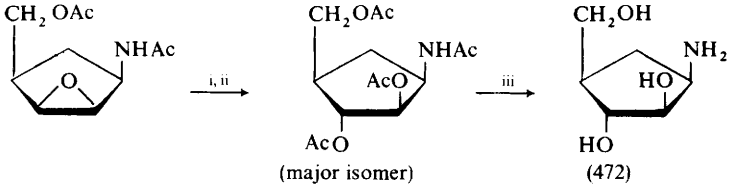
(468)



(469)



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



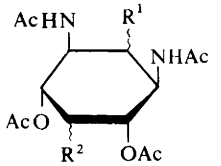
(major isomer)

(472)

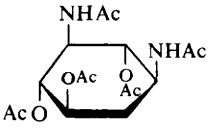
Reagents: i, H_3O^+; ii, Ac_2O–py; iii, HCl–H_2O

Scheme 82

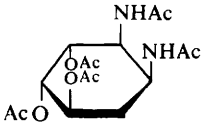
Acetylated positional isomers (473)—(477) of deoxystreptamine have been synthesized from cyclohexanepentaol tosylates and related epoxides by conventional displacement and ring-opening reactions, respectively.[649] The triaminocyclohexanediol penta-acetates (478) and (479) were also prepared by way of azidolysis of 1,2,5-tri-*O*-methanesulphonyl-1,3,4/2,5-cyclohexanepentaol.



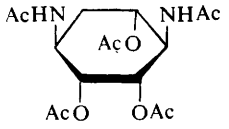
(473) R^1 = H, R^2 = OAc
(474) R^1 = OAc, R^2 = H



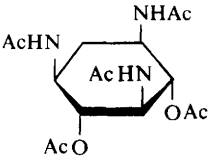
(475)



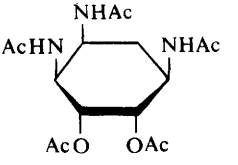
(476)



(477)



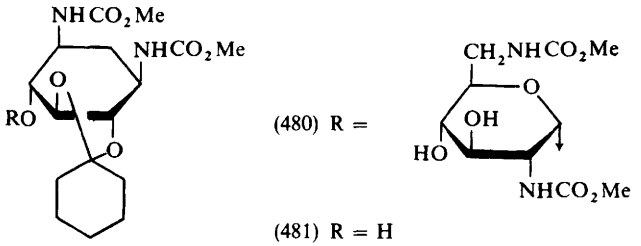
(478)



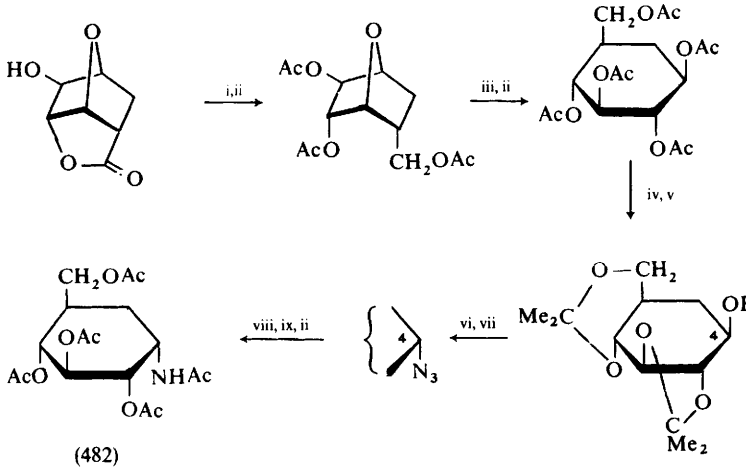
(479)

Oxidation of 5,6-*O*-cyclohexylidenetetra-*N*-(methoxycarbonyl)neamine (480) with sodium periodate and reaction of the resulting dialdehyde with triethylamine (or with catalytic amounts of sodium methoxide) effected β-elimination to give the

[649] S. Ogawa, T. Ueda, Y. Funaki, Y. Hongo, A. Kasuga, and T. Suami, *J. Org. Chem.*, 1977, **42**, 3083.



2-deoxystreptamine derivative (481), which is a suitable substrate for glycosylation at O-4, leading to aminocyclitol glycosides.[650] The racemic penta-acetyl derivative (482) of validamine, a component of the validamycins produced by *Streptomyces hygroscopicus* var. *limoneus*, has been synthesized by the route shown in Scheme 83.[651]



(482)

All compounds are racemates

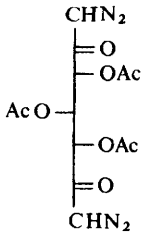
Reagents: i, $LiAlH_4$; ii, Ac_2O–py; iii, AcOH–H_2SO_4; iv, MeONa–MeOH; v, $Me_2C(OMe)_2$–TsOH; vi, TsCl–py; vii, NaN_3–DMF; viii, H_2–Ni; ix, H_3O^+

Scheme 83

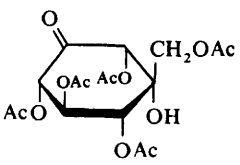
The bis(diazoketone) (483) prepared from 2,3,4-tri-*O*-acetylxylaryl dichloride cyclized to give the branched-chain cyclitol (484) on heating in acetic acid containing copper(II) acetate.[652] Reduction of (484) and acetylation of the products gave the DL-2-*C*-hydroxymethyl-*epi*-inositol hexa-acetate (485).

3-Amino-4,5-*O*-isopropylidene-3/4,5-cyclopentene-4,5-diol has been coupled to a 7-deazapurine derivative to furnish, after appropriate transformations, the 9-

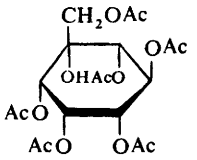
[650] A. Canas-Rodriguez, S. G. Ruiz-Poveda, A. Gomez Sanchez, and E. B. Gonzalez, *Carbohydrate Res.*, 1977, **59**, 240.
[651] T. Suami, S. Ogawa, K. Nakamoto, and I. Kasahara, *Carbohydrate Res.*, 1977, **58**, 240.
[652] C. E. Cantrell, D. E. Kiely, G. J. Abruscato, and J. M. Riordan, *J. Org. Chem.*, 1977, **42**, 3562.



(483)



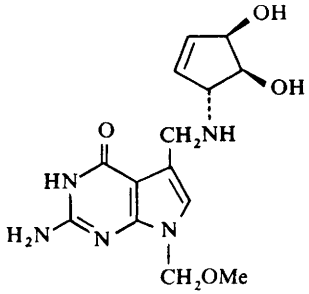
(484)



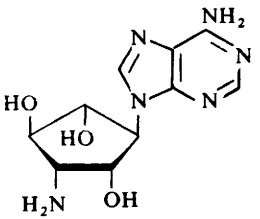
(485)

methoxymethylated derivative (486) of the basic component of nucleoside Q[653] (see Vol. 9, p. 139).

9-[DL-(1,4/2,3,5)-3-Amino-2,4,5-trihydroxycyclopentyl]adenine (487) and its DL-(1,2,4/3,5)- and DL-(1,2,5/3,4)-isomers have been synthesized by condensation of the corresponding 3-*N*-acetyl-1,3-diaminocyclopentanetriol with 4-amino-6-chloro-5-nitropyrimidine, reduction of the nitro-group, and ring-closure with

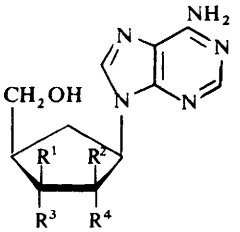


(486)



(487)

formamide, *etc.*[654] Aristeromycin (488) reacted with acetyl bromide in acetonitrile (see Vol. 8, p. 149) to give, after deacetylation, a 1:5 mixture of the brominated derivatives (489) and (490), respectively, which yielded 2′- and 3′-deoxyaristeromycin on catalytic hydrogenolysis.[655]

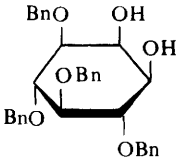


(488) $R^1 = R^2 = H$, $R^3 = R^4 = OH$
(489) $R^1 = R^4 = H$, $R^2 = Br$, $R^3 = OH$
(490) $R^1 = Br$, $R^2 = R^3 = H$, $R^4 = OH$

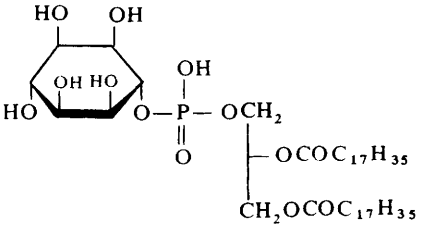
[653] T. Ohgi, T. Kondo, and T. Goto, *Nucleic Acids Res., Special Publ.*, 1976, 2 (4th Symposium Nucleic Acids Chem., 1976), 83 (*Chem. Abs.*, 1977, **86**, 190 394c).

[654] K. Tanado, Y. Emori, M. Ayabe, and T. Suami, *Bull. Chem. Soc. Japan*, 1977, **50**, 1572.

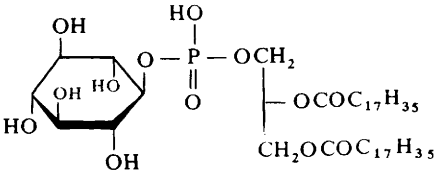
[655] R. Marumoto, Y. Yoshioka, Y. Furukawa, and M. Honjo, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 2624.



(491)



(492)



(493)

Derivatives prepared from inositols include both enantiomers of the tetra-*O*-benzyl-*myo*-inositol (491), which were resolved after their conversion into diastereoisomeric α-D-mannopyranosyl or β-D-glucopyranosyl derivatives,[656, 656a] 2-*O*-α-D-mannopyranosyl-*myo*-inositol,[657] and the phosphatidyl *muco*- and *scyllo*-inositols (492)[658] and (493),[659] respectively.

The γ-radiolysis of *myo*-inositol hexasulphate has been accounted for by a radical-initiated chain process that results in a high *G*-value for the sulphate anion and a low yield of hydrogen peroxide.[660] The substrate reacted slowly with hydroxy-radicals.

[656] A. E. Stepanov, V. I. Shvets, and R. P. Evstigneeva, *Bioorg. Khim.*, 1976, **2**, 1618 (*Chem. Abs.*, 1977, **86**, 121 654a).

[656a] A. E. Stepanov, B. A. Klyashchitskii, V. Shvets, and R. P. Evstigneeva, *Bioorg. Khim.*, 1976, **2**, 1627 (*Chem. Abs.*, 1977, **86**, 121 655b).

[657] A. E. Stepanov, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1977, **13**, 1410 (*Chem. Abs.*, 1977, **87**, 201 950e).

[658] V. P. Shevchenko, T. Yu. Lazurkina, Yu. G. Molotkovskii, and L. D. Bergel'son, *Bioorg. Khim.*, 1976, **2**, 923 (*Chem. Abs.*, 1977, **86**, 16 856v).

[659] V. P. Shevchenko, T. Yu. Lazurkina, Yu. G. Molotkovskii, and L. D. Bergel'son, *Bioorg. Khim.*, 1977, **3**, 252 (*Chem. Abs.*, 1977, **86**, 190 385a).

[660] M. Balakrishnan, W. J. Criddle, and B. J. Jones, *Internat. J. Radiation Phys. Chem.*, 1976, **8**, 549 (*Chem. Abs.*, 1977, **86**, 16 858x).

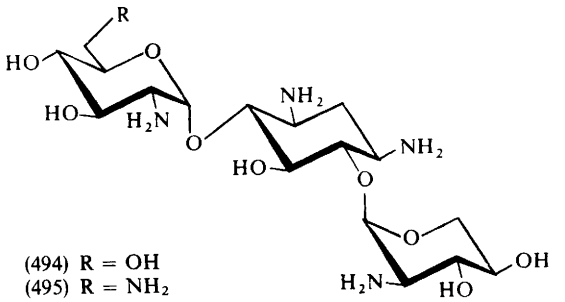
18
Antibiotics

A general review covering sugar-containing antibiotics, including aminoglycoside, nucleoside, and macrolide antibiotics, has appeared.[661]

Aminoglycoside Antibiotics

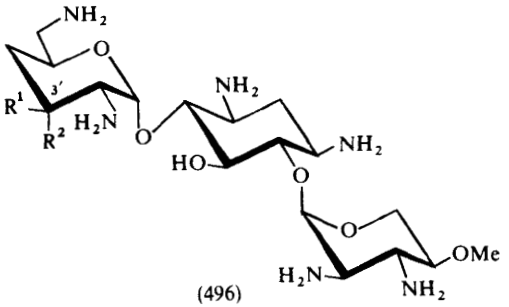
The aminoglycoside antibiotics[662] and chemical transformations on them [663] have been reviewed.

Several components have been separated from the seldomycin complex elaborated by *Streptomyces hofunensis*.[664, 665] Seldomycin factors 1 and 2 were identified as 6-*O*-(2-amino-2-deoxy-α-D-xylopyranosyl)paromamine (494) and 4′-deoxyneamine, respectively; factor 3 as, probably, (495) (*i.e.* the 6′-amino-6′-deoxy analogue of factor 1); and factor 5, which is the most active of these antibiotics, as 6-*O*-(2,3-diamino-2,3-dideoxy-4-*O*-methyl-α-D-xylopyranosyl)-4′-deoxyneamine (496; R^1 = OH, R^2 = H). Seldomycin factor 5 has been converted into its 3′-epimer (496; R^1 = H, R^2 = OH)[666] and its 3′-deoxy analogue (496; R^1 = R^2 = H)[667] by conventional transformations involving the *N*-protected 3′-tosylate.



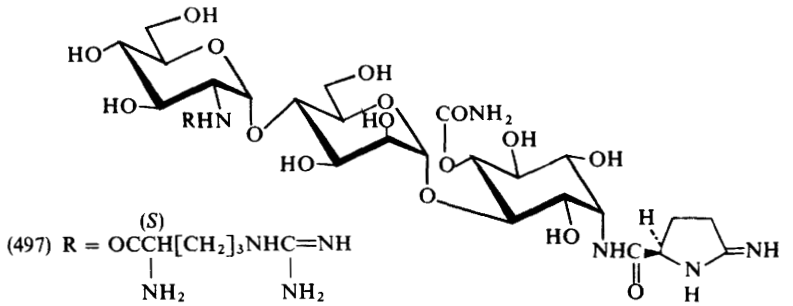
(494) R = OH
(495) R = NH_2

[661] S. Umezawa, *M.T.P. Internat. Rev. Sci., Org. Chem., Ser. Two*, 1976, **7**, 149.
[662] D. A. Cox, K. Richardson, and B. C. Ross, *Topics Antibiotic Chem.*, 1977, **1**, 2 (*Chem. Abs.*, 1977, **87**, 177 326h).
[663] B. N. Strunin, *Uspekhi Khim.*, 1977, **46**, 1445 (*Chem. Abs.*, 1977, **87**, 184 788p).
[664] R. S. Egan, A. C. Sinclair, R. L. de Vault, J. B. McAlpine, S. L. Mueller, P. C. Goodley, R. S. Stanaszek, M. Cirovic, R. J. Mauritz, L. A. Mitscher, K. Shirahata, S. Sato, and T. Iida, *J. Antibiotics*, 1977, **30**, 31.
[665] J. B. McAlpine, A. C. Sinclair, R. S. Egan, R. L. de Vault, R. S. Stanaszek, M. Cirovic, S. L. Mueller, P. C. Goodley, R. J. Mauritz, N. E. Wideberg, L. A. Mitscher, K. Shirahata, H. Matsushima, S. Sato, and T. Iida, *J. Antibiotics*, 1977, **30**, 39.
[666] H. Matsushima, K. Kitaura, and Y. Mori, *Bull. Chem. Soc. Japan*, 1977, **50**, 3039.
[667] H. Matsushima, Y. Mori, and K. Kitaura, *J. Antibiotics*, 1977, **30**, 890.

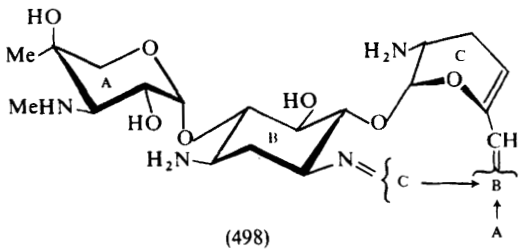


(496)

Among other new aminoglycoside antibiotics isolated during the past year were the pseudotrisaccharide–peptide derivative LL-BM123α (497), a remarkably non-toxic antibiotic, from an unidentified species of *Nocardia*;[668] the dimeric pseudotrisaccharide (498), a minor component of antibiotic 66-40C elaborated by



(497) R = (*S*)-OCCH(NH$_2$)[CH$_2$]$_3$NHC(=NH)NH$_2$



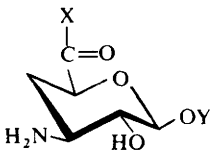
(498)

Micromonospora inyoensis[669] [the principal component of the complex is sisomicin (see Vol. 9, p. 134)]; fortimicins A (499) and B (500), which are glycosides of 6-*epi*-purpurosamine B containing the novel aminocyclitol fortamine (see also p. 147).[642] ^{1}H N.m.r. spectroscopy showed that the *N*-glycylfortamine unit of fortimicin A adopts the alternative chair conformation to that depicted in (499). Degradative and spectroscopic studies have established that ezomycins B$_1$, B$_2$,

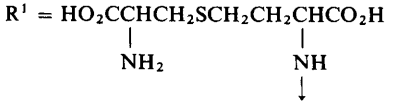
[668] G. A. Ellestad, J. H. Martin, G. O. Morton, M. L. Sassiver, and J. E. Lancaster, *J. Antibiotics*, 1977, **30**, 678.

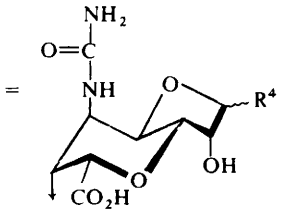
[669] D. H. Davies, A. K. Mallams, J. McGlotten, J. B. Morton, and R. W. Tkach, *J.C.S. Perkin I*, 1977, 1407.

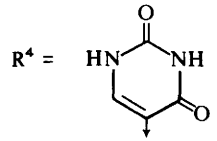
C_1, C_2, D_1, and D_2 from fermentation broths of a *Streptomyces* species (see also Vol. 8, p. 130) possess the structures shown in Scheme 84.[670] Ezomycins B_1, C_1, and D_1, as well as ezomycins B_2, C_2, and D_2, interconvert under mild acidic conditions.

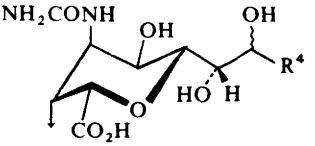


ezomycin	X	Y	R^4
B_1	R^1	R^2	β-linked
B_2	HO	R^2	β-linked
C_1	R^1	R^2	α-linked
C_2	HO	R^2	α-linked
D_1	R^1	R^3	
D_2	HO	R^3	

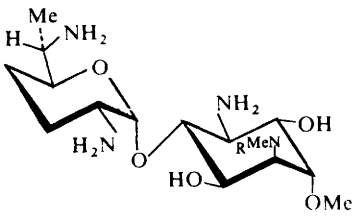




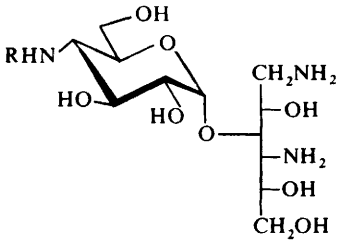




Scheme 84



(499) R = $COCH_2NH_2$
(500) R = H

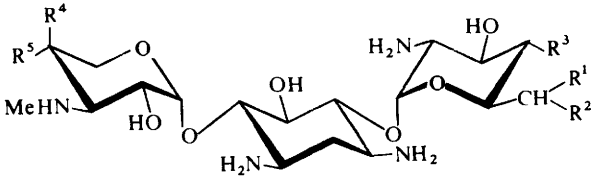


(501) R = Ac or H
(502) R = COEt

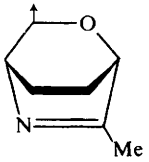
The structures of antibiotics P-2563 (A) and (P) elaborated by *Pseudomonas fluorescence* P-2563 have been identified as (501; R = Ac) and (502).[671,671a] Antibiotics (501; R = Ac) and (502), as well as the *N*-deacylated derivative (501; R = H), were also found among the components of the antibiotic complex LL-AM31 produced by a *Streptoverticillium* species.[672] The structure of the aglycone

[670] K. Sakata, A. Sakurai, and S. Tamura, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 2033.
[671] K. Nara, Y. Sumino, K. Katamoto, S. Akiyama, and M. Asai, *Chem. Letters*, 1977, 33.
[671a] K. Nara, K. Katamoto, S. Suzuki, S. Akiyama, and E. Mizuta, *Chem. Letters*, 1977, 229.
[672] J. P. Kirby, G. E. van Lear, G. O. Morton, W. E. Gore, W. V. Curran, and D. B. Borders, *J. Antibiotics*, 1977, **30**, 344.

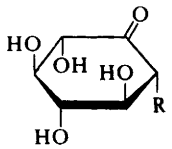
of (501) and (502) was established as 1,4-diamino-1,4-dideoxy-D-glucitol by chemical evidence[671a] and *X*-ray crystallography.[672] The antibiotic LL-BM408 (isolated from an unidentified *Streptomyces* species that also produces paromomycins I and II) has been identified as 5-*O*-β-D-ribofuranosylparomamine, previously recognized as a degradation product of paromomycin and related antibiotics.[673] Fermentation broths of *Micromonospora purpurea* var. *nigrescens* have yielded twenty-five aminoglycoside antibiotics, including seven new antibiotics (503) belonging to the gentamicin family and another having the hexosamine residue of the pseudotrisaccharide in the form of a bicyclic ketimine (504).[674]



(503) R^1 = Me or H; R^2 = OH, NH_2, or NHMe
R^3 = OH or H; R^4 = OH or H; R^5 = OH, H, or Me



(504)

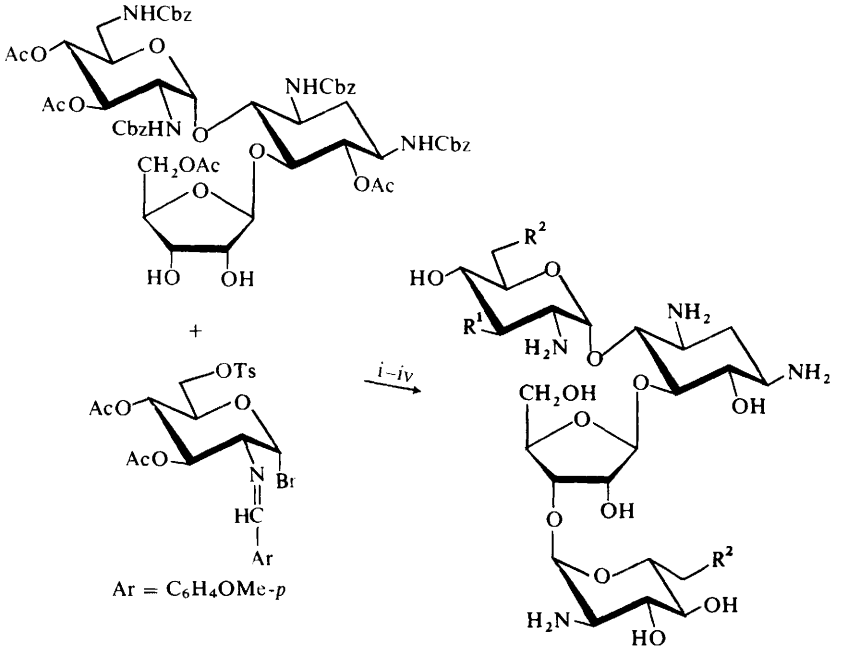


(505) R = OH
(506) R = H

A mutant strain of *Micromonospora purpurea* synthesized gentamicin antibiotics on being fed with the inososes (505) and (506), which are presumably converted into streptamine and 2-deoxystreptamine, respectively, prior to incorporation into the intact antibiotics.[675] A mutant strain of *S. fradiae* synthesized the pseudotrisaccharide ribostamycin (see Vol. 10, p. 131) instead of the pseudotetrasaccharide neomycin.[676] After a second and reverse mutation, new colonies again synthesized neomycin, indicating that ribostamycin may be an intermediate along the biosynthetic pathway leading to neomycin. A chemical synthesis of neomycin C (507) from a derivative of ribostamycin is shown in Scheme 85.[677]

The pseudotetrasaccharide (508), which contains 2-amino-2-deoxy-α-D-glucopyranose instead of the 2,6-diamino-2,6-dideoxy-β-L-idopyranosyl residue of lividomycin B, has been synthesized by way of glycosylation of a protected 3′-deoxyparomamine derivative.[678] The lividomycin B analogue (508) displayed

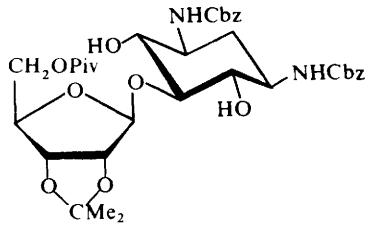
[673] J. P. Kirby, D. B. Borders, and G. E. van Lear, *J. Antibiotics*, 1977, **30**, 175.
[674] J. Berdy, J. K. Pauncz, Z. M. Vajna, G. Horváth, J. Gyimesi, and I. Koczka, *J. Antibiotics*, 1977, **30**, 945.
[675] S. J. Daum, D. Rosi, and W. A. Goss, *J. Amer. Chem. Soc.*, 1977, **99**, 283.
[676] H. Baud, A. Betencourt, M. Peyre, and L. Penasse, *J. Antibiotics*, 1977, **30**, 720.
[677] S. Umezawa and Y. Nishimura, *J. Antibiotics*, 1977, **30**, 189.
[678] I. Watanabe, T. Tsuchiya, T. Takase, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1977, **50**, 2369.



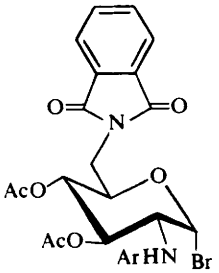
Scheme 85

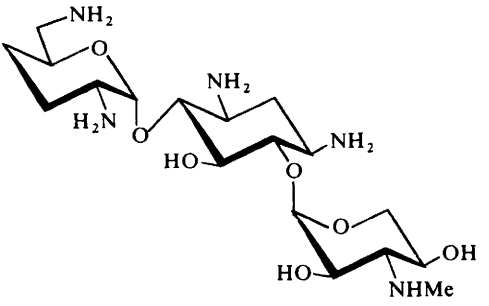
much weaker antibacterial activity than did lividomycin B, indicating the importance of the 6‴-amino-group in determining the antibacterial activity of lividomycin B. New syntheses of ribostamycin (see Vol. 10, p. 131) have been described. In one, condensation of the 5-*O*-β-D-ribofuranosyl-2-deoxystreptamine derivative (509) with the glycosyl bromide (510) gave only a minor proportion (6%) of the required α-(1 → 4)- and β-(1 → 5)-linked pseudotrisaccharide derivative among the products,[679] whereas condensation of 3′,4′,6-tri-*O*-acetyl-1,2′,3,6′-tetra-*N*-ethoxycarbonylneamine (511) with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride gave the required pseudotrisaccharide derivative (75%), which furnished ribostamycin in 47% overall yield after removal of the protecting groups.[680] 5-*O*-β-D-Glucopyranosylneamine, also prepared from (511), was much less active than the corresponding α-D-anomer against several micro-organisms. Condensation of appropriately protected derivatives of 3′,4′-dideoxyneamine and 3-deoxy-3-dimethylamino-D-xylopyranose has been used to prepare an analogue (512) of gentamicin A.[681] The mass-spectral analysis of gentamicin components is referred to in Chapter 21.

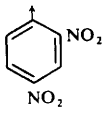
[679] H. Fukami, S. Ikeda, K. Kitahara, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 1689.
[680] T. Suami, S. Nishiyama, Y. Ishikawa, and S. Katsura, *Carbohydrate Res.*, 1977, **56**, 415.
[681] R. D. Sitrin, D. J. Cooper, and J. A. Weisbach, *J. Antibiotics*, 1977, **30**, 836.



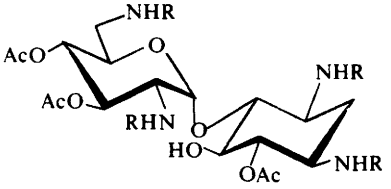
(509) Piv = COBu^t





(510) Ar = 

(512)



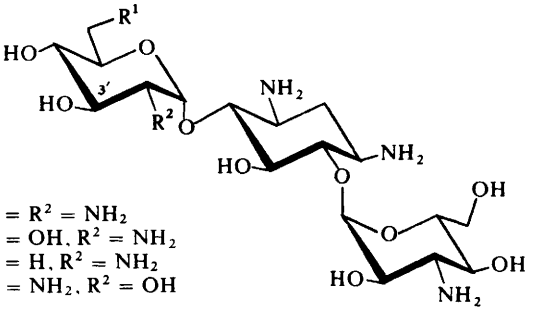
(511) R = CO_2Et

Oxidation of 1,2′,2‴,3,6‴-penta-*N*-acetyl-lividomycin B with sodium periodate and reaction of the resulting dialdehyde with triethylamine effected β-elimination of the pseudotrisaccharide moiety[682] (see also Vol. 8, p. 136).

Kanamycin B (513) has been converted, by essentially standard procedures, into kanamycin C (514), 6′-deoxykanamycin C (515), [683] 3′,4′-dideoxykanamycin B[684] (see Vol. 5, p. 131; Vol. 10, p. 133), tobramycin (3′-deoxykanamycin B),[685] and 4′-deoxykanamycin B,[686, 687] and kanamycin A (516) into 4′-deoxykanamycin A (see Vol. 9, p. 138) by way of an iodide displacement on the 4′-mesylate prepared by selective sulphonylation of 2′,2″,3′-tri-*O*-benzoyl-tetra-*N*-benzyloxycarbonyl-

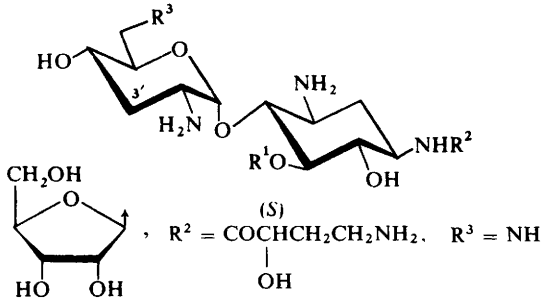
[682] T. Yamaguchi, K. Kamiya, T. Mori, and T. Oda, *J. Antibiotics*, 1977, **30**, 332.
[683] S. Toda, S. Nakagawa, and T. Naito, *J. Antibiotics*, 1977, **30**, 1002.
[684] T. Nishimura, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1977, **50**, 1580.
[685] Y. Takagi, T. Miyake, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1976, **49**, 3649.
[686] Y. Abe, S. Nakagawa, K.-I. Fujisawa, T. Naito, and H. Kawaguchi, *J. Antibiotics*, 1977, **30**, 1004.
[687] T. Miyake, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1977, **50**, 2362.

4″,6″-*O*-cyclohexylidenekanamycin A.[687] Neither HO-3′ nor HO-4′ of *N*-protected butirosin and kanamycin derivatives was benzoylated in an aqueous medium, thereby providing a route to partially blocked derivatives of the antibiotics.[688] New procedures for selective deoxygenation of aminoglycoside antibiotics, including kanamycin B, are mentioned[492,493] in Chapter 11.



(513) $R^1 = R^2 = NH_2$
(514) $R^1 = OH$, $R^2 = NH_2$
(515) $R^1 = H$, $R^2 = NH_2$
(516) $R^1 = NH_2$, $R^2 = OH$

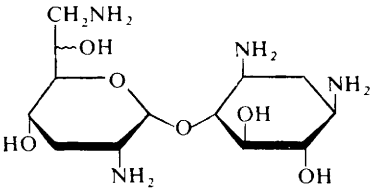
3′-Deoxybutirosin B (517) has been obtained by two routes that involved the D-ribofuranosylation, *etc.*, of derivatives of 3′-deoxyparomamine (518), whereas D-xylofuranosylation, *etc.*, afforded 3′-deoxybutirosin A.[689,690] 3′-Deoxybutirosin B (517) has also been synthesized from ribostamycin by introducing the 3′-deoxy and N^1-[(*S*)-4-amino-2-hydroxybutyryl] groups.[691] Neomycin and paromomycin have been converted into their 5″-amino-5″-deoxy analogues, *via* the corresponding 5″-bromides,[692] and 3′-deoxyparomamine (available by hydrolysis of lividomycin) has been transformed, by way of the action of nitromethane on its 6′-aldehyde derivative, into isomeric 6′-*C*-aminomethyl-3′-deoxyparomamines (519).[693] The use of the Tipson–Cohen procedure to prepare 3′,4′-unsaturated derivatives of butirosin and kanamycin is mentioned[522] in Chapter 12.



(517) $R^1 =$, $R^2 = COCHCH_2CH_2NH_2$ (*S*), $R^3 = NH_2$

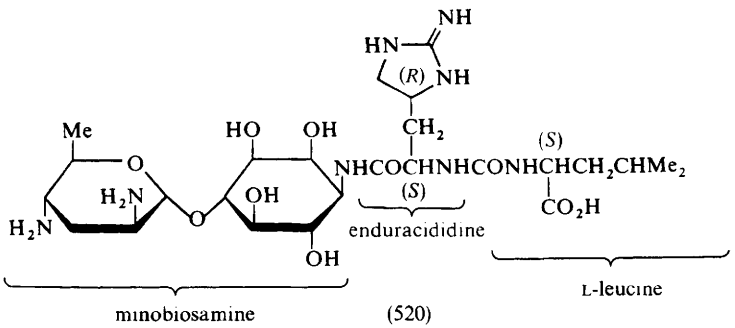
(518) $R^1 = R^2 = H$, $R^3 = OH$

[688] H Saeki, T. Hayashi, Y. Shimada, N. Takeda, and E. Ohki, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 2089.
[689] I. Watanabe, A. Ejima, T. Tsuchiya, D. Ikeda, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1977, **50**, 487.
[690] I. Watanabe, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1977, **50**, 972.
[691] D. Ikeda, F. Nagaki, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1976, **49**, 3666.
[692] S. Hanessian, R. Massé, and M.-L. Capmeau, *J. Antibiotics*, 1977, **30**, 893.
[693] T. Yamaguchi, T. Tsuchiya, and S. Umezawa, *J. Antibiotics*, 1977, **30**, 71.



(519)

(−)-1D-1-Amino-1-deoxy-*myo*-inositol, a component of the antibiotic minosaminomycin (520), has been synthesized by selective catalytic oxidation of D-inositol and reduction of the oxime prepared from the resulting L-*myo*-inosose-1.[694] Minobiosamine (1D-1-amino-1-deoxy-4-*O*-α-D-kasugaminyl-*myo*-inositol) was prepared in the same way from 2′,4′-di-*N*-acetylkasuganobiosamine. A total synthesis of minosaminomycin (520) was accomplished by coupling 2′,4′-di-*N*-benzyloxycarbonylminobiosamine to a protected *N*-leucine-enduracididine derivative and removal of the protecting groups from the product.



(520)

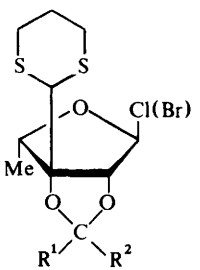
Included among the modified components of aminoglycoside antibiotics reported during the past year were 3′,4′,5,6-tetradeoxy-[695] and 5- and 6-deoxy- and 5,6-dideoxy-neamine;[695, 696] removal of HO-6 from the 2-deoxystreptamine residue of neamine decreased the antimicrobial activity relative to that of the parent neamine, whereas removal of HO-5 enhanced the activity, especially against kanamycin-resistant strains. 1,2′,3,6′-Tetra-*N*-benzyloxycarbonyl-5,6-*O*-cyclohexylideneneamine has been synthesized and converted into nebramine derivatives for use in the synthesis of tobramycin and 4″-*epi*-tobramycin.[697] 2′,4′,6′-Triacetyl-3,3[1]-*O*-carbonyl-3′-deoxydihydrostreptobiosamine, a precursor for the synthesis of 3″-deoxydihydrostreptomycin, has been synthesized from benzyl α-dihydrostreptobiosaminide.[698] The synthesis involved chlorination of the key

[694] K. Iinuma, S. Kondo, K. Maeda, and H. Umezawa, *Bull. Chem. Soc. Japan*, 1977, **50**, 1850.
[695] A. Canas-Rodriguez and S. G. Ruiz-Poveda, *Carbohydrate Res.*, 1977, **58**, 379.
[696] T. Saumi, S. Nishiyama, Y. Ishikawa, and S. Katsura, *Carbohydrate Res.*, 1977, **53**, 239.
[697] M. Tanabe, D. M. Yasuda, and G. Jetre, *Tetrahedron Letters*, 1977, 3607.
[698] H. Sano, T. Tsuchiya, S. Kobayashi, H. Umezawa, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1977, **50**, 975.

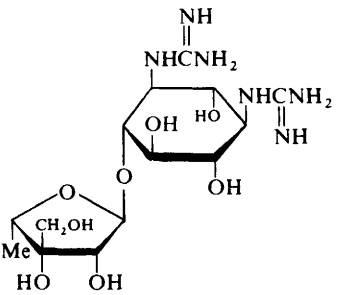
intermediate benzyl 2-*O*-(2-acetamido-2-deoxy-4,6-*O*-isopropylidene-2-*N*-methyl-α-L-allopyranosyl)-3,3^1-*O*-isopropylidene-α-dihydrostreptoside with sulphuryl chloride, followed by dechlorination of the product with tributyltin hydride. The 3′,4′,5- and 3′,4′,6-tri-*O*-acetyl derivatives of 1,2′,3,6′-tetra-*N*-ethoxycarbonylneamine have been prepared by random hydrolysis of 3′,4′-di-*O*-acetyl-1,2′,3,6′-tetra-*N*-ethoxycarbonyl-5,6-*O*-ethoxyethylideneneamine.[699] Condensation of the 3′,4′,5-triacetate and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride gave, after removal of the protecting groups, 6-*O*-β-D-ribofuranosylneamine, which showed much less antimicrobial activity compared with that of neamine. 6-*O*-β-D-Glucopyranosyl- and 6-*O*-β-D-galactopyranosyl-neamines and their α-anomers were also prepared by analogous reactions; the introduction of a β-D-hexopyranosyl residue at O-6 also decreased the antimicrobial activity compared with that of neamine. Similar condensations have furnished 4-, 5-, and 6-*O*-β-D-ribofuranosyl-2-deoxystreptamines from appropriately protected 2-deoxystreptamine precursors.[700]

Paulsen's group has transformed the protected streptosyl chloride (or bromide) (521; R^1, R^2 = Ph,H) into dihydrostreptosylstreptidine (522) and related pseudodisaccharides.[701,702] The 2,3-*O*-benzylidene derivatives (521; R^1,R^2 = Ph,H) yielded α-linked glycosides of L-streptose preferentially,[701,702] whereas the 2,3-carbonates (521; R^1R^2 = O) gave β-linked glycosides (*e.g.* with cyclohexanol and 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose).[703] This work culminated in the synthesis of D-streptobiosamine (523) and its L-enantiomer (a disaccharide unit of streptomycin) by a route that involved condensation of methyl α-D(or L)-streptoside 3^1-(trimethylene dithioacetal) with the nitrosyl chloride adduct of 3,4,6-tri-*O*-acetyl-D-(or L)-glucal.[704]

The quantitative nature of the reactions between aminoglycoside antibiotics (*e.g.* kanamycin) and polyanionic detergents has been investigated by classical tube and gel-precipitation techniques, either of which could be used for the rapid determination of aminoglycoside antibiotics.[705]

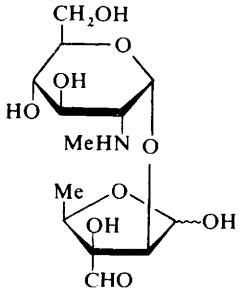


(521) R^1,R^2 = Ph, H or R^1R^2 = O



(522)

[699] T. Suami, S. Nishiyama, Y. Ishikawa, and S. Katsura, *Carbohydrate Res.*, 1976, **52**, 187.
[700] H. Fukami, H. Kohno, K. Kitahara, and M. Nakajima, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 1685.
[701] H. Paulsen, P. Stadler, A. Banaszek, and F. Tödter, *Chem. Ber.*, 1977, **110**, 1908.
[702] H. Paulsen, F. Tödter, A. Banaszek, and P. Stadler, *Chem. Ber.*, 1977, **110**, 1916.
[703] H. Paulsen, P. Stadler, and F. Tödter, *Chem. Ber.*, 1977, **110**, 1896.
[704] H. Paulsen, P. Stadler, and F. Tödter, *Chem. Ber.*, 1977, **110**, 1925.
[705] S. C. Edberg, D. Savino, and J. M. Singer, *Experientia*, 1977, **33**, 323.



(523)

Macrolide Antibiotics

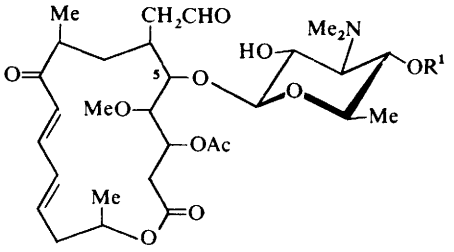
The recent chemistry and biochemistry of macrolides, including the macrolide antibiotics and their component sugars, have been reviewed.[706]

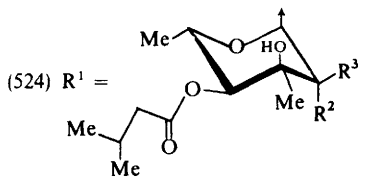
Several minor antibiotics related to lankamycin have been isolated from fermentation broths of *Streptomyces violaceoniger*; two of them, 15-deoxy-5-oxo- and 15-*O*-(4-*O*-acetyl-α-L-arcanbsyl)-lankamycin, showed weak antibacterial activity.[707] The former of these antibiotics could be obtained in high yield by oxidation of lankamycin with Jones's reagent.[707]

The antibiotic complex M-4365 elaborated by *Micromonospora capillata* MCRL 0940 contains at least six components having β-desosamine (3,4,6-trideoxy-3-dimethylamino-D-*xylo*-hexopyranose) attached to C-5 of a 16-membered macrolide ring, whose structure shows minor differences among the various components.[708] The macrolide ring (as its ethylene acetal) of carbomycin B (524; $R^2 = R^3 = H$) has been condensed with 2,4-di-*O*-acetyl-α-mycaminosyl bromide to give, after deacetylation, the 5-*O*-β-mycaminosyl derivative (525) (as its ethylene acetal).[709] This derivative was transformed into 2″-bromocarbomycin B (524; $R^2 = Br$, $R^3 = H$), following its reaction with the glycal derived from 4-*O*-isovalerylmycarose in the presence of the brominating reagent 1,3-dibromo-5,5-dimethylhydantoin, and carbomycin B (524; $R^2 = R^3 = H$) (by radical-initiated debromination of 2″-bromocarbomycin B with tributyltin hydride). The cladinose (3-*O*-methylmycarose) analogue of carbomycin B has also been prepared from demycarosylcarbomycin B ethylene acetal.[710] Erythromycin is composed of erythronolide (a 14-membered lactone that also occurs in erythromycin B), mycarose, and desosamine.[711]

The revised structure (526) has been proposed for the tetraene antibiotic rimocidin on the basis of chemical, ^{13}C n.m.r., and mass-spectral evidence.[712] The gross

[706] S. Masamune, G. S. Bates, and J. W. Corcoran, *Angew. Chem. Internat. Edn.*, 1977, **16**, 585.
[707] J. R. Martin, R. S. Egan, A. W. Goldstein, R. S. Stanaszek, J. Tadanier, and W. Keller-Schierlein, *Helv. Chim. Acta*, 1977, **60**, 2559.
[708] A. Kinumaki, K.-I. Harada, T. Suzuki, M. Suzuki, and T. Okuda, *J. Antibiotics*, 1977, **30**, 450.
[709] K. Tatsuta, A. Tanaka, K. Fujimoto, M. Kinoshita, and S. Umezawa, *J. Amer. Chem. Soc.*, 1977, **99**, 5826.
[710] K. Tatsuta, A. Tanaka, M. Kinoshita, and S. Umezawa, *Chem. Letters*, 1977, 769.
[711] J. Majer, J. R. Martin, R. S. Egan, and J. W. Corcoran. *J. Amer. Chem. Soc.*, 1977, **99**, 1620.
[712] R. S. Pandey and K. L. Rinehart, jun., *J. Antibiotics*, 1977, **30**, 146.

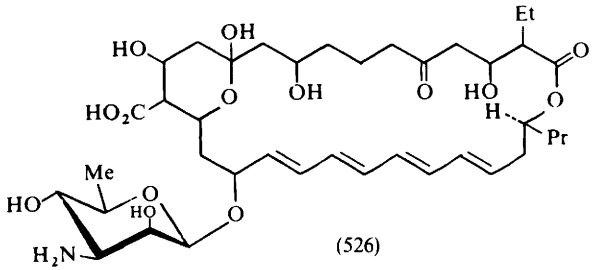




(524) R^1 =

(525) R^1 = H

structures of the heptaene macrolide antibiotics 67-121-A and -C have been reported.[713] Antibiotic 67-121-A, like most other polyene macrolides, contains mycosamine, whereas antibiotic 67-121-C contains 4-*O*-β-D-mannopyranosylmycosamine—this is the first example of a disaccharide occurring as a component of a heptaene antibiotic. Chromatography of the candihexin complex of polyene macrolides elaborated by *S. viridoflavus* has afforded eight components (A—H).[714] Candihexins A and B, which displayed antifungal activity against yeasts and filamentous fungi, yielded mycosamine on hydrolysis with acid, whereas hydrolysis of candihexins E and F did not yield any sugars.



(526)

Anthracycline Antibiotics

The gross structure of nogalamycin (527) has been reported.[715] It contains nogalose (6-deoxy-3-*C*-methyl-2,3,4-tri-*O*-methyl-L-mannose) and a 3,6-dideoxy-3-dimethylaminohexose that is linked both glycosidically and through C-5 to ring A of the tetracycline system.

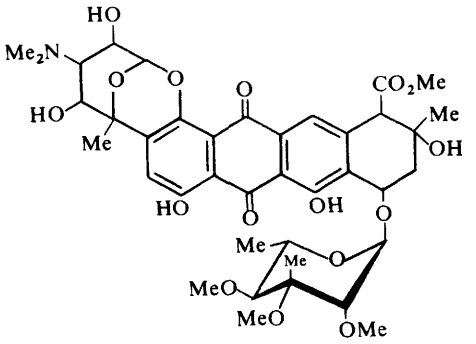
Nineteen anthracycline-related antibiotics have been found in fermentation broths of *Streptomyces galilaeus* strain MA144-M1.[716] They contain rhodosamine or *N*-methyldaunosamine attached to ring D of the tetracycline system, as in

[713] J. J. Wright, D. Greeves, A. K. Mallams, and D. H. Picker, *J.C.S. Chem. Comm.*, 1977, 710.

[714] J. F. Martin and L. E. McDaniel, *Antimicrob. Agents Chemother.*, 1975, **8**, 200 (*Chem. Abs.*, 1977, **87**, 102 580c).

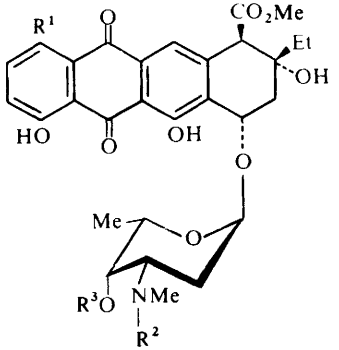
[715] P. F. Wiley, R. B. Kelly, E. L. Caron, V. H. Wiley, J. H. Johnson, F. A. MacKellar, and S. S. Mizsak, *J. Amer. Chem. Soc.*, 1977, **99**, 542.

[716] T. Oki, N. Shibamoto, Y. Matsuzawa, T. Ogasawara, A. Yoshimoto, I. Kitamura, T. Inui, H. Naganawa, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1977, **30**, 683.

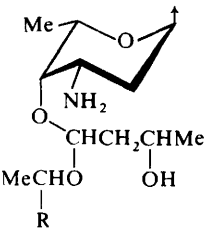


(527)

(528); the amino-sugar residues are usually substituted at O-4 with 2-deoxy-L-fucose (2,6-dideoxy-L-*lyxo*-hexose), which, in most of the antibiotics, carries a residue of amicetose or rhodinose or cinerulose at O-4. These antibiotics are closely related to musettamycin and marcellomycin, new antitumour antibiotics elaborated by *Actinosporangium* sp. strain C-36145, which have the structures [528; R^1 = OH, R^2 = Me, R^3 = 2-deoxy-α-L-fucopyranosyl or 2-deoxy-4-*O*-(2-deoxy-α-L-fucopyranosyl)-α-L-fucopyranosyl, respectively].[717] Baumycins A_1, A_2, B_1, B_2, C_1, and C_2 have been identified as derivatives of daunorubicin (Vol. 10, p. 134) having the amino-sugar residue either substituted at O-4, as in (529), or *N*-formylated.[718]



(528) R^1 = H or OH, R^2 = H or Me
R^3 = H or dideoxy-sugar



(529) R = CH_2OH or CO_2H

A series of ε-rhodomycinone glycopyranosides, including those derived from L-fucose, L-rhamnose, and D-ribose and their 2-deoxy analogues, has been synthesized; the 3,4-di-*O*-acetyl-2-deoxy-D-*erythro*-pentopyranosyl derivative showed

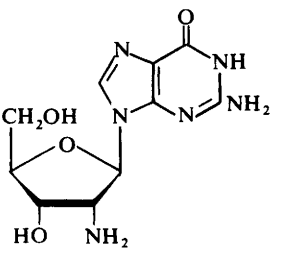
[717] D. E. Nettleton, jun., W. T. Bradner, J. A. Bush, A. B. Coon, J. E. Moseley, R. W. Myllymaki, F. A. O'Herron, R. H. Schreiber, and A. L. Vulcano, *J. Antibiotics*, 1977, **30**, 525.

[718] Y. Takahashi, H. Naganawa, T. Takeuchi, H. Umezawa, T. Komiyama, T. Oki, and T. Inui, *J. Antibiotics*, 1977, **30**, 622.

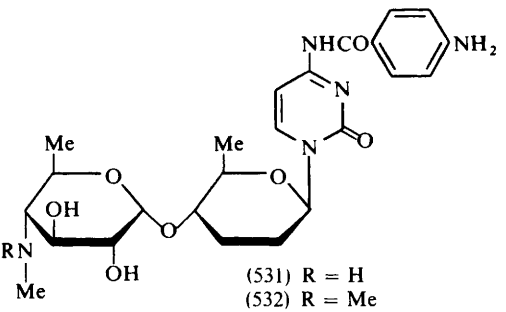
some biological activity.[719] 7-*O*-(2,6-Dideoxy-α-L-*lyxo*-hexopyranosyl)daunomycinone, a functional analogue of daunorubicin having the amino-group in the sugar residue replaced by a hydroxy-group, has been synthesized by condensation of daunomycinone with 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl chloride and deacetylation of the product.[720] ^{13}C n.m.r. measurements on daunorubicin and its derivatives are mentioned in Chapter 20.

Nucleoside Antibiotics

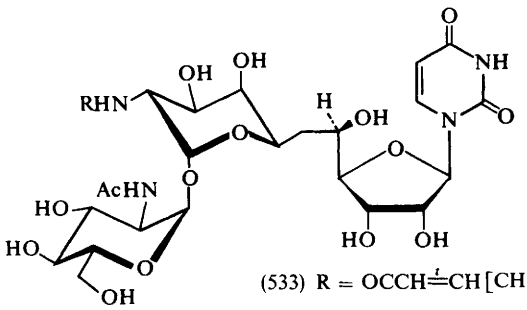
A naturally occurring 2′-aminonucleoside, which exhibited antitumour activity as well as antibacterial activity against some strains of *E. coli*, has been identified as 2′-amino-2′-deoxyguanosine (530).[721] Norplicacetin (531) has been found among the components, which include plicacetin (532), of the antibiotic complex produced by *Streptomyces plicatus*.[722] Components of the tunicamycin complex (533) of antibiotics from *S. lysosuperficus* have been shown to differ in the chain-length of the fatty acid attached to one of the amino-sugar residues.[723] Degradative studies have confirmed that thuringiensin, the exotoxin produced by *Bacillus thuringiensis*, has the structure previously assigned to it[724] (see Vol. 9, p. 136; Vol. 10, p. 139).



(530)



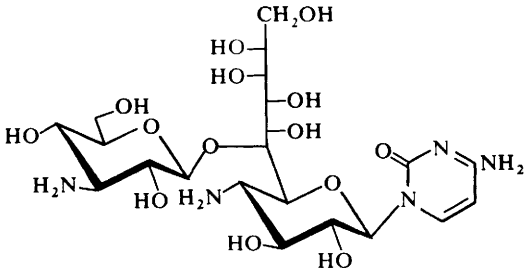
(531) R = H
(532) R = Me



(533) R = $OCCH{=}^{t}CH[CH_2]_{8-11}CHMe_2$

[719] H. S. El Khadem, D. L. Swartz, and R. C. Cermak, *J. Medicin. Chem.*, 1977, **20**, 957.
[720] E.-F. Fuchs, D. Horton, and W. Weckerle, *Carbohydrate Res.*, 1977, **57**, C36.
[721] T. Nakanishi, T. Iida, F. Tomita, and A. Furuya, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 2955.
[722] J. R. Evans and G. Weare, *J. Antibiotics*, 1977, **30**, 604.
[723] A. Takatsuki, K. Kawamura, M. Okina, Y. Kodama, T. Ito, and G. Tamura, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 2307.
[724] J. Farkaš, K. Šebestá, K. Horská, Z. Samek, L. Dolejš, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1977, **42**, 909.

Spectroscopic and other studies have indicated that amipurimycin, a nucleoside antibiotic from *S. novoguineensis*, is related to 2-amino-9-β-D-ribofuranosylpurine, although a detailed structure was not assigned.[725] The antibiotics hikizimycin and anthelmycin produced by *Streptomyces* A-5 and *S. longissimus*, respectively, are one and the same compound, having the structure (534)[726,726a] (*cf.* Vol. 9, p. 134).



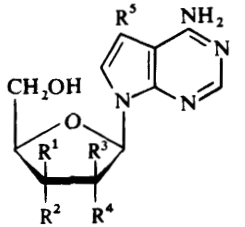
(534)

The structures of the ezomycins, which contain 5-substituted uracil residues, are noted on p. 155.[670] 5-β-D-Ribofuranosyluracil (ψ-uridine) has been converted into its N^1-methyl derivative, which proved to be identical with an antiviral and antibacterial antibiotic (U-44590) isolated from fermentation broths of *S. platensis* var. *clarensis*.[727] Analogues of septacidin containing a 4-amino-4-deoxy- or 4-amino-4,6-dideoxy-L-glucopyranosyl residue instead of the natural 4-amino-4-deoxy-L-*glycero*-L-*gluco*-heptopyranosyl residue displayed antitumour activity comparable to that of septacidin.[728]

Tubercidin (7-deaza-adenosine) (535) has been converted, *via* treatment of its 2′,3′-*O*-methoxyethylidene derivative with pivaloyl chloride in refluxing pyridine, into 4′-*epi*-, 3′-deoxy-, and 2′,3′-dideoxy-tubercidin.[729] 2′,3′-Anhydrotubercidin was also prepared and converted into its 2′,3′-anhydro-β-D-lyxofuranosyl analogue.[730] These epoxides were then used to prepare D-*xylo* and D-*arabino* analogues [(536) and (537), respectively] of tubercidin. 7-Methyltubercidin (538) and its α-anomer have been prepared from the products obtained on condensation of 2,3,5-tri-*O*-benzyl-D-ribofuranosyl bromide and an appropriately protected 7-methylpyrrolo[2,3-*d*]pyrimidine.[731]

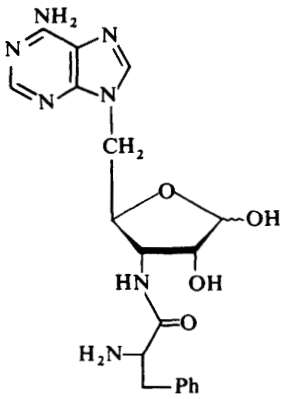
The reverse nucleoside (539), a structural analogue of puromycin, has been derived from the product obtained when adenine reacted with the 5-tosylate (540) in the presence of sodium hydride.[732] Syntheses of 9-(5-deoxy-β-D-*erythro*-

[725] S. Harada and T. Kishi, *J. Antibiotics*, 1977, **30**, 11.
[726] S. Ennifar, B. C. Das, S. M. Nash, and R. Nagarajan, *J.C.S. Chem. Comm.*, 1977, 41.
[726a] M. Vuilhorgne, S. Ennifar, B. C. Das, J. W. Paschal, R. Nagarajan, E. W. Hagaman, and E. Wenkert, *J. Org. Chem.*, 1977, **42**, 3289.
[727] U. Reichman, K. Hirota, C. K. Chu, K. A. Watanabe, and J. J. Fox, *J. Antibiotics*, 1977, **30**, 129.
[728] E. M. Acton, K. J. Ryan, and A. E. Luetzow, *J. Medicin. Chem.*, 1977, **20**, 1362.
[729] M. J. Robins, R. A. Jones, and R. Mengel, *Canad. J. Chem.*, 1977, **55**, 1251.
[730] M. J. Robins, Y. Fouron, and W. H. Muhs, *Canad. J. Chem.*, 1977, **55**, 1260.
[731] T. Kondo, T. Ohgi, and T. Goto, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 1501.
[732] V. Nair and D. J. Emanuel, *J. Amer. Chem. Soc.*, 1977, **99**, 1571.

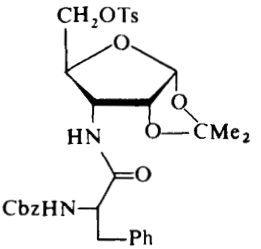


(535) $R^1 = R^3 = R^5 = H$, $R^2 = R^4 = OH$
(536) $R^1 = R^4 = OH$, $R^2 = R^3 = R^5 = H$
(537) $R^1 = R^4 = R^5 = H$, $R^2 = R^3 = OH$
(538) $R^1 = R^3 = H$, $R^2 = R^4 = OH$, $R^5 = Me$

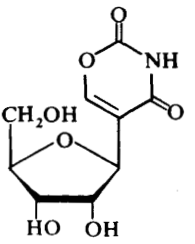
pent-4-enofuranosyl)adenine, an analogue of angustmycin A, and its L-enantiomer have been described[500] (see also Vol. 10, p. 156). Neither the 2′- nor 3′-*O*-methyl derivatives of ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) displayed antiviral activity.[733]



(539)



(540)



(541)

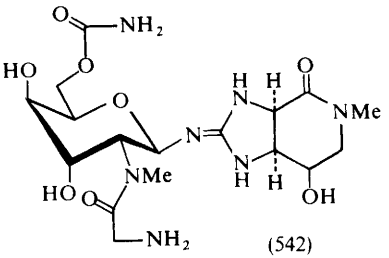
Labelled glutamate and acetate were asymmetrically incorporated by *S. hygroscopicus* into the oxazine ring of the *C*-nucleoside minimycin (oxazinomycin) (541) – other *C*-nucleoside antibiotics (*e.g.* showdomycin and formycin) also incorporate glutamate into their heterocyclic bases.[734] *C*-Nucleosides are also mentioned in Chapters 3, 17, and 19.

[733] L. Dudycz, D. Shugar, E. de Clercq, and J. Deschamps, *J. Medicin. Chem.*, 1977, **20**, 1354.
[734] K. Isono and R. J. Suhadolnik, *J. Antibiotics*, 1977, **30**, 272.

Miscellaneous Antibiotics

The ansamycin antibiotics have been comprehensively reviewed.[735]

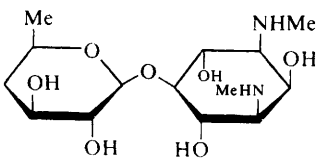
Glycinothricin, a new antibiotic isolated from fermentation broths of a strain of *S. griseus* and belonging to the streptothricin family, has the structure (542) (*i.e.* the deformimino derivative of antibiotic LL-AB664).[736] 2-Amino-2-deoxy-D-[1-^{14}C]glucose was incorporated only into the 2-amino-2-deoxy-D-gulose moiety of racemomycin A, a streptothricin antibiotic, when fed to growing cultures of *S. lavendulae* 1SP-5069[737] (see also Vol. 10, p. 137).



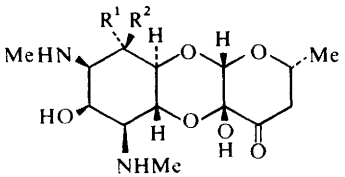
(542)

Tallysomycins A and B, two of the principal components of the antibiotic complex elaborated by an unidentified strain E465-94 of *Actinomycetes*, are glycopeptides containing the disaccharide 2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-L-gulopyranose, which also occurs in the bleomycins, and 4-amino-4,6-dideoxy-L-talose, which has not been encountered before in this group of antibiotics.[738]

Removal of the protecting groups from one of the products obtained in low yield on condensation of 1,3-di-*N*-benzyloxycarbonylactinamine and 2-*O*-chloroacetyl-4,6-dideoxy-3-*O*-(4-nitrobenzoyl)-α-D-*xylo*-hexopyranosyl chloride afforded 5-*O*-(4,6-dideoxy-β-D-*xylo*-hexopyranosyl)actinamine (543), which proved to be identical with one of the four tetrahydrospectinomycins obtained on exhaustive hydrogenation of spectinomycin (544).[739] 9-Chloro-9-deoxy derivatives of



(543)



(544) R^1 = OH; R^2 = H
(545) R^1 = H; R^2 = Cl

[735] M. Brufani, *Topics Antibiotic Chem.*, 1977, **1**, 91 (*Chem. Abs.*, 1977, **87**, 177 327j).
[736] Y. Sawada, S. Kawakami, H. Taniyama, K. Hamano, R. Enokita, S. Iwado, and M. Arai, *J. Antibiotics*, 1977, **30**, 460.
[737] Y. Sawada, S. Nakashima, H. Taniyama, and Y. Inamori, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 1478.
[738] M. Konishi, K.-I. Saito, K.-I. Numata, T. Tsuno, K. Asama, H. Tsukiura, T. Naito, and H. Kawaguchi, *J. Antibiotics*, 1977, **30**, 789.
[739] T. Suami, S. Nishiyama, H. Ishikawa, H. Okada, and T. Kinoshita, *Bull. Chem. Soc. Japan*, 1977, **50**, 2754.

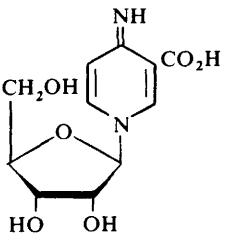
dihydrospectinomycin and of 9-*epi*-spectinomycin [*e.g.* (545)] have been prepared, but they did not display antibacterial activity.[740]

Reference is made to the sugar components of steffimycin in Chapter 3, to a synthesis of prumycin in Chapter 8, and to ^{13}C n.m.r. measurements on lincomycin and related compounds in Chapter 20.

[740] R. E. Carney and W. Rosenbrook, jun., *J. Antibiotics*, 1977, **30**, 960.

19
Nucleosides

Reviews have appeared on 5-substituted pyrimidine nucleosides and nucleotides[741] and on the synthesis of non-glycosidic analogues of nucleosides, including carbocyclic analogues and reverse nucleosides.[742] Several benzo[*a*]pyrene–nucleoside adducts have been isolated in a study of the covalent binding of 9,10-anhydro-7,8,9,10-tetrahydro-7,8,9,10-tetrahydroxybenzo[*a*]pyrene and a related arene epoxide to nucleic acids in aqueous acetone[743] (see Vol. 10, p. 140). Two cross-linked nucleosides have been isolated following enzymic hydrolysis of a DNA that had been treated with nitrous acid.[744] Three ribonucleosides responsible for the cytokinin activity of the tRNA of *Euglena gracilis* var. *bacillaris* have been isolated and identified as N^6-(3-methylbut-2-enyl)-, N^6-[(*E*)-4-hydroxy-3-methylbut-2-enyl]-, and N^6-(4-hydroxy-3-methylbut-2-enyl)-2-methylthioadenosine.[745] The structure and synthesis of clitidine (546), a physiologically active pyridine ribonucleoside from the toadstool *Clitocybe acromelalga*, have been reported.[746] The revised structure of hikizimycin (anthelmycin) is noted[726, 726a] in Chapter 18, which also covers other nucleoside antibiotics.



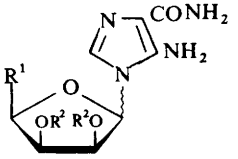
(546)

Synthesis

1-β-D-Arabinofuranosylcytosine has been prepared in one step in 47.5% yield by reaction of cytidine with diphenyl carbonate in DMF at 120 °C in the presence

[741] T. K. Bradshaw and D. W. Hutchinson, *Chem. Soc. Rev.*, 1977, **6**, 43.
[742] A. M. Kritzyn and V. L. Florentiev, *Bioorg. Khim.*, 1977, **3**, 149.
[743] K. W. Jennette, A. M. Jeffrey, S. H. Blobstein, F. A. Beland, R. G. Harvey, and I. B. Weinstein, *Biochemistry*, 1977, **16**, 932.
[744] R. Shapiro, S. Dubelman, A. M. Feinberg, P. F. Crain, and J. A. McCloskey, *J. Amer. Chem. Soc.*, 1977, **99**, 302.
[745] S. Swaminathan and R. M. Bock, *Biochemistry*, 1977, **16**, 1355.
[746] K. Konno, K. Hayano, H. Shirahama, H. Saito, and T. Matsumoto, *Tetrahedron Letters*, 1977, 481.

of a molar equivalent of water, followed by chromatography of the products on an anion-exchange resin.[747] A new synthesis of 9-β-D-arabinofuranosyladenine has involved the selective toluene-*p*-sulphonylation of adenosine 5′-phosphate at O-2′ and conversion of the 2′-tosylate into the 2′,8-cyclonucleoside, *etc.*[748] The 2′,8-cyclonucleoside also reacted with alkyl amines to give 8-alkylamino-9-β-D-arabinofuranosyladenines. The hydroxycyclopentylamine (472) (Scheme 82) has been converted into the carbocyclic analogue of adenosine in an effort to overcome the hydrolytic inactivation of 9-β-D-arabinofuranosyladenine (an antiviral agent) *in vivo*.[648] The carbocyclic analogue resisted hydrolysis *in vivo* and displayed both antiviral and antitumour activities. Standard transformations have yielded 5-fluorouridine,[749] which was converted into 2′-deoxy-5-fluorouridine (an antitumour agent) by a commercially viable process.[750] Glycosylamine derivatives of D-gluco-, D-ribo-, and D-xylo-furanose have been used to prepare substituted uracil nucleosides[751] (see Vol. 8, p. 141 ff), and 1-β-D-erythrofuranosyl-uracil and -cytosine, *etc.*, have been prepared by reaction of 1,2,3-tri-*O*-acetyl-D-erythrofuranose with the trimethylsilylated derivatives of the bases in the presence of tin(IV) chloride.[752] Ethyl 5-amino-1-(2,3-*O*-isopropylidene-α- and -β-D-mannofuranosyl)imidazole-4-carboxylates have been prepared from 2,3:5,6-di-*O*-isopropylidene-D-mannofuranosylamine and, after *N*-acetylation, were converted by oxidation with periodate and borohydride reduction into the corresponding ethyl 5-acetamido-1-(2,3-*O*-isopropylidene-D-lyxofuranosyl)imidazole-4-carboxylates.[753] The imidazole-4-carboxamides (547) and (548) were also prepared and

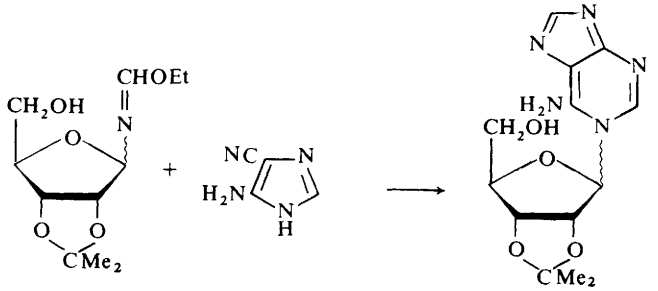


(547) $R^1 = Me_2C$ (O–CH_2, O) ; $R^2, R^2 = Me_2C$

(548) $R^1 = CH_2OH$; $R^2 = H$

converted into the corresponding adenine nucleosides. The reaction outlined in Scheme 86 has yielded the 1-α- and 1-β-D-ribofuranosyladenine derivatives (549) from ethyl *N*-(2,3-*O*-isopropylidene-D-ribofuranosyl)formimidate.[754] The ribonucleosides (549) anomerized in basic media to give an αβ-equilibrium in which the α-anomer preponderated, whereas they isomerized, presumably by opening and reclosure of the pyrimidine ring, to N^6-(2,3-*O*-isopropylidene-α- and -β-D-ribofuranosyl)adenine in neutral aqueous media.

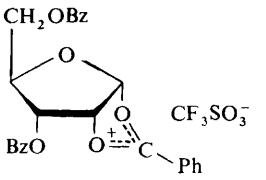
[747] J. Beránek, T. J. Delia, and P. Drašar, *Coll. Czech. Chem. Comm.*, 1977, **42**, 1588.
[748] M. Kaneko, M. Kimura, T. Nishimura, and B. Shimizu, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 2482.
[749] T. P. Nedorezova, S. Ya. Mel'nik, and M. N. Preobrazhenskaya, *Bioorg. Khim.*, 1976, **2**, 1205 (*Chem. Abs.*, 1977, **86**, 55 653w).
[750] S. Ozaki, T. Katakami, and M. Saneyoshi, *Bull. Chem. Soc. Japan*, 1977, **50**, 2197.
[751] R. Lofthouse, G. Shaw, P. S. Thomas, G. Mackenzie, D. H. Robinson, and P. W. Rugg, *J.C.S. Perkin I*, 1977, 997.
[752] G. L. Szekeres, J. T. Witkowski, and R. K. Robins, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 147.
[753] G. Mackenzie and G. Shaw, *J.C.S. Chem. Comm.*, 1977, 753.
[754] B. Rayner, C. Tapiero, and J.-L. Imbach, *Carbohydrate Res.*, 1977, **59**, 111.



(549)

Scheme 86

1-Deazapurine and its TMS derivative reacted with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in acetonitrile in the presence of tin(IV) chloride to give 9- and 3-D-ribofuranosyl derivatives, respectively, while the TMS derivative of adenine gave a mixture containing roughly equal amounts of 7-β-, 7-α-, and 9-β-D-ribofuranosyl derivatives.[755] A careful study of the fusion of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose with indazole has revealed that the distribution of products depends on the amount of catalyst used.[755a] The transglycosylation procedure for converting pyridine nucleosides into purine nucleosides has been improved by using TMS derivatives of purines as glycosyl acceptors in the presence of TMS perchlorate or triflate as a catalyst.[756] The silyl modification of the Hilbert–Johnson reaction has been used to synthesize 1-(2,3,5-tri-*O*-acyl-β-D-ribo- and -xylo-furanosyl)-2-pyrimidinones and benzoylated 1-β-D-gluco- and 1-β-D-ribo-pyranosyl-2-pyrimidinones.[290] ^{1}H N.m.r. measurements have indicated that 2,3,5-tri-*O*-benzoyl-1-*O*-trifluoromethanesulphonyl-D-ribofuranose exists predominantly as the dioxolenium triflate (550) in nitromethane.[757] Reaction of the triflate (550) with, for



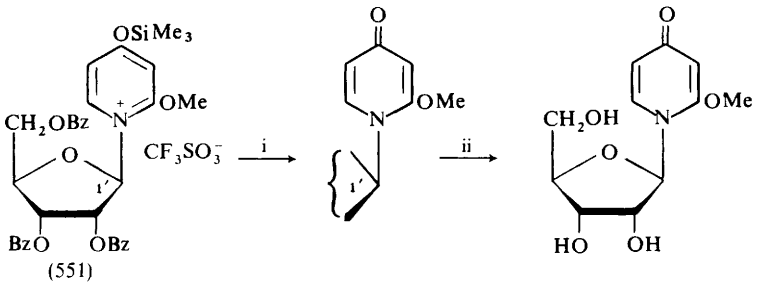
(550)

example, 2,4-bis(trimethylsilyloxy)pyrimidine at −18 °C gave, after methanolysis of the product, 2′,3′,5′-tri-*O*-benzoyluridine. This triflate was also used in a synthesis of 2-*O*-methyl-3-deazauridine by a route (Scheme 87) involving selective cleavage

[755] T. Itoh and Y. Mizuno, *Heterocycles*, 1976, **5**, 285 (*Chem. Abs.*, 1977, **86**, 106 950a).
[755a] J.-L. Barascut, B. Kam, and J.-L. Imbach, *Bull. Soc. chim. France*, 1977, 1983.
[756] T. Azuma, K. Isono, and J. A. McCloskey, *Nucleic Acids Res., Special Publ.*, 1976, 2 (4th Symposium Nucleic Acids Chem., 1976), 19 (*Chem. Abs.*, 1977, **86**, 121 671d).
[757] R. L. Shone, *Tetrahedron Letters*, 1977, 993.

of the *para*-substituent from an intermediate (551) that also contained a reactive *ortho*-substituent.[758]

Syntheses of 3,6-anhydro-1-deoxy-1-(5-halogenouracil-1-yl)-D-mannitols[759] and 5-cyclopropyl-uridine and -6-azauridine[760] have been described. Carbenes added to the 5,6-double bond of uridine derivatives to give a mixture of *endo*- and *exo*-adducts [*e.g.* (552)] in modest to good yields.[761] The adducts (552) or those obtained



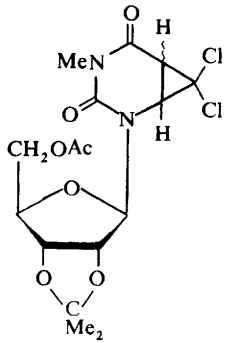
Reagents: i, MeOH–basic resin; ii, NH_3–MeOH

Scheme 87

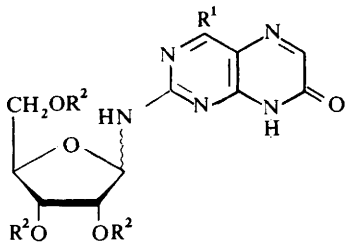
with dibromocarbene reacted with alcohols (*e.g.* t-butanol) at $\geqslant 110$ °C to give diazepine ribonucleosides by thermal opening of the cyclopropane ring.[762] Syntheses of 5-alloxymethyl-2′,3′,5′-tri-*O*-benzoyluridine,[763] of 5-ethynylcytidine,[764] and of (+)- and (−)-*cis*-6-hydroxy-5,6-dihydrothymidines[765] have also been reported. Cytidine-5-carboxylic acid and some related 5-carboxamide derivatives have been prepared, but none of them showed antitumour activity.[766] Various 2,6-disubstituted 4-amino-5-nitropyrimidines have been D-ribosylated by fusion with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of zinc chloride.[767] D-Ribosylation occurred at the 4-amino-group, although the 2-amino-group of 6-substituted 2,4-diamino-5-nitropyrimidines reacted preferentially. Catalytic reduction of the 6-substituted 4-amino-5-nitro-2-αβ-D-ribofuranosylaminopyrimidines and subsequent condensation with ethyl glyoxalate gave 4-substituted 2-D-ribofuranosylamino-7(8*H*)-pteridinones [*e.g.* (553) and (554)].

N^2-Methylguanosine has been prepared by treating the protected guanosine derivative (555) with diazomethane and solvolysis of the protecting methanesulphonyloxy and benzoyl groups.[768] The methanesulphonyloxy group could be

[758] R. L. Shone, *Tetrahedron Letters*, 1977, 4017.
[759] G. Giovanninetti, L. Garuti, V. Cavrini, M. Amorosa, and J. Defaye, *Farmaco, Ed. Sci.*, 1976, **31**, 871 (*Chem. Abs.*, 1977, **87**, 68 558q).
[760] I. Basnak and J. Farkas, *Coll. Czech. Chem. Comm.*, 1976, **41**, 3635.
[761] H. P. M. Thiellier, G. J. Koomen, and U. K. Pandit, *Tetrahedron*, 1977, **33**, 1493.
[762] H. P. M. Thiellier, G. J. Koomen, and U. K. Pandit, *Tetrahedron*, 1977, **33**, 2609.
[763] V. Eschenfelder and R. Brossmer, *Annalen*, 1977, 1969.
[764] A. S. Jones, P. Serafinowski, and R. T. Walker, *Tetrahedron Letters*, 1977, 2459.
[765] J. Cadet, R. Ducolomb, and R. Teoule, *Tetrahedron*, 1977, **33**, 1603.
[766] S. P. Gupta, S. S. Chatterjee, P. C. Jain, and N. Anand, *Indian J. Chem.*, 1977, **15B**, 463 (*Chem. Abs.*, 1977, **87**, 201 990t).
[767] D. Autenrieth, H. Schmid, M. Ott, and W. Pfleiderer, *Annalen*, 1977, 1194.
[768] P. K. Bridson, W. Markiewicz, and C. B. Reese, *J.C.S. Chem. Comm.*, 1977, 447.

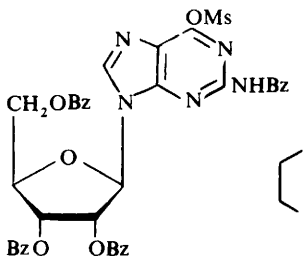


(552)

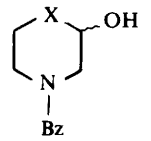


(553) $R^1 = OC_5H_{11}$, R^2 = Bz or H
(554) $R^1 = NMe_2$, R^2 = Bz or H

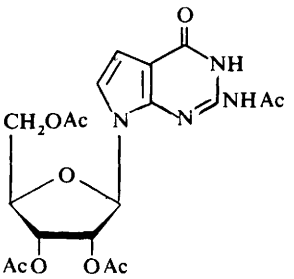
smoothly displaced from (555) with dimethylamine (see also p. 197). 2′,3′,5′-Tri-*O*-acetyladenosine reacted with phenoxycarbonyltetrazole to give only the N^6-phenylcarbamate, which furnished an *NN*′-disubstituted urea on aminolysis with L-threonine 4-nitrobenzyl ester.[769] The 4-benzoyl-2-hydroxy-1-oxa(or thia)-4-azacyclohexanes (556) have been converted into analogues of 9-substituted purine nucleosides by treatment with 6-chloropurine, methyldiphenylphosphine, and



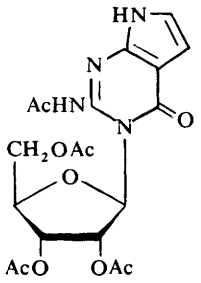
(555)



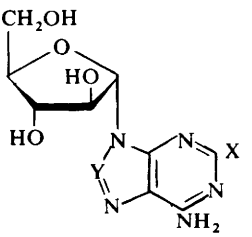
(556) X = S or O



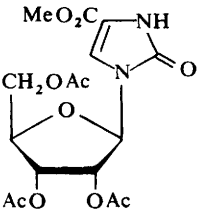
(557)



(558)



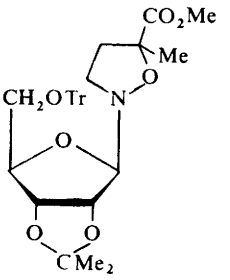
(559) X = OMe, SMe, or $NHNH_2$
Y = CH or N



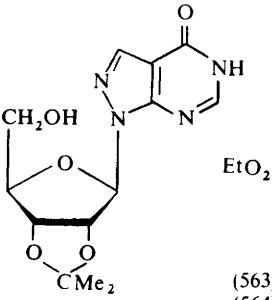
(560)

[769] R. W. Adamiak and J. Stawinski, *Tetrahedron Letters*, 1977, 1935.

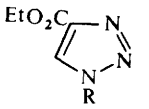
diethyl azodicarboxylate in refluxing THF.[770] The silyl and fusion procedures for the preparation of 3-deazapurine ribonucleosides have been compared,[771] and the synthesis and the biological activity of 5′-*S*-methyl-5′-thio-7-deaza-adenosine have been reported.[772] 2,3,5-Tri-*O*-acetyl-D-ribofuranosyl bromide reacted with 2-acetamidopyrrolo[2,3-*d*]pyrimidin-4-one in the presence of mercury(II) oxide and bromide to give the protected ribonucleoside (557), which was converted into 7-deazaguanosine, and (558).[773] 1-Deaza-8-azaguanosine,[774] analogues of 8-azainosine,[775] 8-aza-adenosine,[775] and 2-substituted derivatives (559) of 9-α-D-arabinofuranosyl-adenine (Y = CH) and -8-aza-adenine (Y = N)[776] have been synthesized by conventional procedures. Diazotization of methyl 5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxylate with sodium nitrite in 11M-nitric acid at −20 °C yielded the 2-oxo-Δ^4-imidazoline derivative (560), which was also synthesized by condensation of the bis(trimethyl-



(561)



(562)

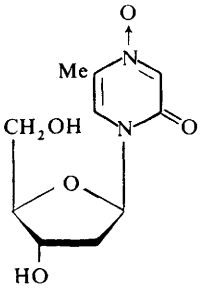


(563) R = H
(564) R = $SiMe_3$

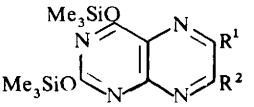
silyl) derivative of methyl 2-oxo-Δ^4-imidazoline-4-carboxylate with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of tin(IV) chloride.[777] The isoxazolidine ribonucleoside derivative (561) has been prepared by treating 2,3-*O*-isopropylidene-5-*O*-trityl-D-ribose oxime with methyl methacrylate and formaldehyde.[778] *O*-Substituted D-ribose hydrazones (*e.g.* 2,3-*O*-isopropylidene-D-ribose hydrazone) reacted regio- and stereo-selectively with alkoxy- or amino-methylenecyanoacetates [*e.g.* $EtOCH{=}C(CN)CO_2Me$ or $NH_2CH{=}C(CN)CO_2Me$] to form pyrazole nucleosides [*e.g.* methyl 5-amino-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-pyrazole-4-carboxylate],[779, 780] which could be elaborated to give pyrazolo[3,4-*d*]-pyrimidine ribonucleosides [*e.g.* (562)]. Partially or fully protected D-ribose hydrazones also reacted with β-dicarbonyl compounds (*e.g.* pentane-2,4-dione) to give, depending on the size and position of the protecting group, either a mixture

[770] B. M. Pinto, D. M. Vyas, and W. A. Szarek, *Canad. J. Chem.*, 1977, **55**, 937.
[771] J. A. Montgomery, A. T. Shortnacy, and S. D. Clayton, *J. Heterocyclic Chem.*, 1977, **14**, 195.
[772] J. K. Coward, N. C. Motola, and J. D. Moyer, *J. Medicin. Chem.*, 1977, **20**, 500.
[773] L. B. Townsend, R. L. Tolman, R. K. Robins, and G. H. Milne, *J. Heterocyclic Chem.*, 1976, **13**, 1363.
[774] B. L. Cline, R. P. Panzica, and L. B. Townsend, *J. Heterocyclic Chem.*, 1976, **13**, 1365.
[775] R. D. Elliott and J. A. Montgomery, *J. Medicin. Chem.*, 1977, **20**, 116.
[776] J. A. Montgomery, A. T. Shortnacy, G. Arnett, and W. M. Shannon, *J. Medicin. Chem.*, 1977, **20**, 401.
[777] P. C. Srivastava, R. J. Jousseau, and R. K. Robins, *J.C.S. Chem. Comm.*, 1977, 151.
[778] A. Vasella, *Helv. Chim. Acta*, 1977, **60**, 426.
[779] R. R. Schmidt, J. Karg, and W. Guilliard, *Chem. Ber.*, 1977, **110**, 2433.
[780] R. R. Schmidt, W. Guilliard, and J. Karg, *Chem. Ber.*, 1977, **110**, 2445.

of α- and β-pyrazole ribonucleosides or the β-anomer only, whereas unprotected D-ribose hydrazone did not form pyrazole ribonucleosides with β-dicarbonyl compounds.[779] Condensation of peracylated aldo-furanose and -pyranose 1-acetates with pyrazole derivatives has been used to prepare variously substituted 1-α- and 1-β-D-arabinofuranosyl-,[781, 782] 1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-,[782] 1-β-D-glucopyranosyl-, and 1-β-D-ribofuranosyl-pyrazole-carboxylates.[783] Glycosylation of the triazole (563) has been accomplished using either 1-*O*-acetyl-2,3,5-tri-*O*-benzyl-D-arabinofuranose or 1-*O*-acetyl-2-deoxy-3,5-di-*O*-(4-nitrobenzoyl)-D-*erythro*-pentofuranose.[784] Nucleoside derivatives were also obtained by condensation of the TMS derivative (564) with corresponding glycofuranosyl chlorides. The synthetic 2′-deoxyribonucleoside (565) displayed some antileukaemic activity.[785]



(565)

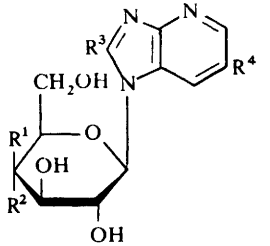


(566) R^1 or R^2 = H, Me, Ph, *p*-ClC_6H_4

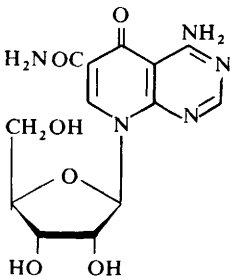
The TMS derivatives (566) of various 6- and 7-substituted lumazines have been converted into ribo- and 2′-deoxyribo-nucleosides using several methods of glycosylation.[786] For example, they reacted rapidly with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of boron trifluoride etherate to give N^1-β-D-ribofuranosyl derivatives and, depending on the solvent used, N^3-mono- and, in some cases, $N^{1,3}$-di-β-D-ribofuranosyl derivatives. 3,4-*O*-Isopropylidene-D-arabinopyranosylamine condensed with α-cyano-β-ethoxycarbonyl-*N*-methylacrylamide to give, after acidic hydrolysis, 1-α- and 1-β-D-arabinopyranosyl-5-cyano-3-methyluracil, and with ethyl *N*-(carbamoylcyanomethyl)formimidate, *etc.*, to give 5-amino-1-α- and -β-D-arabinopyranosylimidazole-4-carboxamide.[787] On treatment with DMF dimethyl acetal, D-ribo- and D-arabino-pyranosylamines yielded the corresponding *NN*-dimethyl-*N*′-α- and -β-pentopyranosylformamidine, which on heating with methanolic acetic acid and condensation of the product with α-amino-α-cyanoacetamide gave a mixture of the anomeric 5-amino-1-D-

[781] I. A. Korbukh, L. M. Abramova, and M. N. Preobrazhenskaya, *Zhur. org. Khim.*, 1977, **13**, 731 (*Chem. Abs.*, 1977, **87**, 39 764y).
[782] O. Makabe, J. Yajima, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1976, **49**, 3552.
[783] A. A. Akhrem, E. I. Kvasyuk, and I. A. Mikhailopulo, *Tezisy Doklady-Vses. Konf. Khimioter. Zlokach. Opukholei. 2nd.*, 1974, 36 (*Chem. Abs.*, 1977, **86**, 190 400b).
[784] O. Makabe, H. Suzuki, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1977, **50**, 2689.
[785] M. Bobek, A. Bloch, P. Berkowitz, and T. J. Bardos, *J. Medicin. Chem.*, 1977, **20**, 458.
[786] G. Ritzmann, K. Ienaga, and W. Pfleiderer, *Annalen*, 1977, 1217.
[787] G. Mackenzie, G. Shaw, and D. H. Robinson, *J.C.S. Perkin I*, 1977, 1094.

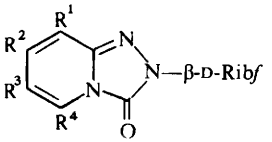
pentopyranosylimidazole-4-carboxamides and, as appropriate, 5-amino-1-α-D-ribofuranosylimidazole-4-carboxamide or 1-(5-amino-4-carbamoylimidazol-1-yl)-1-*O*-methyl-D-arabinitol (possibly by formation and methanolysis of an oxazine intermediate). Syntheses of 4-substituted (*e.g.* 4-alkylthio- and 4-methylamino-) pyrazolo[3,4-*d*]pyrimidine ribonucleosides,[788] 5- and 6-nitro-1-β-D-ribofuranosylindoles,[789] the disubstituted D-gluco- and D-galacto-pyranosylimidazo[4,5-*b*]-pyridines (567),[789a] and the pyrido[2,3-*d*]pyrimidine ribonucleoside (568)[790] have



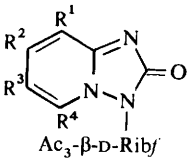
(567) R^1 = H or OH, R^2 = OH or H,
R^3 = Me or CF_3, R^4 = Cl or Br



(568)



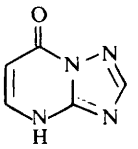
(569) $R^1 = R^2 = R^3 = R^4$ = H
or $R^1 = R^2 = R^3$ = H, R^4 = Me, *etc.*



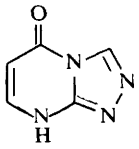
(570) $R^1 = R^2 = R^3 = R^4$ = H
or $R^1 = R^2 = R^3$ = H, R^4 = Me, *etc.*

[788] J.-L. G. Montero, G. A. Bhat, R. P. Panzica, and L. B. Townsend, *J. Heterocyclic Chem.*, 1977, **14**, 483.
[789] V. I. Mukhanov, T. D. Miniker, and M. N. Preobrazhenskaya, *Zhur. org. Khim.*, 1977, **13**, 214 (*Chem. Abs.*, 1977, **86**, 171 776n).
[789a] A. V. Stetsenko and E. V. Goshchulyak, *Ukrain. khim. Zhur.*, 1977, **43**, 165 (*Chem. Abs.*, 1977, **86**, 190 408k).
[790] G. L. Anderson and A. D. Broom, *J. Org. Chem.*, 1977, **42**, 997.

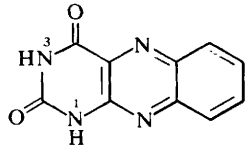
been reported. TMS derivatives of 3-oxo-*s*-triazolo[4,3-*a*]pyridine and its *C*-methyl derivatives have been converted into the ribonucleosides (569) by reaction with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in refluxing 1,2-dichloroethane in the presence of tin(IV) chloride and deacetylation of the products.[791] Rearrangement to the isomeric 2-oxo-3-β-D-ribofuranosyl-*s*-triazolo[1,5-*a*]pyridines (570), by opening of the pyridine ring, sometimes occurred during these reactions. Ribonucleosides substituted in either heterocyclic ring have been prepared from the *s*-triazolopyrimidinones (571) and (572),[792] and D-ribosylation of the 2,4-bis(trimethylsilyl) derivatives of alloxazine (573) and 7,8-dimethylalloxazine in the presence of boron trifluoride etherate gave principally the N^1-β-D-ribofuranosyl



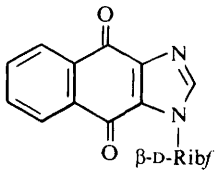
(571)



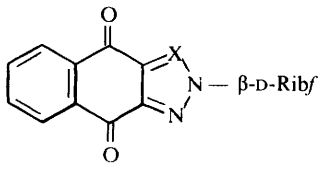
(572)



(573)



(574)



(575) X = N
(576) X = CH

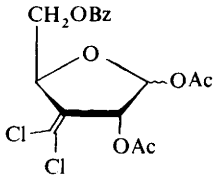
derivatives together with some N^3- and $N^{1,3}$-di-β-D-ribofuranosyl derivatives.[793] The $N^{1,3}$-di-β-D-ribofuranosyl derivatives of lumazine and alloxazine disproportionated in the presence of the corresponding free base and mercury(II) bromide to form N^1- and N^3-β-D-ribofuranosyl derivatives in a ratio of 3:1.[794] Among other nucleosides reported during 1977 were β-D-xylopyranosyl and β-L-fucopyranosyl derivatives of ellipticine and 9-acetoxyellipticine[795] and the naphthoquinone-based ribonucleosides (574) and (575), which showed some activity as inhibitors of hypoxanthine phosphoribosyltransferase, and (576).[796]

Nucleosides containing Branched-chain Components

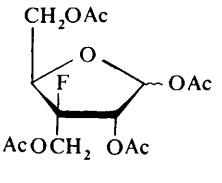
9-(3-Deoxy-3-*C*-dichloromethylene-β-D-*erythro*-pentofuranosyl)adenine has been synthesized from (577), which was obtained straightforwardly from 3-deoxy-3-*C*-

[791] B. M. Lynch and S. C. Sharma, *Canad. J. Chem.*, 1977, **55**, 831.
[792] J. L. Barascut, C. Ollier de Marichard, and J.-L. Imbach, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 281.
[793] K. Ienaga and W. Pfleiderer, *Chem. Ber.*, 1977, **110**, 3449.
[794] K. Ienaga and W. Pfleiderer, *Chem. Ber.*, 1977, **110**, 3456.
[795] M. Bessodes and K. Antonakis, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 215.
[796] M. V. Pickering, P. Dea, D. G. Streeter, and J. T. Witkowski, *J. Medicin. Chem.*, 1977, **20**, 818.

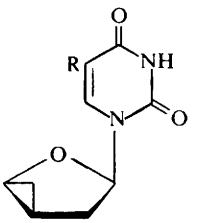
dichloromethylene-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranose,[797] and a similar approach was adopted in preparing the analogous 3′-deoxy-3′-*C*-dibromomethylene nucleoside[798] and 1-(3-deoxy-3-fluoro-3-*C*-hydroxymethyl-α-D-xylofuranosyl)uracil from (403) by way of (578).[562] 1-{(1*R*,3*R*,5*R*)-2-Oxabicyclo-[3.1.0]hexan-3-yl}-thymine and -uracil (579), which contain a fused cyclopropane ring, have been obtained by electrochemical reduction of 3′,5′-dideoxy-3′,5′-diiodothymidine (580; R = Me) and 2′,3′,5′-trideoxy-3′,5′-di-iodouridine (580; R = H), respectively.[799] The 2′,3′-cyclopropanated purine derivatives (581) were obtained on condensation of either the homoallyl iodide (582) or methyl 4,6-*O*-benzylidene-2,3-dideoxy-2,3-*C*-methylene-α-D-allopyranoside with 6-chloropurine in refluxing nitromethane.[800]



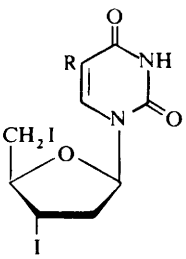
(577)



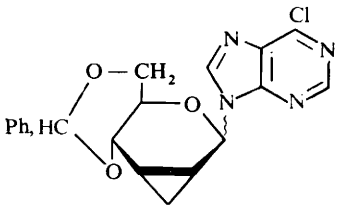
(578)



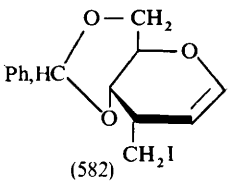
(579) R = H or Me



(580) R = H or Me



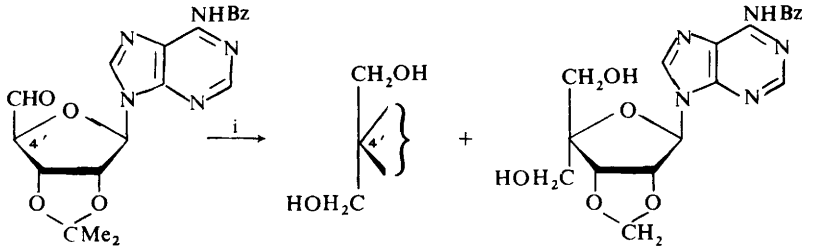
(581)



(582)

The synthetic approach to 4′-(hydroxymethyl)ribonucleosides shown in Scheme 88 is based on aldol coupling of the nucleoside-5′-aldehyde (583) with formaldehyde and accompanying Cannizzaro reduction by the excess of formaldehyde.[541] The 2′,3′-*O*-methylene derivative (584) was obtained as a by-product of the reaction. The branched-chain derivative (363) (see Chapter 13) also condensed with a variety of heterocyclic derivatives [*e.g.* 6-chloropurine and N^6-benzoyl-N^6,9-bis(trimethylsilyl)adenine] to give, after deacetylation, 4′-(hydroxymethyl)ribonucleosides.[536, 541] 6-Chloro-9-(5,5-dimethylfuran-2-yl)purine (585) has been synthesized using the triol (586) derived from L-glutamic acid (see Scheme 65).[538]

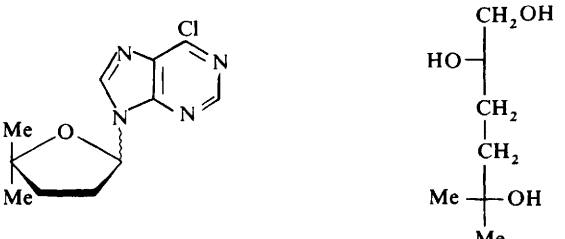
[797] J. M. J. Tronchet and D. Schwarzenbach, *European J. Medicin. Chem.-Chim. Ther.*, 1976, **11**, 489 (*Chem. Abs.*, 1977, **87**, 53 512w).
[798] J. M. J. Tronchet and D. Schwarzenbach, *Helv. Chim. Acta*, 1977, **60**, 1989.
[799] T. Adachi, T. Iwasaki, M. Miyoshi, and I. Inoue, *J.C.S. Chem. Comm.*, 1977, 248.
[800] S. Y.-K. Tam and B. Fraser-Reid, *Canad. J. Chem.*, 1977, **55**, 3996.



(583) (584)

Reagent: i, HCHO–NaOH–aq. dioxan

Scheme 88



(585) (586)

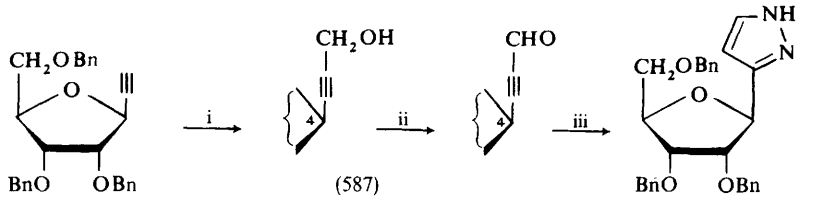
The 2-*C*-nitromethyl precursor of (390) yielded 1-(3-*O*-acetyl-5-*O*-benzoyl-2-deoxy-2-*C*-nitromethyl-α-D-ribofuranosyl)uracil and some of its β-anomer on condensation with bis(trimethylsilyl)uracil in the presence of tin(IV) chloride.[550] 9-(3-*C*-Alkyl-3-deoxy-D-ribofuranosyl)-adenines and a D-*xylo* analogue have been synthesized as potential antimalarial agents.[801]

C-Nucleosides

1-Methylpseudouridine (1-methyl-ψ-uridine) has been prepared by treating 2′,3′,5′-tri-*O*-acetyl-ψ-uridine with bis(trimethylsilyl)acetamide and iodomethane.[802] 2′-Deoxy-ψ-uridine has been obtained by condensing 2,4-di-t-butoxy-5-lithiopyrimidine with 3,5-di-*O*-benzyl- or 3,4-*O*-isopropylidene-2-deoxy-D-*erythro*-pentose, cyclization of the resulting pyrimidin-5-yl polyols with methanolic hydrochloric acid, and removal of the protecting groups. Alternatively, 2′-deoxy-ψ-uridine has been synthesized by reduction (tributyltin hydride) of 2′-chloro-2′-deoxy-ψ-uridine, which was prepared from ψ-uridine using either silicon tetrachloride in hot acetic acid or α-acetoxyisobutyryl chloride in acetonitrile.[803, 804] 2′-Deoxy-ψ-uridine was also converted into 2′-deoxy-1-methyl- and -1,3-dimethyl-ψ-uridine and 2′-deoxy-ψ-isocytidine.[804]

Careful oxidation of 3-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)prop-2-yn-1-ol (587)

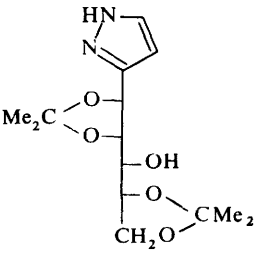
[801] A. Rosowsky and E. J. Modest, *U.S. N.T.I.S., A.D. Report*, 1976, A.D.-A033824, 1977, **77**, 90.
[802] R. A. Earl and L. B. Townsend, *J. Heterocyclic Chem.*, 1977, **14**, 699.
[803] S. D. Bridges, D. M. Brown, and R. C. Ogden, *J.C.S. Chem. Comm.*, 1977, 460.
[804] C. K. Chu, U. Reichman, K. A. Watanabe, and J. J. Fox, *J. Heterocyclic Chem.*, 1977, **14**, 1119.



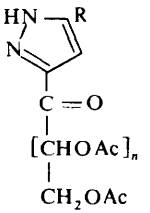
Reagents: i, HCHO–KOH; ii, CrO_3–Me_2CO; iii, NH_2NH_2

Scheme 89

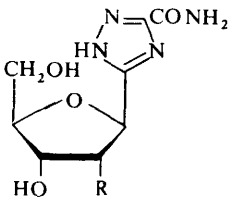
with Jones's reagent in benzene has furnished the corresponding propynal, which yielded 3-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrazole on treatment with hydrazine (Scheme 89).[805] The 3-substituted pyrazole (588) was readily obtained in 93% yield by allowing 1-*C*-ethynyl-2,3:5,6-di-*O*-isopropylidene-D-mannofuranose to react with hydrazine. 3-Phenyl-4-D-ribofuranosylpyrazoles have been prepared by dipolar addition of diazomethane to 1-[2,3,5-tri-*O*-acetyl (or benzoyl)-α- and -β-D-ribofuranosyl]-2-phenylethyne,[806, 807] while dipolar additions of diazo-



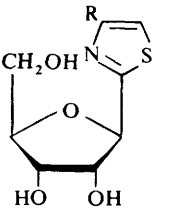
(588)



(589) R = Ph or CO_2Me
n = 3, D-*ribo*
n = 4, D-*galacto*



(590) R = H or OH



(591) R = $CONH_2$ or $CSNH_2$

[805] J. G. Buchanan, A. D. Dunn, A. R. Edgar, R. J. Hutchison, M. J. Power, and G. C. Williams. *J.C.S. Perkin I*, 1977, 1786.

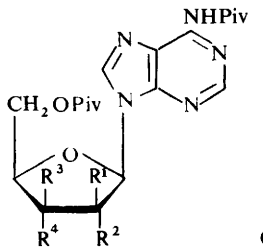
[806] G. Alonso, M. T. Garcia-Lopez, G. Garcia-Munoz, and R. Madronero, *Anales de Quim.*, 1976, **72**, 987 (*Chem. Abs.*, 1977, **87**, 184 819z).

[807] K. Arakawa, T. Miyasaka, and N. Hamamichi, *Nucleic Acids Res., Special Publ.*, 1976, 2 (4th Symposium Nucleic Acids Chem., 1976), 1 (*Chem. Abs.*, 1977, **86**, 140 379f).

ketones derived from acetylated aldonic acids to phenylethyne or methyl prop-2-ynoate have yielded the 3,5-disubstituted pyrazoles (589).[806] Other *C*-nucleosides to be synthesized include ethyl 5-amino-2-β-D-glucopyranosylimidazole-4-carboxylate and its 6′-phosphate,[808] and 5(3)-β-D-ribofuranosyl-, 5(3)-β-D-arabinofuranosyl-, and 5(3)-(2-deoxy-β-D-*erythro*-pentofuranosyl)-1,2,4-triazole-3(5)-carboxamides [*e.g.* (590; R = H)] as structural analogues of ribavirin.[809] Such thiazole *C*-ribonucleosides as (591) and their 5′-deoxy analogues have been synthesized from 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl cyanide; some of them [*e.g.* (591: R = $CONH_2$)] displayed antiviral activity.[810]

Unsaturated Nucleosides

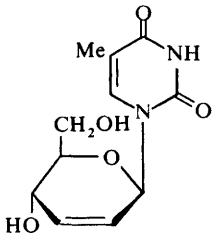
2′,3′-Unsaturated derivatives of adenosine, uridine, N^4-acetylcytidine, formycin, and tubercidin have been prepared by electrochemical reduction of acylated vicinal halohydrin derivatives of nucleosides.[811, 812] For example, electrochemical reduction of the mixture of 2′- and 3′-iodides (592) from the reaction of 2′,3′-*O*-methoxyethylideneadenosine with pivaloyl chloride and sodium iodide in refluxing pyridine (see Vol. 10, p. 159) gave, after deacylation, 9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)adenine.[812] 1-(2,3-Dideoxy-β-D-*erythro*-hex-2-enopyranosyl)thymine (593) has been synthesized from 1-β-D-glucopyranosylthymine by a route involving treatment of 2,2′-anhydro-1-(4,6-*O*-benzylidene-3-*O*-methanesulphonyl-β-D-mannopyranosyl)thymine with sodium iodide–zinc dust in refluxing



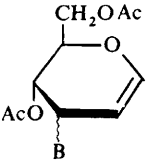
(592) $R^1 = I$, $R^2 = R^3 = H$, $R^4 = O_2CCH{=}CCMe_3$ (OPiv)
or $R^1 = R^4 = H$, $R^2 = O_2CCH{=}CCMe_3$ (OPiv), $R^3 = I$

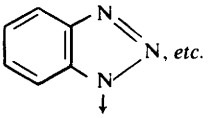
DMF.[813] The acid-catalysed reaction of 3,4,6-tri-*O*-acetyl-D-glucal with benzotriazole or 6-methylthiopurine in acetonitrile at *ca.* 90 °C afforded a mixture of the corresponding 1′,2′-unsaturated isonucleosides and 2′,3′-unsaturated nucleosides in which the former [*e.g.* (594)] predominated.[814] T.l.c. showed that the

[808] V. V. Alenin and V. D. Domkin, *Zhur. obshchei Khim.*, 1976, **46**, 1911 (*Chem. Abs.*, 1977, **86**, 5732a).
[809] T. Huynh-Dinh, J. Igolen, E. Bisagni, J. P. Marquet, and A. Civier, *J.C.S. Perkin I*, 1977, 761.
[810] P. C. Srivastava, M. V. Pickering, L. B. Allen, D. G. Streeter, M. T. Campbell, J. T. Witkowski, R. W. Sidwell, and R. K. Robins, *J. Medicin. Chem.*, 1977, **20**, 256.
[811] T. Adachi, T. Iwasaki, M. Miyoshi, and I. Inoue, *Nucleic Acids Res., Special Publ.*, 1976, 2 (4th Symposium Nucleic Acids Chem., 1976), 93 (*Chem. Abs.*, 1977, **86**, 190 395d).
[812] R. Mengel and J.-M. Seifert, *Tetrahedron Letters*, 1977, 4203.
[813] T. Yamazaki, K. Matsuda, H. Sugiyama, S. Seto, and N. Yamaoka, *J.C.S. Perkin I*, 1977, 1654.
[814] F. G. de las Heras and M. Stud, *Tetrahedron*, 1977, **33**, 1513.



(593)



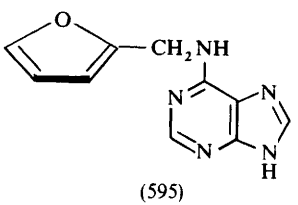
(594) B = 

2′,3′-unsaturated nucleosides were formed initially and then isomerized to the 1′,2′-unsaturated isonucleosides. On further reaction, the 1′,2′-unsaturated isonucleosides gave 2,3-dideoxyhexopyranosides containing heterocyclic bases both at C-1 and C-3.

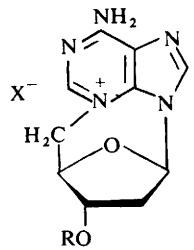
A synthesis of 9-(5-deoxy-β-D-*erythro*-pent-4-enofuranosyl)adenine, an analogue of angustmycin, is referred to in Chapter 18.

Cyclonucleosides

The biosynthesis of kinetin (595) from a 2′-deoxyadenylate unit of DNA may involve an intermediate bridged nucleotide [*e.g.* (596; R = PO_3H_2, X = H_2PO_4]].[815] The feasibility of this intramolecular pathway has been demonstrated by a two-step, chemical conversion of [596; R = $PO(OEt)_2$, X = OTs] into kinetin (595), albeit in very low yield.



(595)

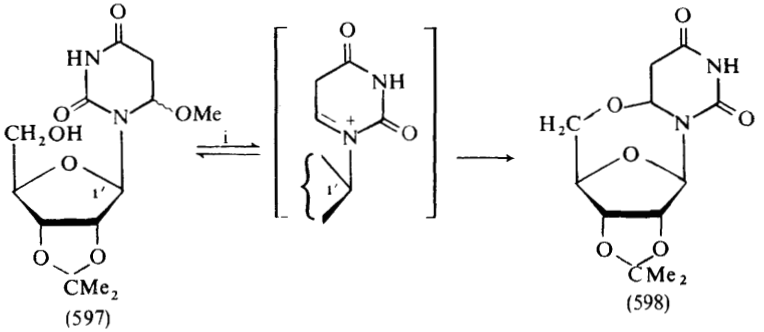


(596)

Treatment of the 5,6-dihydro-6-methoxyuridine derivative (597) with acidified methanol below 10 °C afforded 5′,O^6-cyclo-5,6-dihydro-2′,3′-*O*-isopropylideneuridine (598)(Scheme 90), which reverted to 2′,3′-*O*-isopropylideneuridine upon warming.[816] Deuterium labelling showed that the first step of the conversion of (597) into (598), presumably involving an immonium species, is reversible. Methylation of (598) with methyl iodide–sodium hydride in THF gave 2′,3′-*O*-isopropyl-

[815] D. I. C. Scopes, U. Zarnack, N. J. Leonard, R. Y. Schmitz, and F. Skoog, *Phytochemistry*, 1976, **15**, 1523.

[816] J.-L. Fourrey and P. Jouin, *Tetrahedron Letters*, 1977, 3393.

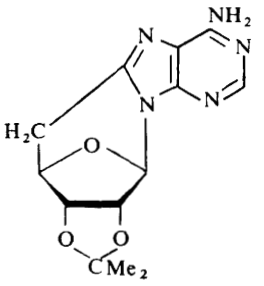


(597) (598)

Reagent: i, H^+–MeOH at < 10°C

Scheme 90

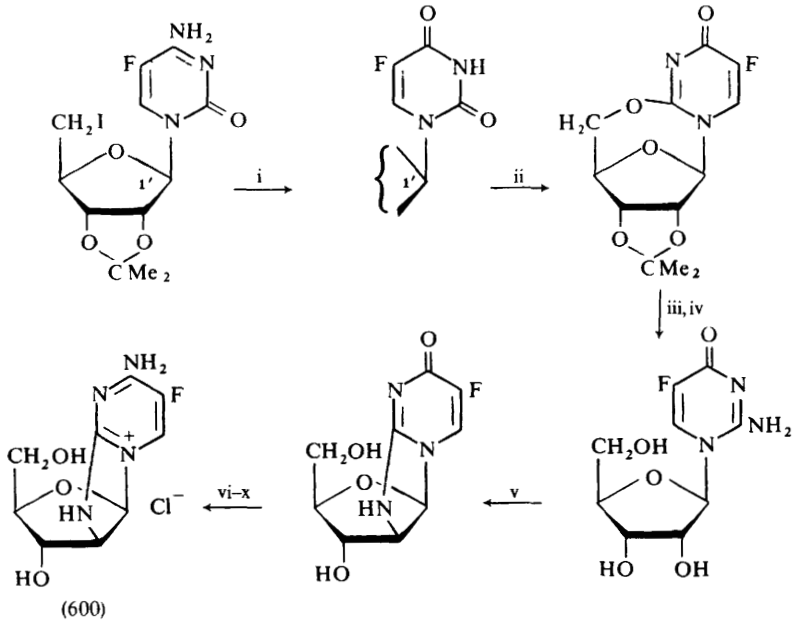
idene-5′-*O*-methyluridine. The 5′,8-cycloadenosine derivative (599) has been prepared in 65% yield by photolysis of 2′,3′-*O*-isopropylidene-5′-*S*-phenyl-5′-thioadenosine in acetonitrile in the presence of triphenylphosphine, and a related cycloinosine derivative was prepared in the same way.[817] Hydroxylation of the cycloadenosine derivative (599) at C-5′ was accomplished by oxidation with selenium dioxide in refluxing dioxan and reduction of the resulting ketone with sodium borohydride to give the (*R*)-5′-hydroxy derivative, which was converted into the (*S*)-5′-hydroxy derivative by displacement of the (*R*)-5′-mesylate with acetate ion, *etc.*[818]



(599)

A nitrogen analogue (600) of the antitumour agent 2,2′-anhydro-1-β-D-arabino-furanosyl-5-fluorocytosine has been prepared by the route outlined in Scheme 91, but neither (600) nor its precursors showed antitumour activity.[819] The reaction between 2′,8-anhydro-9-β-D-arabinofuranosyl-8-hydroxyadenine and hydrazine or alkylamines gave good to high yields of 8-hydrazino- and 8-alkylamino-9-β-D-arabinofuranosyladenines, respectively.[820] Treatment of 9-β-D-arabinofuranosyl-8-hydrazinoadenine with an excess of pentyl nitrite in dilute hydrochloric acid

[817] A. Matsuda, K. Muneyama, T. Nishida, T. Sato, and T. Ueda, *Nucleic Acids Res.*, 1976, **3**, 3349.
[818] A. Matsuda, M. Tezuka, and T. Ueda, *Nucleic Acids Res., Special Publ.*, 1976, 2 (4th Symposium Nucleic Acids Chem., 1976), 13 (*Chem. Abs.*, 1977, **86**, 155 883q).
[819] A. F. Cook, *J. Medicin. Chem.*, 1977, **20**, 344.
[820] J. B. Chattopadhyaya and C. B. Reese, *J.C.S. Chem. Comm.*, 1977, 414.

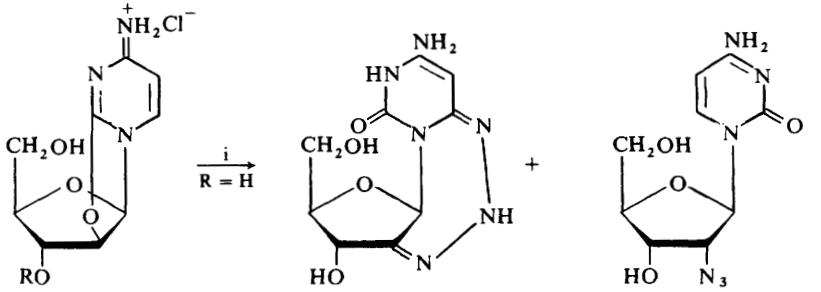


(600)

Reagents: i, $NaNO_2$; ii, AgOAc; iii, NH_3–MeOH; iv, CF_3CO_2H; v, Bz_2O–DMF; vi, Ac_2O–py; vii, P_2S_5; viii, MeI–NaOH; ix, liq. NH_3; x, resin (Cl^-)

Scheme 91

gave the 8-azido derivative, which afforded 8-amino-9-β-D-arabinofuranosyladenine on hydrogenolysis. The 2,2′-cyclonucleoside derivative (601; R = Ac) was obtained in 92% yield following methanolysis of the product obtained when cytidine reacted with 2-acetoxybenzoyl chloride.[821] The deacetylated 2,2′-cyclonucleoside (601; R = H) reacted with lithium azide in DMF to give the novel



(601) R = Ac or H
Reagent: i, LiN_3–DMF

(602) (47%)

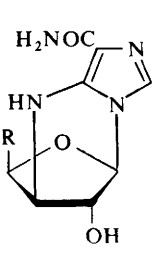
(603) (11%)

Scheme 92

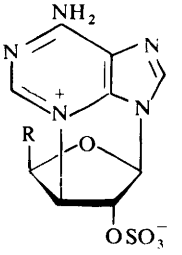
[821] A. A. Akhrem, E. N. Kalinichenko, E. I. Kvasyuk, and I. A. Mikhailopulo, *Bioorg. Khim.*, 1977, 3, 845

2′,6-bridged nucleoside (602) and 2′-azido-2′-deoxycytidine (603) (Scheme 92).[822] Cytidine reacted with aliphatic and aromatic acid anhydrides, or with carboxylic acid chlorides or carboxylic acids, in the presence of boron trifluoride etherate in refluxing acetonitrile to give 1-(3,5-diacyl-β-D-arabinofuranosyl)-2,2′-anhydrocytosine hydrotetrafluoroborates[823] (see also Vol. 10, p. 157). Treatment of uridine with acetic anhydride–boron trifluoride etherate in refluxing acetonitrile gave 1-(3,5-di-*O*-acetyl-β-D-arabinofuranosyl)-2,2′-anhydrouracil (65%) and 2′,3′,5′-tri-*O*-acetyluridine (4%).

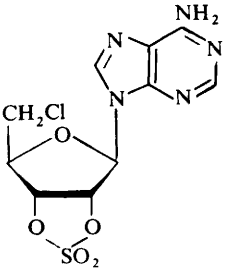
Uridine 2′,3′-episulphide and an analogous D-*lyxo* 2′,3′-episulphide have been prepared from 1-(2,3-anhydro-5-*O*-benzoyl-β-D-lyxofuranosyl)uracil and 2,2′-*S*-anhydro-1-β-D-arabinofuranosyl-2-mercaptouracil, respectively, by conventional procedures.[824] Treatment of 2′,3′-anhydroinosine with dilute sodium hydroxide solution at 80 °C gave the 3′,5-cyclonucleoside-4-carboxamide (604).[825]



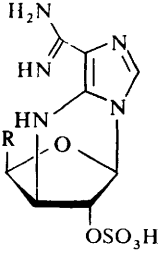
(604) R = CH_2OH



(605) R = CH_2Cl



(606)



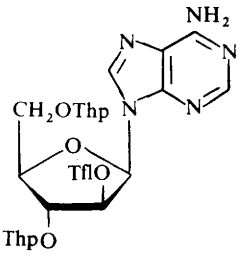
(607) R = CH_2Cl

The 3,3′-cycloadenosine derivative (605) and 5′-chloro-5′-deoxyadenosine 2′,3′-sulphate (606) were formed when adenosine reacted with sulphuryl chloride in acetonitrile.[826] Solvolysis of (606) in aqueous ethanol resulted in opening of the pyrimidine ring and formation of the cyclonucleoside (607). 3,5′-Cycloadenosine was synthesized from 5′-chloro-5′-deoxyadenosine, which was prepared by treating adenosine with thionyl chloride in HMPT.

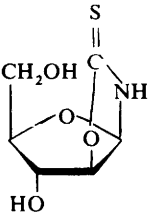
[822] M. Ichino and K. Kikugawa, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 265.
[823] K. Kondo and I. Inoue, *J. Org. Chem.*, 1977, **42**, 2809.
[824] T. Ueda, T. Asano, and H. Inoue, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 365.
[825] R. Mengel and W. Muhs, *Annalen*, 1977, 1585.
[826] K. Kikugawa, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 2181.

Halogeno-sugar Nucleosides

Several halogenated derivatives of nucleosides have been mentioned at the end of the previous section. Adenosine and its 2′,3′-*O*-isopropylidene derivative reacted with thionyl chloride in HMPT to give the corresponding 5′-chloro-5′-deoxy derivatives, while a similar reaction with thymidine furnished 1-(3,5-dichloro-2,3,5-trideoxy-β-D-*threo*-pentofuranosyl)thymine.[827] 2′,3′-*O*-Methoxyethylideneinosine reacted with pivaloyl chloride in refluxing pyridine to give a mixture of 2′(3′),5′-diacylated 9-(3-chloro-3-deoxy-β-D-xylofuranosyl)- and 9-(2-chloro-2-deoxy-β-D-arabinofuranosyl)-hypoxanthines, which were either reduced to the corresponding 3′- and 2′-deoxynucleosides or converted into 2′,3′-anhydroinosine[825] (see Vol. 10, p. 158). 9-(2,5-Di-*O*-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl)adenine has been prepared in 47% yield by treating adenosine with tetra-acetoxysilane, phosphorus tribromide, and boron trifluoride etherate in acetonitrile at room temperature.[828] A mixture of 3′(2′),5′-diacylated 9-(2-chloro-2-deoxy-β-D-arabinofuranosyl)- and 9-(3-chloro-3-deoxy-β-D-xylofuranosyl)-adenines was obtained from the reaction of adenosine with 2-acetoxybenzoyl chloride (see Vol. 7, p. 60).[829] A similar reaction with uridine gave the corresponding halogenated nucleosides and a 2′,3′-*O*-ethylidene derivative. These halogenated nucleosides and those similarly obtained from 5′-*O*-acetyl(or benzoyl)uridine were reduced with tributyltin hydride to 2′- and 3′-deoxyuridine.[829,830] 3′,5′-Di-*O*-acetyl-2′-deoxy-2′-halogenouridines can be deacetylated selectively at *O*-5′ by transesterification with methanol–hydrogen chloride.[831] The resulting 3′-*O*-acetyl derivatives were converted, *via* their 5′-mesylates, into 5′-chloro-5′-deoxy derivatives. The 5′-substituted 2′-halogeno derivatives gave the corresponding 2,2′-anhydrouridine on treatment with sodium methoxide.



(608) Tfl = SO_2CF_3



(609)

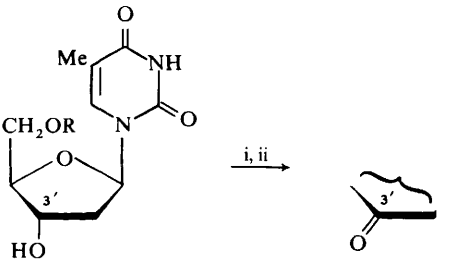
A convenient synthesis of 5′-deoxy-2-fluoroadenosine from guanosine has been reported.[832] Ring-opening of 1-(2,3-anhydro-β-D-lyxofuranosyl)-cytosine and -uracil with ammonium bromide, iodide, azide, and thiocyanate in refluxing ethanol has been used to prepare 1-(3-deoxy-3-halogeno-β-D-arabinofuranosyl)-

[827] D. E. Gibbs and J. G. Verkade, *Synthetic Comm.*, 1976, **6**, 563.
[828] K. Kondo, T. Adachi, and I. Inoue, *J. Org. Chem.*, 1977, **42**, 3967.
[829] A. A. Akhrem, G. V. Zaitseva, E. N. Kalinichenko, and I. A. Mikhailopulo, *Bioorg. Khim.*, 1976, **2**, 1325 (*Chem. Abs.*, 1977, **86**, 73 047f).
[830] A. A. Akhrem, G. V. Zaitseva, I. A. Mikhailopulo, and A. F. Abramov, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 43.
[831] M. Merész, P. Sohár, and J. Kuszmann, *Tetrahedron*, 1977, **33**, 2131.
[832] P. C. Srivastava and R. K. Robins, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 93.

cytosine and -uracil, *etc.*[833] The 2′-triflate (608), prepared from the thionoxazolidine (609), has proved to be a versatile intermediate for effecting nucleophilic displacements at C-2′.[834] For example, it reacted readily with lithium chloride or bromide, sodium iodide or acetate, and potassium thioacetate in HMPT at room temperature and with tetrabutylammonium fluoride in THF at −10°C to give 2′-deoxy-2′-halogenoadenosines, *etc.*, after removal of the protecting groups.

Keto-nucleosides and Nucleosides containing Uronic Acid Components

Photolysis of pyruvic esters (see p. 129) has been used for the selective oxidation of nucleosides.[835] For example, this photochemical process was used to prepare 5′-*O*-trityl(or benzoyl)-3′-ketothymidine (610) from either 5′-*O*-trityl(or benzoyl)-thymidine (Scheme 93) or their 3′-epimers. The 5′-*O*-trityl-3′-ketonucleoside (610;



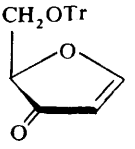
R = Tr or Bz (610)

Reagents: i, MeCOCOCl-py; ii, PhH-*hν*

Scheme 93

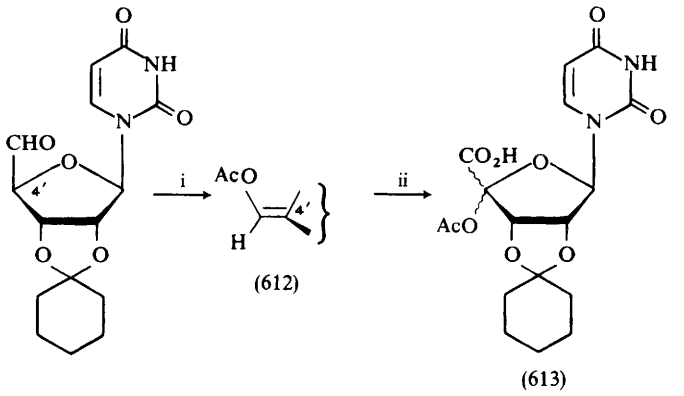
R = Tr) decomposed to thymine and the enone (611) in pyridine or during chromatography on silica gel. ^{1}H N.m.r. measurements have indicated that 1-(6-deoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranosyl-4-ulose)thymine adopts a $^1C_4$ conformation, while 9-(6-deoxy-α-L-*lyxo*-hexopyranosyl-4-ulose)theophylline and its hydrated form adopt $^1C_4$ and $^4C_1$ conformations, respectively.[836]

Nucleoside-5′-aldehydes were converted into 4′,5′-enol acetates [*e.g.* (612)] on heating with potassium carbonate in acetic anhydride (Scheme 94).[837] Treat-



(611)

[833] D. H. Hollenberg, K. A. Watanabe, and J. J. Fox, *J. Medicin. Chem.*, 1977, **20**, 113.
[834] R. Ranganathan, *Tetrahedron Letters*, 1977, 1291.
[835] R. W. Binkley, D. G. Hehemann, and W. W. Binkley, *Carbohydrate Res.*, 1977, **58**, C10.
[836] J. Herscovici and K. Antonakis, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 65.
[837] S. L. Cook and J. A. Secrist, III, *Carbohydrate Res.*, 1976, **52**, C3.

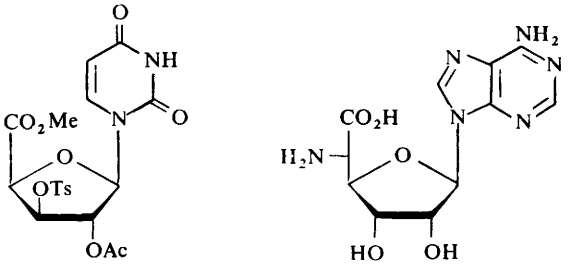


Reagents: i, Ac_2O–K_2CO_3 at 80°C; ii, *m*-$ClC_6H_4CO_3H$

Scheme 94

ment of the (*Z*)-enol acetate (612) with an excess of 3-chloroperbenzoic acid in dichloromethane afforded (613) (a single isomer); this process apparently involves initial epoxidation of (612) followed by rearrangement to a pair of isomeric 5′-aldehydes, one of which is oxidized to (613). Treatment of 1-(methyl 2-*O*-acetyl-3-*O*-toluene-*p*-sulphonyl-β-D-xylofuranosyluronate)uracil (614) with an excess of phthalimide in DMF at 130°C afforded a mixture of 1-(methyl 2- and 3-*O*-acetyl-β-D-ribofuranosyluronate)uracil, presumably by decomposition of a 2′,3′-*O*-phthalimidoethylidene intermediate.[838] Under these reaction conditions, the N^6-benzoyladenin-9-yl analogue of (614) gave N^6-benzoyladenine (71%) and a mixture of acetates (24%) that gave N^6-benzoyl-9-(methyl β-D-ribofuranosyluronate)adenine on deacetylation.

A transglycosylation reaction involving N^6-benzoyl-N^6,9-bis(trimethylsilyl)-adenine and a suitably protected uracil polyoxin C has been used in preparing the adenin-9-yl analogue (615) of the nucleoside skeleton of the polyoxin antibiotics, which contain a labile 5-amino-5-deoxy-β-D-allofuranosyluronic acid residue.[839]



[838] A. A. Akhrem, A. G. Lapko, I. A. Mikhailopulo, and V. A. Timoshchuk, *Carbohydrate Res.*, 1977, **54**, C1.

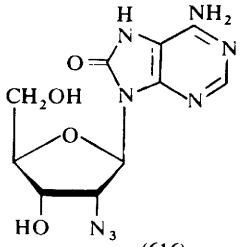
[839] T. Azuma, K. Isono, P. F. Crain, and J. A. McCloskey, *J.C.S. Chem. Comm.*, 1977, 159.

The synthesis of analogues of the octosyl acids[591, 591a] and of derivatives of 5-fluoro-O^4-(β-D-glucopyranosyluronic acid)uracil[125] are mentioned in Chapters 15 and 3, respectively.

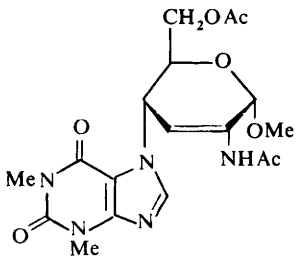
Nucleosides containing Amino-sugar Components

A general method for the preparation of (2-azido- and 2-amino-2-deoxy-D-ribofuranosyl)purines has been described.[840] Thus, condensation of 1,3,5-tri-*O*-acetyl-2-azido-2-deoxy-αβ-D-ribofuranose [derived from 2′-azido-2′-deoxyuridine (Vol. 5, p. 147)] with N^6-octanoyladenine and subsequent deacylation afforded a separable mixture of 2′-azido-2′-deoxyadenosine and its α-anomer. A mixture of 7- and 9-(2-azido-2-deoxy-D-ribofuranosyl)guanines was obtained when N^2-palmitoylguanine was used instead of N^6-octanoyladenine in the condensation step. Reduction of the 2′-azido-2′-deoxynucleosides with triphenylphosphine and ammonia afforded the 2′-amino-2′-deoxynucleosides. Another procedure yielding (2-amino-2-deoxy-β-D-ribofuranosyl)purines is based on the reaction of 2′,8-anhydro-9-β-D-arabinofuranosyl-8-hydroxyadenine with azide ion to give the 2′-azido-2′-deoxynucleoside (616), which could be converted into 2′-amino-2′-deoxy-adenosine and -inosine and related purine nucleosides.[841]

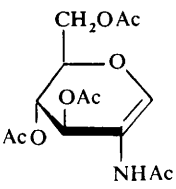
The unsaturated isonucleoside (617), whose structure was established by *X*-ray crystallography, was obtained on fusion of the unsaturated sugar derivative (618) with theophylline in the presence of boron trifluoride etherate.[842]



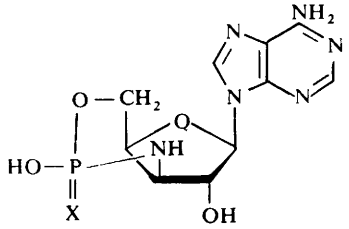
(616)



(617)



(618)



(619) X = O or S

[840] J. B. Hobbs and F. Eckstein, *J. Org. Chem.*, 1977, **42**, 714.

[841] M. Ikehara, T. Maruyama, H. Miki, and Y. Takatsuka, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 754.

[842] B. Kojić-Prodić, B. Danilov, and N. Pravdić, *Carbohydrate Res.*, 1976, **52**, C7.

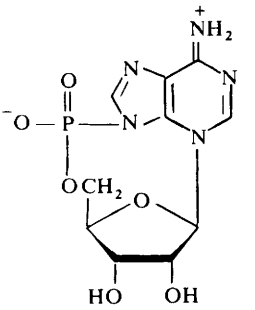
The phosphoramidate analogues (619) of cAMP[842a] and improved syntheses of 5′-azido-5′-deoxythymidine and 5′-azido-2′,5′-dideoxyadenosine and their 3′-phosphates[843] have been reported.

Hydrogen sulphide in aqueous pyridine can be used for the selective reduction of azido-groups on unsaturated sugar moieties of nucleosides.[844]

The daunosaminyl (3-amino-2,3,6-trideoxy-L-*lyxo*-hexopyranosyl) analogue of the antibiotic puromycin and β-daunosaminyl derivatives of adenine, thymine, and cytosine have been synthesized.[845] For example, 9-β-daunosaminyladenine was prepared by condensing 3-*N*,4-*O*-bis(trifluoroacetyl)-α-daunosaminyl chloride with N^6-benzoyl-9-chloromercuriadenine in refluxing xylene, or with N^6-benzoyladenine in dichloromethane at room temperature in the presence of a molecular sieve, and removal of the protecting groups from the product.

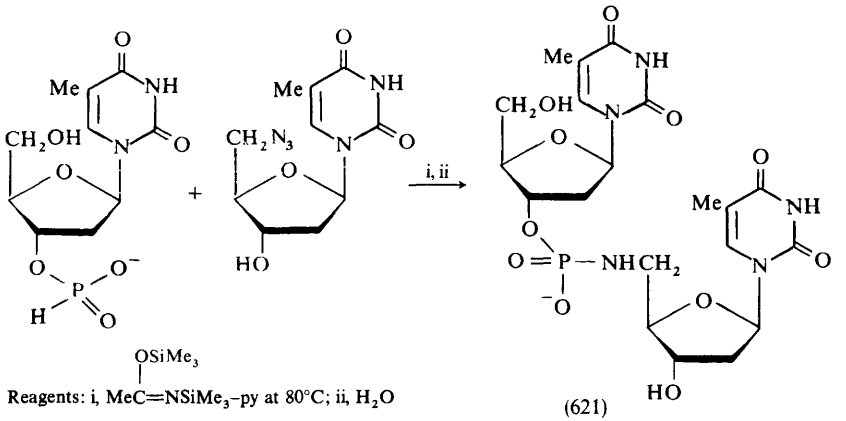
Phosphate and Related Esters of Nucleosides

8-Quinolyl dihydrogenphosphate in pyridine containing anhydrous copper(II) chloride is a useful reagent for the 5′-phosphorylation of (2,3-*O*-isopropylidene-β-D-ribofuranosyl)-purine and -pyrimidine nucleosides.[846] 9-β-D-Arabinofuranosyladenine 3′-phosphate can be conveniently prepared by treating 9-(2,3-anhydro-β-D-lyxofuranosyl)adenine with phosphoric acid in HMPT at 100 °C.[847] 2′,5′-Protected ribonucleosides and 5′-protected 2′-deoxyribonucleosides reacted readily with 4-nitrophenyl phenyl phosphorochloridate in acetonitrile containing a catalytic amount of 5-chloro-1-methylimidazole to give high yields of phosphotriesters, which could be converted quantitatively into phosphodiesters for use in oligonucleotide synthesis by the phosphotriester approach.[848] The 8-quinolyl group has been used as a protecting group for the phosphates of inter-nucleotidic bonds in the synthesis of oligo(thymidylic acids) by the phosphotriester approach, since it is readily hydrolysed with copper(II) chloride in aqueous DMSO.[849] The 2-nitro-4-t-butylphenyl group has also been tested as a protecting group for the



(620)

[842a] M. Morr and R. Mengel, *Chem. Ber.*, 1977, **110**, 3947.
[843] D. E. Gibbs and L. E. Orgel, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 315.
[844] T. Adachi, Y. Yamada, I. Inoue, and M. Saneyoshi, *Synthesis*, 1977, 45.
[845] E. Lazzari, A. Vigevani, and R. Arcamone, *Carbohydrate Res.*, 1977, **56**, 35.
[846] H. Takaku, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 2121.
[847] R. Mengel and H. Wiedner, *Angew. Chem. Internat. Edn.*, 1977, **16**, 317.
[848] C. B. Reese and Y. T. Y. Kui, *J.C.S. Chem. Comm.*, 1977, 802.
[849] H. Takaku, M. Kato, and T. Hata, *J.C.S. Chem. Comm.*, 1977, 190.



Reagents: i, $MeC{=}NSiMe_3$–py at 80°C; ii, H_2O

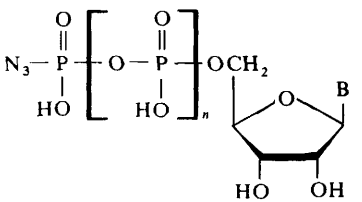
(621)

Scheme 95

phosphates of inter-nucleotidic linkages in related work, but the 2-nitro-4-t-butylphenylated oligonucleotides were extremely sensitive to basic conditions and difficult to purify by chromatography.[850]

Basic hydrolysis of the products from the reaction of 3-β-D-ribofuranosyladenine with phosphorus oxychloride in trimethyl phosphite gave the cyclic phosphoramidate (620) as well as the expected 5′-phosphate.[851] Phosphoramidates [*e.g.* (621)] have been obtained from the reaction between nucleoside 5′-azides and 3′-silylphosphites; the silylation reaction and azide condensation were conducted simultaneously (Scheme 95).[852]

Syntheses of the adenosine and guanosine 5′-phosphoroazidates (622) and (613), which were required as affinity labels,[853] of $P^{1,5}$-di([^{14}C]adenosine-5′)pentaphosphate, an inhibitor of adenylate kinase (E.C. 2.7.4.3),[854] and, by improved procedures, of 3′-deoxyadenosine (cordycepin) 5′-triphosphate[316] have been reported. 2′-Deoxy-2′-deuterio-adenosine (276) (see p. 100) and its 2′-epimer have been converted into the corresponding 3′,5′-phosphates, whose conformers were examined by ^{1}H n.m.r. spectroscopy.[491]

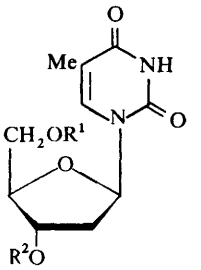


(622) B = guanin-9-yl; n = 0, 1, or 2
(623) B = adenin-9-yl; n = 0 or 1–4

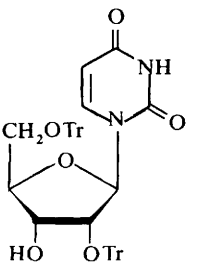
[850] R. W. Adamiak, R. Arentzen, and C. B. Reese, *Tetrahedron Letters*, 1977, 1431.
[851] J. T. Uchic, *Tetrahedron Letters*, 1977, 3775.
[852] D. E. Gibbs, *Tetrahedron Letters*, 1977, 679.
[853] S. Chládek, K. Quiggle, G. Chinali, J. Kohut, III, and J. Ofengand, *Biochemistry*, 1977, **16**, 4312.
[854] J. Köhrle, K. S. Boos, and E. Schlimme, *Annalen*, 1977, 1160.

Other Derivatives

Thymidine 5′-crotonate and related substituted acrylic esters, unlike the corresponding 5′-cinnamate, were readily deacylated on treatment with dilute solutions of hydrazine in methanol–pyridine.[282] The value of the 4-methoxycrotonyl group, for example, in nucleoside chemistry was demonstrated by a synthesis of 3′-*O*-acetylthymidine (624) from 5′-*O*-(4-methoxycrotonyl)thymidine (625) in 94% yield overall. The sterically hindered hydroxy-group at C-3′ of 2′,5′-di-*O*-trityluridine (626) can be acylated using carboxylic acid anhydrides, including pivalic anhydride, in THF containing tetrabutylammonium fluoride as a catalyst.[303] Silyl groups can be replaced by acyl groups using these reagents.



(624) R^1 = H, R^2 = Ac
(625) R^1 = COCH=CHCH$_2$OMe, R^2 = H



(626)

The 2′-*O*-(2-nitrobenzyl) derivatives of adenosine and cytidine, which are normally prepared using 2-nitrophenyldiazomethane and tin(II) chloride (see Vol. 9, p. 163), have now been prepared using 2-nitrobenzyl bromide and sodium hydride in DMF.[855] These ethers are of interest because they are readily removed by photolysis in methanol–DMF.

The reactions outlined in Scheme 96 have been used to prepare 3′-thioadenosine and 9-(3-thio-β-D-xylofuranosyl)adenine.[856] The *O*,*S*-dibenzoate (627), one of the products formed in the displacement reaction with sodium thiobenzoate, arises from an $S \rightarrow O$ benzoyl migration and benzoylation of the liberated 3′-thiol group. The other product isolated from this displacement reaction was 9-(2-*O*-benzoyl-3-deoxy-β-D-*glycero*-pent-3-enofuranosyl)adenine. Hydrogenolysis of the dibenzoate (627) and a related 2′-thiobenzoate [prepared from 5′,N^6-dipivaloyl-9-(2-deoxy-2-iodo-β-D-arabinofuranosyl)adenine] gave 3′- and 2′-deoxyadenosine after removal of the protecting groups.

[5′-^{2}H]Adenosine, as a 2:1 mixture of the (*R*)- and (*S*)-isomers, has been synthesized from D-[5-^{2}H]xylose.[857] The observed spin–spin coupling between the residual 5′-protons and H-4′ showed that the *gauche–gauche* rotamer is preponderant (*ca.* 70%) and the *gauche–trans* rotamer of minor importance (*ca.* 20%) in aqueous solution, contrasting with the preference for the latter rotamer exhibited by adenosine in the solid state. Degradative studies have established that naturally

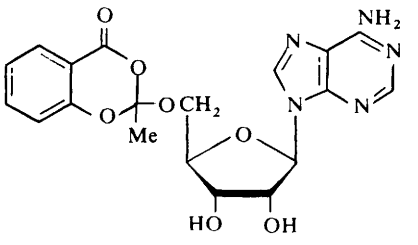
[855] E. Ohtsuka, S. Tanaka, and M. Ikehara, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 949.
[856] R. Mengel and H. Griesser, *Tetrahedron Letters*, 1977, 1177.
[857] R. G. S. Ritchie and A. S. Perlin, *Carbohydrate Res.*, 1977, **55**, 121.

Reagents: i, NaI–BF_3·Et_2O; ii, NaSBz–DMF; iii, MeONa–MeOH; iv, NaSBz; v, BzSH

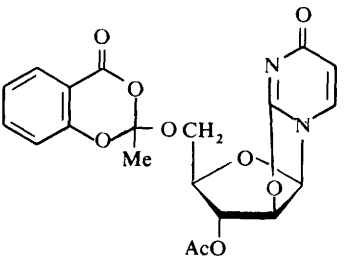
Scheme 96

occurring *S*-adenosylmethionine has the (*S*)-configuration at the sulphur atom.[858] Treatment of 2′,3′-*O*-isopropylideneuridine with sodium chloroacetate–sodium hydride and subsequent acid hydrolysis yielded 5′-*O*-carboxymethyluridine, which reacted with halogens in acidic media to give the corresponding 5-halogeno-derivatives (*e.g.* 5′-*O*-carboxymethyl-5-fluorouridine).[859] The 5-halogeno derivatives are suitable for bonding to proteins for biological studies.

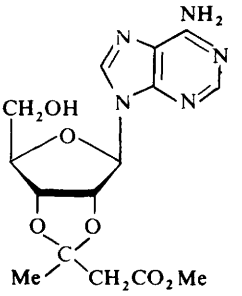
The benzodioxanyl derivatives (628) and (629) were formed when adenosine or uridine, respectively, reacted briefly with 2-acetoxybenzoyl chloride in liquid sulphur dioxide at or below $-15\,^{\circ}C$.[860] On prolonged reaction, followed by methanolysis, adenosine gave the 2′,3′-acetal (630) and 9-(3-chloro-3-deoxy-β-D-xylofuranosyl)adenine.[860a]



(628)



(629)



(630)

Reactions

Guanosine reacted slowly with chloroacetaldehyde in aqueous solution at physiological pH to give 1,N^2-ethenoguanosine (631); this reaction indicates that the action of the mutagen chloroacetaldehyde can damage guanosine derivatives under physiological conditions.[861]

γ-Irradiation of oxygenated aqueous solutions of 2′-deoxyadenosine yielded

[858] J. W. Cornforth, S. A. Reichard, P. Talalay, H. L. Carrell, and J. P. Glusker, *J. Amer. Chem. Soc.*, 1977, **99**, 7292.

[859] A. Holy and H. Pischel, *Coll. Czech. Chem. Comm.*, 1977, **42**, 2261.

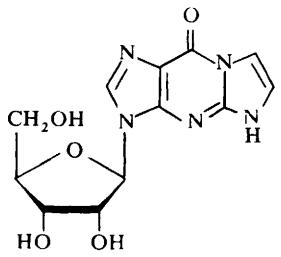
[860] A. A. Akhrem, E. N. Kalinichenko, and I. A. Mikhailopulo, *Vestsi. Akad. Navuk Belorusk.S.S.R., Ser. khim. Navuk*, 1977, 65 (*Chem. Abs.*, 1977, **87**, 53 514y).

[860a] A. A. Akhrem, E. N. Kalinichenko, and I. A. Mikhailopulo, *Zhur. obshchei Khim.*, 1977, **47**, 968 (*Chem. Abs.*, 1977, **87**, 53 515z).

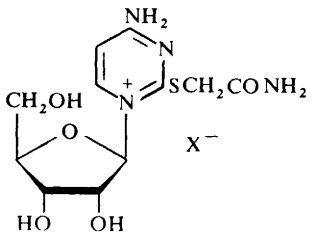
[861] P. D. Sattsangi, N. J. Leonard, and C. R. Frihart, *J. Org. Chem.*, 1977, **42**, 3292.

2′-deoxy-7,8-dihydro-8-oxoadenosine, adenine, and 7,8-dihydro-8-oxoadenine.[862] The proportions of 5,6-dihydrouridine and its 6-methoxy and 6-hydroxymethyl derivatives formed on u.v.-irradiation of uridine in methanol depended on the concentration of the substrate.[863] Substituents on the D-ribofuranosyl moiety of uridine inhibited the formation of the corresponding 6-methoxy derivatives, and 5′,O^6-cyclonucleoside analogues of (598) were obtained on u.v.-irradiation of two uridines with HO-5′ unsubstituted. The photochemical degradation of 2′-deoxyguanosine in oxygenated aqueous solution of pH > 7 appears to occur by two pathways: one of them involves rupture of the purine ring without scission of the glycosidic bond, whereas the other leads to the release of 2-deoxy-D-*erythro*-pentose.[864]

Further studies on the oxidation of adenosine, guanosine, cytidine, and uridine with sodium periodate have confirmed (see Vol. 10, p. 169) that several hydrated species, but no free dialdehydes, are present in aqueous solution, whereas the dialdehydes are completely polymerized in the solid state.[865] The nucleoside dialdehydes gave, with hydrazides, morpholine derivatives possessing rigid, stable structures. Uridine and 2′-deoxyuridine suffered extensive cleavage of the glycosidic bond when attempts were made to nitrate the heterocyclic base at C-5 with nitronium tetrafluoroborate in sulpholane.[344] However, 2′-deoxyuridine 5′-phosphate reacted with this reagent to give some 5-nitrouracil (< 10%) but mainly 2′-deoxy-5-nitrouridine 5′-phosphate and its 3′-nitrate, both of which could be dephosphorylated by an alkaline phosphatase from *E. coli*.



(631)



(632) X = Cl or I

Kinetic studies of the acid-catalysed hydrolysis of such purine nucleosides as guanosine and 8-amino-, 8-methylamino-, 8-dimethylamino-, and 8-bromoguanosine have supported an A-type mechanism in which protonation of the base is followed by cleavage of the glycosidic bond.[866] The much higher rate of hydrolysis of 8-dimethylaminoguanosine, which has a fixed *syn* conformation about the glycosidic bond (see below), was attributed to the relief of steric compression on hydrolysis. ^{1}H N.m.r. studies on these and related 8-substituted adenosine derivatives at various temperatures have indicated that 8-dimethylamino-guanosine and -adenosine have *syn* conformations about the glycosidic bond, whereas the other

[862] N. Mariaggi and R. Teoule, *Bull. Soc. chim. France*, 1977, 1595.
[863] J.-L. Fourrey and P. Jouin, *Tetrahedron Letters*, 1977, 3397.
[864] V. A. Ivanchenko, E. I. Budovskii, N. A. Simukova, N. S. Vul'fson, A. I. Tishchenko, and D. B. Askerov, *Nucleic Acids Res.*, 1977, **4**, 955. (*Chem. Abs.*, 1977, **87**, 136 251m).
[865] F. Hansske and F. Cramer, *Carbohydrate Res.*, 1977, **54**, 75.
[866] F. Jordan and H. Niv, *Nucleic Acids Res.*, 1977, **4**, 697 (*Chem. Abs.*, 1977, **87**, 136 243k).

derivatives have flexible *syn–anti* conformations.[867] The glycosidic bond of the 2-*S*-(carbamoylmethyl)-2-thiocytidine salts (632) was hydrolysed between pH 3 and 8 at a rate almost directly proportional to the concentration of hydroxyl ions.[868] The reaction mechanism changed above pH 9, yielding products (*e.g.* cytidine) having the glycosidic bond intact.

Chemioselective deacetylation of 3′,5′-di-*O*-acetyl-2′-deoxy-2′-halogenouridines at O-5′ with methanol–hydrogen chloride has been noted earlier, although no significant differences were observed among the rates of transesterification of the acetyl groups of 2′,3′,5′-tri-*O*-acetyluridine.[831] Specific *N*-debenzoylation of fully benzoylated adenosine and cytidine has been achieved with a series of phenols and alcohols at 130—135 °C.[869] 2′,3′,5′-Tri-*O*-acetylguanosine reacted with 2,6-dichlorobenzoyl and arenesulphonyl chlorides in pyridine to give the corresponding O^6-substituted derivatives.[870] 2′,3′,5′-Tri-*O*-acetyl-O^6-mesityleneguanosine was particularly susceptible to nucleophilic substitution at O-6, so that it may be used for the synthesis of otherwise inaccessible 6-substituted guanosine derivatives.

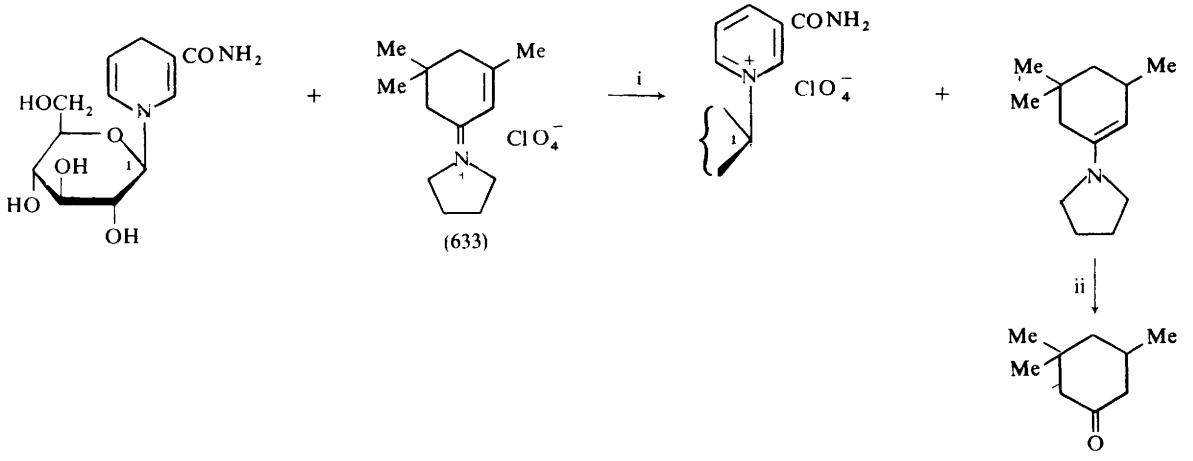
The use of 1-α- and 1-β-D-glucopyranosyl-1,4-dihydronicotinamides and their tetra-acetates in the asymmetric reduction of the αβ-unsaturated iminium perchlorate (633) has been examined.[871] The reaction shown in Scheme 97 gave the best asymmetric reduction [27% excess of the (+)-enantiomer] and best chemical yield (59%) of the chiral product.

The use of iron(III) chloride to catalyse the acetonation of ribonucleosides is mentioned[265a] in Chapter 5.

Physical Measurements

The chemical shift of the signal for the anomeric proton in the ^{1}H n.m.r. spectra of a number of 1-D-manno- and -lyxo-furanosylimidazole derivatives did not accord with the empirical rule stating that H-1′ resonates at lower field when the 1′,2′-substituents are *cis* than when they are *trans*.[753] Nor were differences in the chemical shifts of the methyl signals of the 2′,3′-*O*-isopropylidene derivatives within the limits set for ribonucleoside derivatives (see Vol. 9, p. 167). A useful review of the methods available for assigning the anomeric configuration of nucleosides is given in the introduction to a paper dealing with nucleoside 3′,5′-phosphates, which have $J_{1',2'} \leqslant 0.7$ Hz for 1′,2′-*trans*-nucleosides and $\geqslant 3.5$ Hz for 1′,2′-*cis*-nucleosides.[872] High-resolution ^{1}H n.m.r. spectroscopy has indicated that the furanose ring of several α-ribonucleoside 3′,5′-phosphates in deuterium oxide has a conformation in the range $_2T^3$ to $^3T_2$, with an unusually high distortion from planarity, whereas that of the β-anomers has a conformation in the range ^3E to $_4T^3$ (*cf.* the $^2E \rightleftharpoons {}^3E$ equilibrium found for ribonucleotides).[873] The change in the preferred conformation of the α-ribonucleoside 3′,5′-phosphates was ascribed to a repulsive interaction between HO-2′ and the heterocyclic base,

[867] F. Jordan and H. Niv, *Biochim. Biophys. Acta*, 1977, **476**, 265.
[868] M. Kröger and F. Cramer, *Chem. Ber.*, 1977, **110**, 361.
[869] Y. Ishido, N. Nakazaki, and N. Sakairi, *J.C.S. Perkin I*, 1977, 657.
[870] P. K. Bridson, W. T. Markiewicz, and C. B. Reese, *J.C.S. Chem. Comm.*, 1977, 791.
[871] T. Makino, N. Baba, J. Oda, and Y. Inouye, *Chem. and Ind.*, 1977, 277.
[872] M. J. Robins and M. MacCoss, *J. Amer. Chem. Soc.*, 1977, **99**, 4654.
[873] M. MacCoss, F. S. Ezra, M. J. Robins, and S. S. Danyluk, *J. Amer. Chem. Soc.*, 1977, **99**, 7495.



Reagents: i, DMF at 140°C; ii, H_3O^+

Scheme 97

since removal of HO-2′ produced a less-strained system: 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)adenine 3′,5′-phosphate, for example, adopts a ^3E to $_4E$ pucker. Saturation of the heterocyclic base of α-uridine 3′,5′-phosphate was less effective than removal of HO-2′ in relieving the strain in these rigid systems. An examination of the configuration of *C*-nucleosides derived from formycin by high-resolution ^{1}H n.m.r. spectroscopy has shown that the chemical shifts and ^2J values are not always reliable in distinguishing between the α- and β-anomers.[874] On the other hand, the spin-relaxation times of the protons, particularly that of the anomeric proton, distinguish unambiguously between the α- and β-anomers. The ratio T_1(H-1′)/T_1(H$_i$) (i = 2′, 3′, or 4′) of the β-anomers lies between 2.4 and 3.0, whereas that of the α-anomers is always < 1.5 – this criterion permits unambiguous determination of the anomeric configuration of *C*-nucleosides, even when only one anomer is available. Conditions that enable proton spin–lattice relaxation measurements to be used in conformational studies of nucleosides have been delineated by Bock and Hall in a paper dealing with the conformations of uridine and 2′,3′-*O*-isopropylideneuridine.[875]

360 MHz ^{1}H n.m.r. measurements have indicated that at least eight conformers of puromycin are present in solution, the most abundant conformer (36%) being identical to that of puromycin dihydrochloride in the solid state.[876] High-resolution ^{1}H n.m.r. spectroscopy of a series of 5′-amino-5′-deoxythymidine derivatives has been used to assess the conformational changes that are induced on replacing O-5′ with NH in thymine nucleosides and nucleotides.[877] It was concluded that substitution of NH for O at C-5′ affects neither the shape of the furanose ring nor the *syn–anti* torsional relationship between the heterocyclic base and the furanose ring, whereas the proportion of *gauche–gauche* rotamer in the *X*–C-5′–C-4′ segment decreases, and, in nucleotides, the proportion of *gauche–gauche* rotamer in the P–*X*–C-5′ segment also decreases. The conformations of 2′-, 3′-, and 5′-deoxyadenosine in deuterium oxide at 40—100 °C, in [$^2H_3$]ammonia at −40 to −60 °C, and in pyridine at 10—60 °C have been examined by ^{1}H n.m.r. spectroscopy.[878] The results, which were analysed in terms of the two-state (S ⇄ N) model for the furanose ring (C. Altona and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1972, **94**, 8205), indicated that, in all solvents, 2′-deoxyadenosine has a slight preference for the S state and 3′-deoxyadenosine has a strong preference for the N state, while both states are equally favoured by 5′-deoxyadenosine. The results of molecular-orbital calculations for the chemical shifts of the non-exchangeable protons in pyrimidine and purine ribonucleosides as a function of the torsional angle about the glycosidic bond have been compared with experimental findings.[879, 880] ^{1}H N.m.r. data on the self-association of ATP molecules in basic aqueous solutions are compatible with a monomer–dimer equilibrium, but also

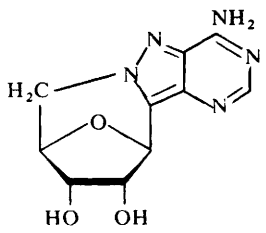
[874] S. Tran-Dinh, J.-M. Neumann, J.-M. Thiéry, T. Huynh-Dinh, J. Igolen, and W. Guschlbauer, *J. Amer. Chem. Soc.*, 1977, **99**, 3267.
[875] K. Bock and L. D. Hall, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 83.
[876] H. P. M. de Leeuw, J. R. de Jager, H. J. Koeners, J. H. van Boom, and C. Altona, *European J. Biochem.*, 1977, **76**, 209.
[877] E. M. Nottoli, J. B. Lambert, and R. L. Letsinger, *J. Amer. Chem. Soc.*, 1977, **99**, 3486.
[878] E. Westhof, H. Plach, I. Cuno, and H. D. Luedemann, *Nucleic Acids Res.*, 1977, **4**, 939 (*Chem. Abs.*, 1977, **87**, 136 250k).
[879] C. Giessner-Prettre and B. Pullman, *J. Theoretical Biol.*, 1977, **65**, 171 (*Chem. Abs.*, 1977, **87**, 136 245n).
[880] C. Giessner-Prettre and B. Pullman, *J. Theoretical Biol.*, 1977, **65**, 189 (*Chem. Abs.*, 1977, **87**, 136 246p).

accord with a model that includes tetramers, pentamers, and species of higher order.[881]

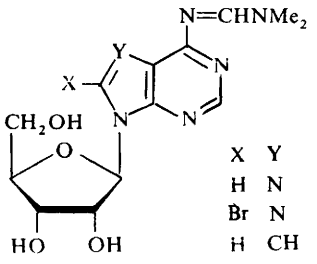
Because of the greater magnetic anisotropy of the C=Se group compared with that of the C=O or C=S group, the anomeric proton of 2-selenopyrimidine and 8-selenopurine nucleosides was observed at a lower field than that of their oxygen- and sulphur-containing analogues.[882]

^{13}C N.m.r. measurements on the ezomycin nucleoside antibiotics are discussed in Chapter 20. ^{13}C N.m.r. measurements on 8-substituted purine nucleosides have shown that many substituents (*e.g.* Br, Cl, SMe, SEt, SH, OMe, OH, and Me) cause characteristic upfield shifts (−2 to −3 p.p.m.) of the C-2′ signal, owing to the proximity of the lone pair of electrons on N-3 of the heterocyclic base to the C-2′—H-2′ bond in the *syn* conformation of these nucleosides.[883]

The ^{1}H noise-decoupled natural-abundance ^{15}N n.m.r. spectra of several pyrimidine and purine ribo- and 2′-deoxyribo-nucleosides have been measured at 18.24 MHz and fully assigned.[884] The use of off-resonance ^{1}H continuous-wave decoupling as an aid to the assignment of signals was discussed. Natural-abundance ^{15}N n.m.r. spectra have also been recorded for several naturally occurring nucleosides (in DMSO) and nucleotides (in water) by Roberts's group, who also studied the effects of protonation on the ^{15}N chemical shifts, which enabled the site of protonation to be detected directly.[885]



(634)



(635)

The fluorescence spectra of several nucleoside derivatives, including (634) and (635), have been measured.[886] The intensity of the fluorescence emission was increased by bridging in such cyclonucleosides as (634), while that of the dimethylaminomethylene derivatives (635) was variable, although such derivatives are potentially useful fluorescent probes.

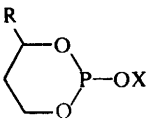
The sequence analysis of oligo(2′-deoxyribonucleotides) by mass spectrometry is mentioned in Chapter 21, which also contains a list of nucleoside derivatives whose structures have been analysed by *X*-ray crystallography. The interaction between Cu^{2+} cations and adenosine, guanosine, cytidine, and uridine in aqueous DMSO has been discussed[620] in Chapter 16.

[881] Y.-F. Lam and G. Kotowycz, *Canad. J. Chem.*, 1977, **55**, 3620.
[882] D. S. Wise and L. B. Townsend, *Tetrahedron Letters*, 1977, 755.
[883] S. Uesugi and M. Ikehara, *J. Amer. Chem. Soc.*, 1977, **99**, 3250.
[884] G. E. Hawkes, E. W. Randall, and W. E. Hull, *J.C.S. Perkin II*, 1977, 1268.
[885] V. Markowski, G. R. Sullivan, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1977, **99**, 714.
[886] H. L. Chung and J. Žemlička, *J. Heterocyclic Chem.*, 1977, **14**, 135.

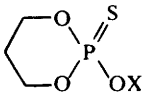
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N.M.R. Spectroscopy and Conformational Features of Carbohydrates

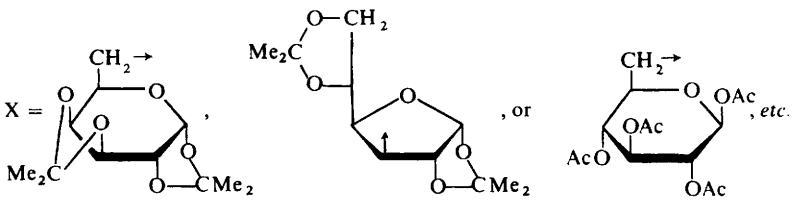
A study of the natural-abundance ^{15}N n.m.r. spectra of 2-amino-2-deoxy-D-hexopyranose hydrochlorides, 2-acetamido-2-deoxy-D-hexopyranoses, and 2-benzamido-2-deoxy-D-glucopyranose has enabled the influence of steric, stereoelectronic, and hydrogen-bonding effects on the chemical shifts to be assessed.[887] The results obtained with these model compounds were used to study substituent effects in polysaccharides and antibiotics containing amino-sugar residues. Deuteriation and lanthanide shift reagents have aided the assignment of ^{29}Si and ^{1}H resonances in the corresponding n.m.r. spectra of methyl 2,3,4,6-tetra-*O*-trimethylsilyl-α-D-glucopyranoside and the related 2,3,4-tri-*O*-trimethylsilyl derivative.[888] ^{31}P N.m.r. spectroscopy has been used to detect diastereoisomeric anisochronism in some of the 2-glyco-1,3,2-dioxaphosphorinans (636) and (637).[889] Details of the assignments of the ^{1}H and natural-abundance ^{13}C n.m.r. spectra of methyl 2-deoxy-2-mercuri-D-aldopyranosides have been reported[890] (see Vol. 10, p. 177). The ^{199}Hg–^{1}H and ^{199}Hg–^{13}C couplings, which were readily obtained from the ^{199}Hg-satellite peaks, showed that the gross angular-dependence of these couplings parallels that for other vicinal ^{1}H—C—C—X couplings. A study of the complexation of Na^+ cations with sugars by using ^{23}Na n.m.r. spectroscopy is referred to[615] in Chapter 16.



(636) R = H or Me



(637)



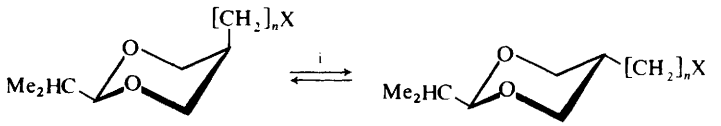
[887] R. E. Botto and J. D. Roberts, *J. Org. Chem.*, 1977, **42**, 2247.
[888] A. H. Haines, R. K. Harris, and R. C. Rao, *Org. Magn. Resonance*, 1977, **9**, 432.
[889] E. E. Nifant'ev, D. A. Predvoditelev, M. K. Grachev, and V. A. Shin, *Doklady Akad. Nauk S.S.S.R.*, 1977, **235**, 595.
[890] V. G. Gibb and L. D. Hall, *Carbohydrate Res.*, 1977, **55**, 239.

A simple modification of a conventional Fourier transform n.m.r. spectrometer has made it feasible to apply selective, radiofrequency pulses at the resonance frequency of one or more proton resonances and to monitor the effects of these perturbations in the usual way.[891] 2′,3′-*O*-Isopropylideneuridine was used to illustrate how this technique can be used to measure selective spin–lattice relaxation rates, to eliminate unwanted resonances by selective saturation or by selective partial relaxation, and to carry out the pulse equivalent of a ^1H–^1H INDOR experiment.

Both ^1H and ^{13}C n.m.r. measurements have indicated that a solution of D-hamamelose (2-*C*-hydroxymethyl-D-ribose) in either deuterium oxide or [$^2H_6$]-DMSO contains two furanose and two pyranose ($^1C_4$) forms.[892]

Monte Carlo techniques have been used to calculate the average energies and free-energy differences between the aldohexopyranoses and between the two chair conformers of each aldohexopyranose.[893] Methods that take into account the deformation of a pyranose ring resulting from steric interactions between substituents were developed. The calculated α:β ratios for the hexopyranoses in aqueous solution were in reasonable overall agreement with experiment, except for some anomers having several equatorial hydroxy-groups; in these cases, the agreement was improved by including a correction for solvent effects (see Vol. 9, p. 174ff.). Free-energy calculations for α- and β-D-glucopyranose have confirmed that the summed energies of intermolecular hydrogen-bonds do not exceed 1.5 kcal mol^{-1} and have little influence on the anomeric equilibrium.[894] Similar calculations have been carried out for other D-aldohexopyranoses and the D-aldopentopyranoses[895] and for intramolecular hydrogen-bonding in aldopyranoses.[895a]

Eliel's group has examined the conformational preferences of 5-substituted 1,3-dioxans with sulphur-containing or ether functions in the side-chain (Scheme 98).[896] The greater the positive charge on the atom attached to C-5, the



X = SMe, SOMe, SO_2Me, SMe_2, or OMe
n = 0, 1, or 2

Reagent: i, H^+

Scheme 98

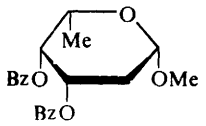
[891] K. Bock, R. Burton, and L. D. Hall, *Canad. J. Chem.*, 1977, **55**, 1045.
[892] G. Schilling and A. Keller, *Annalen*, 1977, 1475.
[893] L. G. Dunfield and S. G. Whittington, *J.C.S. Perkin II*, 1977, 654.
[894] V. P. Panov, V. N. Gritsan, V. I. Dubrovin, and R. G. Zhbankov, *Doklady Akad. Nauk Belorusk.S.S.R.*, 1976, **20**, 799 (*Chem. Abs.*, 1977, **86**, 43 931x).
[895] V. P. Panov, V. N. Gritsan, and R. G. Zhbankov, *Doklady Akad. Nauk Belorusk.S.S.R.*, 1977, **21**, 236 (*Chem. Abs.*, 1977, **86**, 171 751a).
[895a] V. P. Panov, V. N. Gritsan, and R. G. Zhbankov, *Doklady Akad. Nauk Belorusk.S.S.R.*, 1977, **21**, 794 (*Chem. Abs.*, 1977, **87**, 201 928d).
[896] E. L. Eliel, D. Kandasamy, and R. C. Sechrest, *J. Org. Chem.*, 1977, **42**, 1533.

greater is its attraction for the ring-oxygen atoms and, consequently, its preference for an axial orientation. The anomeric effect has been referred to briefly in a general review of phenomenological conformational effects,[897] and *ab initio* molecular-orbital calculations for the chair conformations of 2-chlorotetrahydropyran predict that an equatorial C—H bond next to the ring-oxygen atom has more *s*-character than the other C—H bonds.[898] The preferential attack of electrophiles at the C-1—H bond of pyranoses and $^1J_{^{13}C,\,^1H}$ values were cited in support of this prediction.

Pyranoid Systems

X-Ray analysis of the crystal structure of methyl 3-acetamido-2,3,6-trideoxy-3-*C*,4-*O*-dimethyl-β-L-*arabino*-hexopyranoside (383) has shown that the stereochemistry previously assigned to C-3 of this compound, and, consequently, to C-3 of the branched nitro-sugar evernitrose (a component of the everninomicins), is in error.[545] It seems that caution must be exercised in assigning the stereochemistry at tertiary centres in such compounds as (383) solely on the basis of the chemical shift of the acetamido methyl group.

The chemical shifts of the geminal protons at C-2 of methyl 3,4-di-*O*-benzoyl-2,6-dideoxy-β-L-*lyxo*-hexopyranoside (638) provide another exception to the



(638)

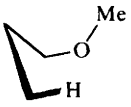
frequently used generalization that the equatorial-proton signals lie downfield from those of axial protons in pyranose systems.[502] The reversal of the usual order was ascribed to the deshielding (or shielding) effect of the neighbouring *O*-benzoyl group upon the axial (or equatorial) hydrogen atom at C-2. Analyses of the high-resolution ^{1}H n.m.r. spectra of 4-*O*-α- and -β-D-xylopyranosyl-D-xylopyranose hexa-acetates[152] and of D-glucopyranose and 2-acetamido-2-deoxy-D-glucopyranose and its derivatives (in deuterium oxide)[899] have been reported. In the latter study, the implications of the systematic errors from assuming first-order conditions for the ^{1}H n.m.r. spectra of sugars were discussed in relation to measuring shift changes of sugar–enzyme complexes.

Increment rules that predict the chemical shifts of protons in the ^{1}H n.m.r. spectra of aldohexopyranoses to within ±0.09 p.p.m. have been presented.[900] 300 MHz ^{1}H n.m.r. spectroscopy has shown that both α- and β-D-ribopyranose exist as a mixture of $^1C_4$ and $^4C_1$ conformations in deuterium oxide, while β-D-ribo- and β-D-xylo-pyranosylamines prefer the $^4C_1$ conformation.[901] The con-

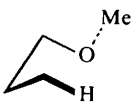
[897] N. S. Zefirov, *Tetrahedron*, 1977, **33**, 3193.
[898] J. Auge and S. David, *Nouv. J. Chim.*, 1977, **1**, 57 (*Chem. Abs.*, 1977, **87**, 23 682j).
[899] S. J. Perkins, L. N. Johnson, D. C. Phillips, and R. A. Dwek, *Carbohydrate Res.*, 1977, **59**, 19.
[900] A. de Bruyn, M. Anteunis, and J. van Beeumen, *Bull. Soc. chim. belges*, 1977, **86**, 259 (*Chem. Abs.*, 1977, **87**, 184 792k).
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formational preferences of aryl 2,3,4-tri-*O*-acetyl-α- and -β-D-ribopyranosides and some of their 1-thio analogues have also been determined.[902] Complete analyses, with the help of computer-simulated spectra, have been reported for the high-resolution ^{1}H n.m.r. spectra of 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine and its 6-*O*-α-L-fucopyranosyl derivative[903] and of methyl β-D-glucopyranoside tetranitrate and β-cellobiose octanitrate.[904] The ^{1}H n.m.r. spectra of numerous D-galactopyranose derivatives have been measured to aid in determining the main structural features of fragments released on partial hydrolysis of agar-type polysaccharides.[905]

A study of the rotameric behaviour of methoxy-groups in several partially methylated aldopyranos(id)es has shown that an axial proton at C-5 is much less deshielded by an axial methoxy-group at C-1 than by an axial hydroxy-group at the same position.[906] A small shielding (δ) effect is experienced by a proton in the arrangement (639) (g^+, g^-, g^-) or (640) (g^-, g^+, a). Chemical shifts and H—C—O—H couplings have been measured for the hydroxylic protons of methyl 4,6-*O*-benzylidene-α-and-β-D-allo-and-gluco-pyranosides in [$^2H_6$]DMSO.[907,908] The H—C—O—H coupling is larger for HO-2 than for HO-3 in the anomers of both series; since a similar correlation holds for an 'isolated' hydroxy-group in 2- and 3-substituted derivatives, such information could be used to locate a substituent in monosubstituted derivatives.



(639)



(640)

Analyses of the ^{13}C and ^{1}H n.m.r. spectra of 2-substituted methylenecyclohexanes (*e.g.* 2-methoxy-1-methylenecyclohexane) and 3-substituted cyclohexenes have demonstrated that a double bond stabilizes the axial or pseudoaxial conformer, respectively, when an electronegative substituent is present at the allylic position.[909] Stabilization of the (pseudo)axial conformer by the double bond in both series is adequately explained in terms of double bond–no bond resonance [*e.g.* (641) ↔ (642)]. This effect is enhanced by a methoxy-group on the double bond, as in 2-methoxy-1-(methoxymethylene)cyclohexane.



(641) (642)

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[907] Y. Kondo and K. Kitamura, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 907.
[908] Y. Kondo and K. Kitamura, *Canad. J. Chem.*, 1977, **55**, 141.
[909] J. Lessard, P. V. M. Tan, R. Martino, and J. K. Saunders, *Canad. J. Chem.*, 1977, **55**, 1015.

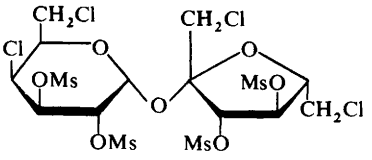
Furanoid Systems

Analyses of the ^1H n.m.r. spectra of solutions of erythrose, threose, and the 5-*O*-methylpentoses in deuterium oxide showed that only erythrose exists to an appreciable extent in a form other than the furanose form; at least 55% of the hydrated monomer was detected in the tautomeric equilibrium of erythrose.[910] The configurations and conformations of several D-xylo-, D-gluco-, and L-ido-furanose derivatives, particularly 6-deoxy-6-nitrohexofuranose derivatives,[911] and of 2,5-anhydro-D-xylose and -lyxose 4-nitrophenylhydrazones and their 3,4-diacetates[912] have been determined using ^1H n.m.r. spectroscopy. The orientation at the C-4—C-5 bond of 1,2-*O*-isopropylidenepentodialdo-1,4-furanoses having α-D-*ribo*, β-L-*lyxo*, and β-L-*arabino* configurations, as well as analogues having one (C-3) or two (C-3 and C-4) hybrid sp^2 carbon atoms, is virtually independent of modification of the substitution at C-3 and seems to be directed by orbital factors favouring a conformer having the carbonyl group and the O—C-4 bond nearly eclipsed.[569]

^1H N.m.r. studies have indicated that the introduction of a methyl group at O-2′ of adenosine has little effect on the molecular conformation of the nucleoside in deuterium oxide.[913] Other ^1H n.m.r. studies on nucleosides are included in Chapter 19.

Di-, Oligo-, and Poly-saccharides

^1H N.m.r. spectroscopy has been used to determine the structure of the disaccharide 4-*O*-β-D-gulopyranosyl-D-glucopyranose.[900] The use of 360 MHz ^1H and 91 MHz ^{13}C n.m.r. spectroscopy, including measurements of the ^1H and ^{13}C spin–lattice relaxation rates, in structural and conformational studies of carbohydrates and their derivatives has been discussed.[914] Such measurements showed that the furanose ring of the *galacto*-sucrose derivative (643) adopts the $^3T_4$ conformation. Changes in the conformation of the D-fructofuranosyl ring of 2,3,3′,4,4′,6-hexa-*O*-acetyl-1′,6′-diacetamido-1′,6′-dideoxysucrose on changing the solvent from deuterium oxide to [$^2H_6$]benzene–[$^2H_6$]acetone were ascribed to changes in the orientation of the acetamido-groups arising from changes in intramolecular attractive forces.[915]



(643)

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The use of ^1H and natural-abundance ^{13}C n.m.r. spectroscopy in structural investigations of oligo- and poly-saccharides is now commonplace, and these techniques are particularly helpful in assigning the configurations of inter-sugar linkages. They were readily applied to gentiodextrins and their derivatives[916] and *Klebsiella* serotype K36[917] and K62[918] polysaccharides, all of which are composed of regular repeating-units, although ^1H n.m.r. spectroscopy also showed that a structurally complex antitumour D-glucan from *Coriolus versicolor* contains α- and β-(1 → 3)-, α- and β-(1 → 4)-, and α- and β-(1 → 6)-linkages.[919]

^{13}C N.M.R. Spectroscopy

The applications of ^{13}C n.m.r. spectroscopy to carbohydrate chemistry have been ably reviewed by Perlin.[920]

Both ^{13}C and high-resolution ^1H n.m.r. spectroscopy have been used in determining the structure and conformation of the polyene macrolide antibiotic pimaricin.[921] The value of the anomeric coupling constant, $J_{^{13}C\text{-}1,\,^1H\text{-}1} = 156$ Hz, for the mycosaminyl residue of *N*-acetylpimaricin indicated that the sugar is β-linked to the macrolide ring.

The high resolving-power of ^{13}C n.m.r. spectroscopy, in conjunction with the high sensitivity of a 20 mm probe, has been used to determine the percentage of β-D-glucofuranose in aqueous solutions of D-glucose.[51] The results indicated that a dilute aqueous solution of D-glucose at 43 °C contains 0.14 ± 0.02% of β-D-glucofuranose and, possibly, ≤0.05% of α-D-glucofuranose; the values of 37.3 ± 1 and 62.6 ± 1% obtained for the proportions of α- and β-D-glucopyranose, respectively, are in agreement with values reported previously. Similar examination of a dilute aqueous solution of D-mannose revealed the presence of 0.6 ± 0.1 and 0.3 ± 0.1% of α- and β-D-mannofuranose, respectively.[50] Catalytic deuterium exchange of carbon-bonded hydrogen atoms geminal to a hydroxy-group (see p. 132) has been used to simplify the splitting patterns and to assign resonances in the ^{13}C n.m.r. spectra of carbohydrate derivatives (*e.g.* methyl α-D-glucopyranoside, methyl 3,6-anhydro-α-D-galactopyranoside, and methyl β-maltoside).[922] With ketopyranoses, for example, the C-1 and C-6 signals are frequently close together: deuteriation readily distinguished between these signals in methyl α-L-sorbopyranoside because the hydrogen atoms on C-1, but not those on C-6, were readily exchangeable, thereby removing the signal for C-1 from the ^1H-decoupled ^{13}C n.m.r. spectrum. This approach showed that earlier assignments made for C-3′ and C-5′ and for C-2 and C-5 of methyl β-maltoside, and also for C-3 and C-5 and for C-3′ and C-5′ of methyl β-cellobioside, should be reversed. As noted earlier (p. 132), inversion of configuration can occur at a centre undergoing deuterium exchange, but it is likely to become prominent only during prolonged treatment or unless it is relatively facile (*e.g.* at C-4 of methyl α-D-galactopyrano-

[916] D. Bassieux, D. Gagnaire, and M. Vignon, *Carbohydrate Res.*, 1977, **56**, 19.
[917] G. G. S. Dutton and K. L. Mackie, *Carbohydrate Res.*, 1977, **55**, 49.
[918] G. G. S. Dutton and M. Yang, *Carbohydrate Res.*, 1977, **59**, 179.
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[921] O. Ceder, B. Hansson, and U. Rapp, *Tetrahedron*, 1977, **33**, 2703.
[922] F. Balza, N. Cyr, G. K. Hamer, A. S. Perlin, H. J. Koch, and R. S. Stuart, *Carbohydrate Res.*, 1977, **59**, C7.

side). Off-resonance decoupling experiments and the use of specifically deuteriated samples have allowed the individual resonances in the ^{13}C n.m.r. spectrum of D-glucitol to be assigned unambiguously, and earlier assignments were shown to be incorrect.[923]

The effect of the trichloroacetyl group on the chemical shifts of geminal and adjacent carbon atoms can be used in assigning the ^{13}C resonances of sugar derivatives.[924] For 2-, 3-, 4-, and 6-*O*-trichloroacetyl-β-D-glucopyranose tetra-acetates and 3′-, 4′-, and 6′-*O*-trichloroacetyl-β-cellobiose hepta-acetates, the 'α-effect' was +4 to +5 p.p.m. (deshielding), whereas the 'β-effect' had smaller and negative values. ^{13}C N.m.r. data for diastereoisomeric *O*-benzylidene derivatives of pyranoid vicinal diols (*e.g.* methyl 2,3-*O*-benzylidene-α-L-rhamnopyranoside and benzyl 3,4-*O*-benzylidene-β-D-arabinopyranoside) have indicated that the configuration at the acetal carbon atom can be determined, even if only one isomer is available.[925] The chemical shift of the acetal carbon atom of the dioxolan ring resonates in the range 102.8—103.4 or 103.8—104.7 p.p.m., depending on whether the phenyl group has an *exo* or *endo* configuration, respectively; *O*-substitution (benzylation or acetylation, *etc.*) has only a small effect on the chemical shift of the acetal carbon atom; the phenyl carbon atom attached to the acetal carbon atom always resonates at a lower field in the *exo* isomers (139 p.p.m.) than in the *endo* isomers (137.5 p.p.m.); the difference ($\Delta\delta$) between the chemical shifts of the acetal carbon atom and the phenyl carbon atom attached to it is >35.4 p.p.m. for *exo* isomers and <33.8 p.p.m. for *endo* isomers. Resonances in the ^{13}C n.m.r. spectra of methyl 2,3-anhydro-4,6-*O*-benzylidene-α- and -β-D-mannopyranosides, methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside, and 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose have been assigned.[926]

Changes in the chemical shifts of the carbon atoms of 1,2-*O*-isopropylidene-aldofuranoses resulting from epimerization or branching at C-3 have been measured.[927] The ^{13}C n.m.r. spectra of D-glucal and D-allal and several of their derivatives have been assigned,[928] and two- and three-bond ^{13}C—^{1}H couplings have been measured in the proton-coupled 67.9 MHz ^{13}C n.m.r. spectra of 1,6-anhydro-β-D-galactopyranose and other hexose derivatives.[929] The three-bond couplings show a Karplus-type dependence, while the values of two-bond couplings are determined by the orientation of oxygen atoms directly connected to the coupling pathway. ^{13}C N.m.r. data for 1,6-anhydro-β-D-glucopyranose[930] and methyl 3,6-anhydro-α-D-galactopyranoside[931] and their *O*-methyl ethers have been reported.

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[926] T. E. Kolosova, T. A. Tsetsokho, and V. P. Panov, *Tezisy Doklady Vses. Konf. Khim. Fiz. Tsellyul., Ist*, **2**, 32 (*Chem. Abs.*, 1977, **86**, 43 937*d*).

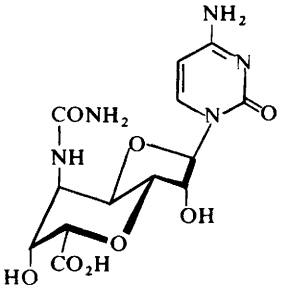
[927] A. S. Shashkov, A. I. Shienok, M. Islomov, A. F. Sviridov, and O. S. Chizhov, *Bioorg. Khim.*, 1977, **3**, 1021 (*Chem. Abs.*, 1977, **87**, 184 822v).

[928] A. I. R. Burfitt, R. D. Guthrie, and R. W. Irvine, *Austral. J. Chem.*, 1977, **30**, 1037.

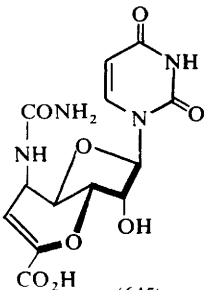
[929] K. Bock and C. Pedersen, *Acta Chem. Scand.* (*B*), 1977, **31**, 354.

[930] N. Gullyev, A. Ya. Shmyrina, A. F. Sviridov, A. S. Shashkov, and O. S. Chizhov, *Bioorg. Khim.*, 1977, **3**, 50 (*Chem. Abs.*, 1977, **86**, 121 647a).

[931] A. S. Shashkov, A. I. Usov, and S. V. Yarotskii, *Bioorg. Khim.*, 1977, **3**, 46 (*Chem. Abs.*, 1977, **86**, 106 923u).



(644)



(645)

^{13}C N.m.r. data have been used in assigning structures to such components as (644) and (645) of the ezomycin complex of antibiotics[932] (see p. 155) and in demonstrating that 5′-amino-5′-deoxy analogues of thymidine 5′-phosphate have predominantly an *anti* conformation about the glycosidic bond.[933] Changes in the chemical shifts of the carbon atoms in the ^{13}C n.m.r. spectrum of adenosine 3′,5′-phosphate on protonation of the heterocyclic base or on ionization of HO-2′ have been monitored; the values of vicinal $^{13}C—^{31}P$ couplings indicated that the chair conformation of the cyclic phosphate ring is retained on ionization of HO-2′.[934] The pH-dependence of the chemical shifts in the ^{13}C n.m.r. spectrum of L-ascorbic acid in deuterium oxide has also been measured and interpreted.[935] The transition of the dimer of dehydro-L-ascorbic acid into the hydrated monomer was also monitored by using ^{13}C n.m.r. spectroscopy. ^{13}C N.m.r. data have revealed that a solution of 3-deoxy-D-*erythro*-hexulosonic acid 6-phosphate in deuterium oxide at 25 °C contains a mixture of α- and β-furanose forms (41 and 50%, respectively) and the open-chain form (9%),[936] and, in conjunction with ^{1}H n.m.r. data, that *N*-acetylneuraminic acid complexes strongly, probably at O-7 and O-8, with Ca^{2+} cations.[937] The ^{13}C n.m.r. spectra of lincomycin hydrochloride and some related compounds have been assigned.[938]

The ^{13}C n.m.r. spectra of the following glycosides have been assigned: isoprenoid β-D-gluco- and -manno-pyranosides[939] (see also Vol. 10, p. 182), various steroidal and alkyl α- and β-D-glucopyranosides and their tetra-acetates,[940] two new β-D-glucopyranosides, arvenins I and II, from *Anagallis arvensis* L. (Primulaceae),[941] the plant glycoside rutin,[942] the triterpenoid glycosides saiko-

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[939] R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Letters*, 1977, 175.
[940] K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Letters*, 1977, 179.
[941] Y. Yamada, K. Hagiwara, and K. Iguchi, *Tetrahedron Letters*, 1977. 2099.
[942] J. Y. Lallemand and M. Duteil, *Org. Magn. Resonance*, 1977, **9**, 179.

saponin E and acetylsaikosaponins, minor components from *Bupleurum falcatum* L.,[943] multiflorin A, a flavonoid glycoside from *Rosa multiflora*,[944] sennoside E, one of the bianthrone β-D-glucopyranosides from rhubarb rhizomes,[944] the glycoalkaloids solasonine and tomatine, which contain the oligosaccharides *O*-β-D-glucopyranosyl-(1 → 3)-[*O*-α-L-rhamnopyranosyl-(1 → 2)]-β-D-galactopyranose and *O*-β-D-xylopyranosyl-(1 → 3)-[*O*-β-D-glucopyranosyl-(1 → 2)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranose, respectively,[945] *N*-acetyldaunorubicinol and daunorubicin,[945a] and the sarsasapogenin disaccharides filiferins A and B and 8-methoxycarbonyloctyl 2-*O*-β-D-glucopyranosyl-β-D-galactopyranoside (67).[207]

The $^{3}J_{^{13}C,\,^{1}H}$ couplings (across the glycosidic bond) of four peracetylated D-glucobioses selectively labelled with ^{13}C at C-1′ and having α- and β-(1 → 4)- and α- and β-(1 → 3)-linkages have provided information on the conformations about the inter-sugar bond in solution.[946] The values obtained for the inter-residue angles (ψ) were compared with known *X*-ray data for similar compounds. The structure of the diammonium salt of 9-*O*-α-D-*N*-acetylneuraminyl-β-D-*N*-acetylneuraminic acid (obtained by partial hydrolysis of the homopolymer from *Neisseria meningitidis* serogroup C) has been confirmed by using ^{13}C n.m.r. spectroscopy, and it was established that it is necessary to use the carboxylate-ion form, rather than the free-acid form, of model sialic acid monomers in order to obtain accurate assignments, because formation of the carboxylate anion produced large downfield displacements of the chemical shifts of the carboxylic acid carbon atom (C-1) and neighbouring carbon atoms (C-2—C-4).[947] Formation of the sodium salt of methyl α-D-*N*-acetylneuraminic acid also produced a significant chemical-shift difference (0.9 p.p.m.) at the remote C-8 position, possibly due to hydrogen-bonding between the axial carboxylate anion and HO-8.

^{13}C N.m.r. data have established that β-gentiobiose is linked to C-4 of thevetose (6-deoxy-3-*O*-methyl-L-glucopyranose) in the steroidal trisaccharides thevetins A and B.[948]

The applications of ^{13}C n.m.r. spectroscopy to macromolecules of biological interest, including carbohydrates and nucleotides, have been reviewed.[949] The structures of the following oligo- and poly-saccharides have been examined during the past year, using natural-abundance ^{13}C n.m.r. spectroscopy: *Klebsiella* serotype K5, K36, and K70 polysaccharides, which are composed of three to six sugar residues, some of which may carry acetyl or 1-carboxyethylidene substituents,[950] the capsular antigen from *Haemophilus influenzae* type a,[951] lichenin, a regular D-glucan containing three types of glycosidic linkage in the repeating-

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[945] R. J. Weston, H. E. Gottlieb, E. W. Hagaman, and E. Wenkert, *Austral. J. Chem.*, 1977, **30**, 917.
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[946] G. Excoffier, D. Y. Gagnaire, and F. R. Taravel, *Carbohydrate Res.*, 1977, **56**, 229.
[947] H. J. Jennings and A. K. Bhattacharjee, *Carbohydrate Res.*, 1977, **55**, 105.
[948] K. Tori, T. T. Thang, M. Sangare, and G. Lukacs, *Tetrahedron Letters*, 1977, 717.
[949] S. N. Rosenthal and J. H. Fendler, *Adv. Phys. Org. Chem.*, 1976, **13**, 279.
[950] J. M. Berry, G. G. S. Dutton, L. D. Hall, and K. L. Mackie, *Carbohydrate Res.*, 1977, **53**, C8.
[951] P. Branefors-Helander, C. Erbing, L. Kenne, and B. Lindberg, *Carbohydrate Res.*, 1977, **56**, 117.

unit,[952] two highly branched arabinans from the inner bark of young stems of *Rosa glauca*,[953] alginates from *Laminaria digitata* and *L. hyperborea*,[954] oligosaccharides released by enzymic hydrolysis of agarose,[955] the rhamnomannans and acidic rhamnomannans of *Sporothrix schenckii* and *Ceratocystis stenoceras*,[956] and cellulose *O*-methyl, *O*-carboxymethyl, and *O*-hydroxyethyl ethers.[957] Details of the structures of these polymers are included in Part II of this Report.

The ^{13}C n.m.r. spectra of mono- and di-nucleotides have been measured in a study concerned with the conformation of the sugar–phosphate backbone of nucleic acids.[958] In mononucleotides, the distribution of rotamers about the C-5′—O-5′ bond of 5′-phosphates, unlike that about the C-3′—O-3′ bond of 3′-phosphates, was not affected by secondary ionization of the phosphate group.

Lanthanide Shift Reagents

Complexes of $Eu(fod)_3$ with permethylated aldohexosylaldohexoses consisting of D-gluco- and D-galacto-pyranosyl residues in (1 → 2)- or (1 → 4)- or (1 → 6)-linkage have been studied by using ^1H n.m.r. spectroscopy.[959] It was found that $Eu(fod)_3$ binds preferentially to two neighbouring MeO-oxygens having an *ax.-eq.* relationship and that steric hindrance disfavours binding at certain sites. The signals for most of the methoxy-groups in the permethylated disaccharides were assigned on the basis of $Eu(fod)_3$-effects on the methoxy-groups and by comparison of the chemical shifts of corresponding groups in permethylated mono- and di-saccharides. The shift increments of the signals for these methoxy-groups, with respect to those for the corresponding groups in the permethylated monosaccharides, were related to the type and the configuration of the inter-sugar linkage. The ^1H n.m.r. spectra of β-D-galactopyranose penta-acetate, α- and β-D-glucopyranose penta-acetates, and several aryl 2,3,4,6-tetra-*O*-acetyl-α- and -β-D-glucopyranosides have been recorded in the presence of $Eu(fod)_3$.[960] Comparisons of the chemical-shift data for acetylated derivatives in the D-galacto-pyranose and D-glucopyranose series suggested that the acetoxy-groups at C-4 and C-6 of β-D-galactopyranose penta-acetate complex with $Eu(fod)_3$.

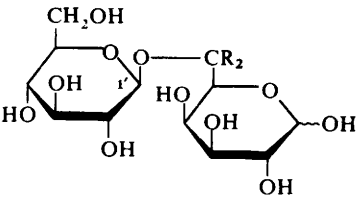
^{13}C N.m.r. spectroscopy has indicated that D-glucitol forms tridentate complexes involving O-2, -3, and -4, with lanthanide(III) cations.[923]

Spin–Lattice Relaxation Times

A quantitative measure of the aglycone–sugar proton-relaxation contributions of glycosides (see Vol. 10, p. 183) has been achieved by comparing the spin–lattice relaxation rates (R_1-values) of the anomeric protons of methyl α- and β-D-glucopyranosides with those of the anomeric protons of the corresponding tri-

[952] D. Gagnaire and M. Vincendon, *Bull. Soc. chim. France*, 1977, 479.
[953] J.-P. Joseleau, G. Chambat, M. Vignon, and F. Barnoud, *Carbohydrate Res.*, 1977, **58**, 165.
[954] H. Grasdalen, B. Larsen, and O. Smidsrød, *Carbohydrate Res.*, 1977, **56**, C11.
[955] G. K. Hamer, S. S. Bhattacharjee, and W. Yaphe, *Carbohydrate Res.*, 1977, **54**, C7.
[956] P. A. J. Gorin, R. H. Haskins, L. R. Travassos, and L. Mendonca-Previato, *Carbohydrate Res.*, 1977, **55**, 21.
[957] A. Parfondry and A. S. Perlin, *Carbohydrate Res.*, 1977, **57**, 39.
[958] J. L. Alderfer and P. O. P. Ts'o, *Biochemistry*, 1977, **16**, 2410.
[959] D. G. Streefkerk and A. M. Stephen, *Carbohydrate Res.*, 1977, **57**, 25.
[960] K. Izumi, *J. Biochem. (Japan)*, 1977, **81**, 1605.

deuteriomethyl D-glucopyranosides.[961] According to the formalism of the dipole–dipole relaxation mechanism, the relaxation contributions made by a deuterium nucleus should be roughly 6% of that of a proton nucleus located at the same position: thus, comparison of the proton R_1-values of a normal sugar with those of its specifically deuteriated analogues provides a direct method for identifying and measuring specific, inter-proton, relaxation contributions. It was pointed out that the relaxation contribution (159 ms^{-1}) received from the methoxy protons by H-1 of methyl β-D-glucopyranoside is nearly twice that (85 ms^{-1}) received by H-1 of the α-glycoside, indicating that the distribution of rotamers about the C-1—O-1 bond of a glycoside can be very dependent on the anomeric configuration. In extending this approach to the disaccharide (646; R = H) and its 6,6-



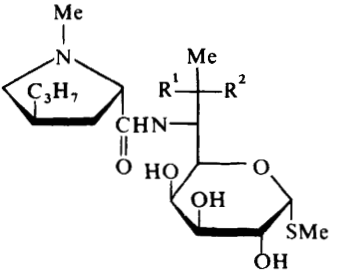
(646) R = H or ^2H

dideuterio analogue (646; R = ^2H), it was calculated that H-1′ of (646; R = H) receives 26% (480 ms^{-1}) of its relaxation from the protons at C-6. ^{13}C Spin–lattice relaxation rates and ^{13}C–^1H nuclear Overhauser enhancement factors have indicated that the tumbling motions of lactose, methyl β-lactoside, and methyl β-cellobioside in aqueous solution have some anisotropic character.[962] The data also confirmed that differences in the R_1-values of H-1 and H-1′ of disaccharides (see Vol. 10, p. 183) arise principally from inter-ring relaxation contributions, with anisotropic tumbling making only a minor contribution. The ^{13}C spin–lattice relaxation rates of the diastereoisomeric methyl 2,3-*O*-benzylidene-α-L-rhamnopyranosides in [^2H]chloroform at 55°C indicated that the molecules tumble isotropically (the dipole–dipole relaxation mechanism being dominant).[925] Since the tumbling motions of the diastereoisomers are similar, the high degree of regioselectivity found for hydrogenolytic cleavage of the acetal ring (see Vol. 10, p. 33) cannot be influenced by the tumbling motion of the phenyl groups.

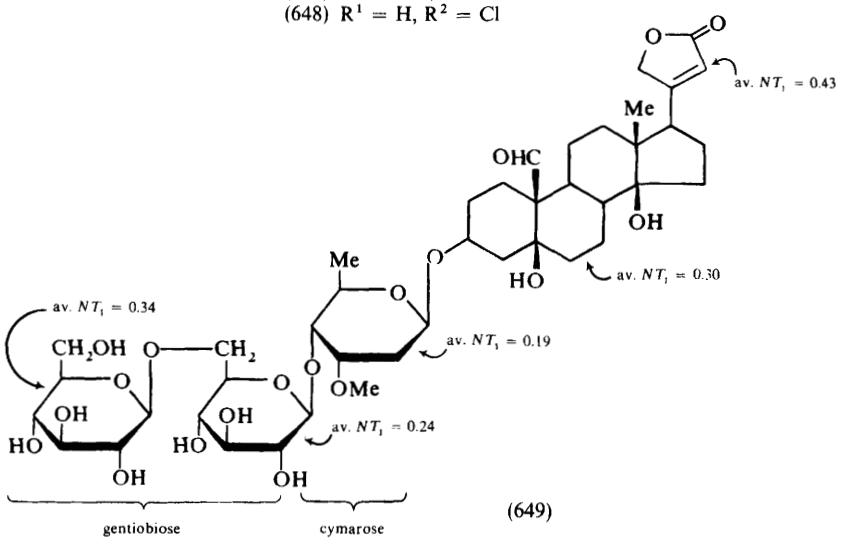
The ^{13}C spin–lattice relaxation times for the hydrochlorides of lincomycin (647) and clindamycin (648) in deuterium oxide suggested that (i) the pyranose ring tumbles isotropically without a preferred axis of rotation, (ii) the tumbling of C-6 and C-7 is isotropic with that of the sugar ring, (iii) the pyrrolidine ring is more mobile than the sugar ring, and (iv) the *C*-, *N*-, and *S*-methyl groups and the propyl side-chain are free to rotate.[938] The principal difference between the tumbling of (647) and (648) was shown by the shorter ^{13}C spin–lattice relaxation rates for (648), which may be associated with the presence of the heavy chlorine atom. ^{13}C Spin–lattice relaxation times have been measured for lankamycin and

[961] J. M. Berry, L. D. Hall, D. W. Welder, and K. F. Wong, *Carbohydrate Res.*, 1977, **54**, C22.
[962] J. M. Berry, L. D. Hall, and K. F. Wong, *Carbohydrate Res.*, 1977, **56**, C16.

other 14-membered macrolide antibiotics—but longer relaxation times do not appear to be associated simply with the presence of a higher number of 1,3-diaxial Me//H interactions in the diamond-lattice-type structure of the macrolide ring[963]—and for the individual carbon atoms of the steroidal oligosaccharide *k*-strophanthoside (649) in [$^2H_5$]pyridine at 90°C.[964] This technique may be useful for determining the sequence of sugars attached to a steroidal aglycone, since the average NT_1 values (N = the number of hydrogens attached to a particular carbon atom) of the sugar residues in *k*-strophanthoside reflect their position with respect to the aglycone.



(647) R^1 = OH, R^2 = H
(648) R^1 = H, R^2 = Cl



(649)

The ^{13}C spin–lattice relaxation times of the carbon atoms of certain 1,2-di-*O*-acyl-3-*O*-β-D-galactopyranosyl- and 1,2-di-*O*-acyl-3-*O*-(6-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-*sn*-glycerols have been measured in [$^2H_4$]methanol, [^2H]-chloroform, and deuterium oxide with a view to understanding the role of chloroplast lipids in the structure and the function of membranes.[965] The T_1-values were

[963] A. Neszmelyi, S. Ōmura. T. T. Thang, and G. Lukacs, *Tetrahedron Letters*, 1977, 725.
[964] A. Neszmelyi, K. Tori, and G. Lukacs, *J.C.S. Chem. Comm.*, 1977, 613.
[965] S. R. Johns, D. R. Leslie, R. I. Willing, and D. G. Bishop, *Austral. J. Chem.*, 1977, **30**, 823.

interpreted in terms of different secondary structures in these solvents; namely, a monomeric structure in [$^2H_4$]methanol, an inverted micellar structure in [^2H]-chloroform, and a bilayer structure in deuterium oxide.

21
Other Physical Methods

I.R. Spectroscopy

I.r. spectra of β-D-glucopyranose have been measured in the 400—4000 cm^{-1} region at room temperature and in the 50—500 cm^{-1} region at liquid-helium temperature.[966] The normal co-ordinates were treated by using the Urey–Bradley force-field, and vibrational assignments to the observed bands were made on the basis of the potential-energy distributions. In the region above 250 cm^{-1}, the observed spectra may generally be interpreted by the one-molecule model, but the results of calculations based on this model do not always explain the observed spectra in the region below 250 cm^{-1}, owing to intermolecular interactions. Studies of intramolecular hydrogen-bonding in mono- and di-saccharides in carbon tetrachloride–DMSO solutions have indicated that five-membered rings formed by hydrogen-bonding persist in solutions containing $\leqslant 25\%$ by volume of DMSO.[967] The positions of the carbonyl and hydroxyl stretching vibrations in the i.r. spectra of the isomeric 4,6-dideoxy-L-hexono-1,5-lactones and several of their derivatives have been discussed in terms of the structures of the molecules and steric factors.[968]

Mass Spectrometry

Field desorption (f.d.) mass spectrometry of underivatized steroid and triterpenoid oligoglycosides has revealed their molecular weights and provided structural information.[969] Salt impurities (mostly alkali-metal halides) in samples isolated from plant materials were found to promote the attachment of the metal cation to the intact molecules and their fragment ions. Related work has shown that underivatized di- and tri-saccharides can be ionized under the conditions of f.d. mass spectrometry by the attachment of alkali-metal cations, using alkali-metal tetraphenylborates as cation-donors.[970] The f.d. mass spectra exhibited only $[M + \text{cation}]^+$ ions, permitting the determination of molecular weights and the analysis of mixtures. Sodium (or potassium) iodide has also been used as a cation-donor in the f.d. mass spectrometry of oligosaccharides containing up to six monosaccharide residues and of partially methylated αα-trehaloses.[971] A new technique, which could be useful for determining the molecular weights of carbohydrates,

[966] M. Hineno, *Carbohydrate Res.*, 1977, **56**, 219.
[967] M. Fialeyre, F. Lafuma, and C. Quivoron, *J. Chim. Phys., Phys.-Chim. Biol.*, 1977, **74**, 701 (*Chem. Abs.*, 1977, **87**, 184 810q).
[968] K. Kefurt, P. Adámek, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1977, **42**, 867.
[969] H.-R. Schulten, T. Komori, and T. Kawasaki, *Tetrahedron*, 1977, **33**, 2595.
[970] H. J. Veith, *Tetrahedron*, 1977, **33**, 2825.
[971] J.-C. Prome and P. Puzo, *Org. Mass Spectrometry*, 1977, **12**, 28.

has been applied to a number of involatile natural products (*e.g.* vitamins and alkaloids); bombardment of the sample on a silver disc with 2.5 keV argon ions causes the emission of secondary ions that include those having masses, usually $(M - H)^-$ or $(M + H)^+$, close to that of the molecular ion of the sample.[972]

Many of the stereoisomers of methylated hexitol acetates having the same arrangement of *O*-methyl and *O*-acetyl groups yielded markedly different chemical-ionization (c.i.) mass spectra; for example, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol and the corresponding galactitol gave clearly distinguishable and reproducible c.i. mass spectra.[973] Hence, c.i. mass spectrometry, in combination with analytical g.l.c. and electron-impact (e.i.) mass spectrometry, can be used in identifying the methylated alditol acetates obtained during methylation analysis of polysaccharides, *etc.*, and in characterizing previously unidentified methylated hexitol acetates.

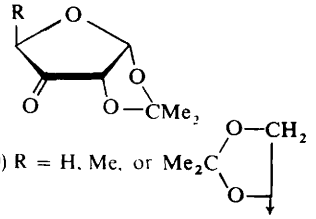
E.i. mass spectra of several hexopyranose penta-acetates,[974] peracetylated pento- and hexo-pyranosides,[975] and methyl 2,3,4,6-tetra-*O*-methyl-α- and -β-D-gluco- and -galacto-pyranosides[976] have been published and interpreted. Detailed studies of methyl 2,3,4,6-tetra-*O*-methyl-α- and -β-D-galactopyranosides and specific trideuteriomethyl derivatives by c.i. mass spectrometry have provided information on the nature and kinetics of the fragmentations.[977,977a] Similar work has been carried out on permethylated methyl 2-, 3-, and 4-deoxyhexopyranosides.[978] Comparisons of fragments in the e.i. mass spectra of methyl *O*-methyl-α-L-rhamnopyranosides with the fragmentation pattern of permethylated methyl 6-deoxyhexopyranosides have permitted the number and the positions of methyl groups in the partially methylated α-L-rhamnopyranosides to be determined.[979] Further, methyl 2,3- and 3,4-di-*O*-methyl-α-L-rhamnopyranosides could be distinguished by the presence or absence of a peak at *m/e* 72, which is characteristic of substitution on O-4.

The molecular ion in the e.i. mass spectra of 4,6-*O*-benzylidene derivatives of hexopyranosides is usually observed as part of a triplet $[(M + 1)^+$, M^+, and $(M - 1)^+]$ and, in most cases, the fragmentation patterns allow substituents to be located.[980]

The e.i. mass spectra of *O*-acetyl and *O*-TMS derivatives of D-glucal and D-xylal have been interpreted with the aid of deuterium labels.[981] Isotopic (^{18}O and ^2H) labels have also been used in elucidating the e.i. mass-spectral fragmentations of

[972] A. Benninghoven and W. Sichtermann, *Org. Mass Spectrometry*, 1977, **12**, 595.
[973] M. McNeil and P. Albersheim, *Carbohydrate Res.*, 1977, **56**, 239.
[974] V. I. Khvostenko, L. A. Baltina, V. S. Fal'ko, and G. A. Tolstikov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 1672 (*Chem. Abs.*, 1977, **86**, 5699v).
[975] G. A. Tolstikov, V. S. Fal'ko, L. A. Baltina, and V. I. Khvostenko, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1977, 1051 (*Chem. Abs.*, 1977, **87**, 136 192t).
[976] L. A. Baltina, V. S. Fal'ko, V. I. Khvostenko, and G. A. Tolstikov, *Zhur. obshchei Khim.*, 1977, **47**, 1429 (*Chem. Abs.*, 1977, **87**, 184 886u).
[977] A. A. Solov'ev, V. I. Kadentsev, and O. S. Chizhov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 2256 (*Chem. Abs.*, 1977, **86**, 106 974m).
[977a] A. A. Solov'ev, V. I. Kadentsev, and O. S. Chizhov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 2261 (*Chem. Abs.*, 1977, **86**, 106 975n).
[978] A. A. Solov'ev, V. I., Kadentsev, and O. S. Chizhov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 2500 (*Chem. Abs.*, 1977, **86**, 90 197x).
[979] V. Kováčik, V. Mihálov, S. Karácsonyi, and R. Toman, *Carbohydrate Res.*, 1977, **58**, 203.
[980] C. Bosso, J. Defaye, A. Gadelle, and J. Ulrich, *Org. Mass Spectrometry*, 1977, **12**, 493.
[981] J. Szafranek, A. Wisniewski, and P. Vouros, *Carbohydrate Res.*, 1977, **56**, 11.

the keto-sugar derivatives (650) and some related 3-*C*-cyanomethylene derivatives.[982, 983]



(650) R = H, Me, or Me_2C

The mass-spectral fragmentation of acetylated hexopyranose methyl sulphates is similar to that of hexose penta-acetates and reflects the position of the sulphate group.[343] The mass-spectral fragmentation of the corresponding acetylated chlorosulphates was also examined. E.i. mass spectrometry, in conjunction with g.l.c., has been used for determining the position of the linkage to 2-acetamido-2-deoxy-D-galactosyl and -glucosyl residues in oligosaccharides and glycoproteins.[984] The method is based on borohydride reduction of the terminal reducing residue of an oligosaccharide (or a glycoprotein) to the corresponding alditol derivative, periodate oxidation, borohydride reduction, hydrolysis (eventually followed by borohydride reduction), separation of the fragments as per-*O*-TMS or per-*O*-trifluoroacetyl derivatives, and identification of the fragments as derivatives of 2-acetamido-2-deoxy-glycerol, -L-threitol, -L-arabinitol, -D-xylitol, -D-galactitol, and -D-glucitol.

The mass spectra of the peracetylated methyl esters of 5-acylamino-3,5-dideoxynononic acids (prepared by reduction of the keto-group in sialic acids) can be easily interpreted because the fragmentation patterns are very simple.[985] Cleavage of the bonds (C-5—C-6 and C-4—C-5) adjacent to the nitrogen-bearing carbon atom leads to the most characteristic and most abundant primary fragments containing nitrogen, which decompose further through elimination of acetic acid and CH_2CO. The fragmentations of permethylated methyl glycoside methyl esters of *N*-acetyl- and *N*-glycolyl-neuraminic acids and the corresponding derivative of 8-*O*-acetyl-*N*-acetylneuraminic acid have also been studied by e.i. mass spectrometry.[986] The results were successfully applied to the methylation analysis of neuraminic acids from brain and kidney gangliosides.

The mass-spectral fragmentation of methyl (methyl *O*-acetyl-*O*-methyl-α-D-gluco- and -galacto-pyranosid)uronates has been studied at 70 and 12 eV.[987] The production of ions from secondary and further processes was greatly diminished at 12 eV, so that the spectra were simpler and easier to interpret. The number and the positions of methyl groups in methyl (methyl *O*-acetyl-*O*-methylhexopyranosid)uronates can be determined by mass spectrometry, which, in combina-

[982] A. Glangetas, A. Buchs, and J. M. J. Tronchet, *Biomed. Mass Spectrometry*, 1976, **3**, 278 (*Chem. Abs.*, 1977, **87**, 23 619u).
[983] A. Glangetas, A. Buchs, and J. M. J. Tronchet, *Org. Mass Spectrometry*, 1977, **12**, 402.
[984] M. A. E. Shaban, V. N. Reinhold, and R. W. Jeanloz, *Carbohydrate Res.*, 1977, **59**, 213.
[985] G. P. Smirnova, N. V. Chekareva, O. S. Chizhov, B. M. Zolotarev, and N. K. Kochetkov, *Carbohydrate Res.*, 1977, **59**, 235.
[986] H. Rauvala and J. Kärkkäinen, *Carbohydrate Res.*, 1977, **56**, 1.
[987] V. Kováčik, V. Mihálov, and P. Kováč, *Carbohydrate Res.*, 1977, **54**, 23.

tion with g.l.c., is particularly suitable for analysing the uronic acid portion of methanolysates of methylated biopolymers and other substances containing hexuronic acids. Per-*O*-TMS derivatives of hexuronic acids and their lactones have also been examined by g.l.c.–mass spectrometry.[590] Reports have also appeared on the mass spectrometry of the following: permethylated aldo- and pseudoaldobiouronic acids,[988] acetylated aldononitriles of *O*-methyl-D-glucoses,[989] the TMS derivatives of aldosulose and dialdose methyl oximes,[990] gentamicins C_1, C_{1a}, and C_2 (using e.i., f.d., and c.i. techniques),[991] the TMS derivatives of flavonoid *O*-glycosides,[992] peracetylated daunorubicin,[993] di-, tri-, and tetra-nucleotides,[994] and underivatized di-(2′-deoxyribonucleoside) monophosphates.[995]

X-Ray and Neutron-diffraction Crystallography

Neutron-diffraction studies on the crystal structures of three sugars with related configurations and conformations, namely methyl α-D-gluco-, α-D-manno-, and α-D-altro-pyranosides, have revealed an unusually systematic relationship between the hydrogen-bond lengths and the type of O—H ··· O interactions.[996] Five different types of interaction between donor hydroxy-groups and acceptor oxygen atoms were distinguished in the crystal structures: namely, (i) that in which a hydroxy-group is both a hydrogen-bond acceptor and a hydrogen-bond donor (this is the shortest hydrogen-bond and this type of bonding is responsible for the frequent occurrence of chains of hydrogen-bonds); (ii) that in which a hydroxy-group serves only as a hydrogen-bond donor; (iii) hydrogen-bonds from hydroxy-groups to the ring- or glycosidic oxygen atom; (iv) bifurcated hydrogen-bonds (which are relatively rare); (v) non-bonding interactions that indicate some specific H ··· O interactions not necessarily directional enough to be designated as bonds. The structural consequences of the co-operative strengthening of hydrogen-bonds was discussed in an accompanying paper.[997] These theoretical studies led to additional structural predictions regarding the role of the anomeric oxygen atom in intermolecular hydrogen-bonding—namely, that both α- and β-pyranoses should exhibit similar H ··· O distances when serving as proton-donors to a given type of acceptor, and that the anomeric oxygen atom should be a relatively poor proton-acceptor. The latter prediction is consistent with the fact that in twelve simple pentoses and hexoses for which *X*-ray or neutron-diffraction data are available, the anomeric oxygen atom serves as a proton-acceptor in only three of them, and the hydrogen-bonds are weak ($r_{H\cdots O} > 2.0$ Å) in these cases. The foregoing theoretical observations were used to interpret the hydrogen-bond geometries for two

[988] V. Kováčik, V. Mihálov, T. Higuchi, J. Hirsch, and P. Kováč, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 223.
[989] F. R. Seymour, M. E. Slodki, R. D. Plattner, and A. Jeanes, *Carbohydrate Res.*, 1977, **53**, 153.
[990] M. Dizdaroglu, D. Henneberg, C. von Sonntag, and M. N. Schuchmann, *Org. Mass Spectrometry*, 1977, **12**, 772.
[991] R. T. Parfitt, D. E. Games, M. Rossiter, M. S. Rogers, and A. Weston, *Biomed. Mass Spectrometry*, 1976, **3**, 232 (*Chem. Abs.*, 1977, **87**, 201 997a).
[992] H. Schels, H. D. Zinsmeister, and K. Pfleger, *Phytochemistry*, 1977, **16**, 1019.
[993] P. P. Roller, M. Sutphin, and A. A. Aszalos, *Biomed. Mass Spectrometry*, 1976, **3**, 166 (*Chem. Abs.*, 1977, **86**, 121 688q).
[994] D. R. Burgard, S. P. Perone, and J. L. Wiebers, *Biochemistry*, 1977, **16**, 1051.
[995] J. L. Wiebers and J. A. Shapiro, *Biochemistry*, 1977, **16**, 1044.
[996] G. A. Jeffrey, M. E. Gress, and S. Takagi, *J. Amer. Chem. Soc.*, 1977, **99**, 609.
[997] Y.-C. Tse and M. D. Newton, *J. Amer. Chem. Soc.*, 1977, **99**, 611.

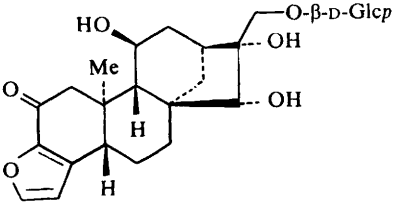
crystal-structure determinations of α-D-galactopyranose (see Vol. 10, p. 188), which, although in agreement regarding the positions of the carbon and oxygen atoms, displayed large differences in the positions assigned to hydroxylic hydrogen atoms.[998] The system of hydrogen-bonding suggested for one of the crystal structures agreed better with theoretical predictions than the other, although final resolution of this question must await a neutron-diffraction study on α-D-galactopyranose. An analysis of the hydrogen-bonding in the crystal structures of amino-acids, peptides, and oligosaccharides has appeared.[999]

The following crystal structures have been determined by *X*-ray or neutron-diffraction (n.d.) analysis or both during the past year.

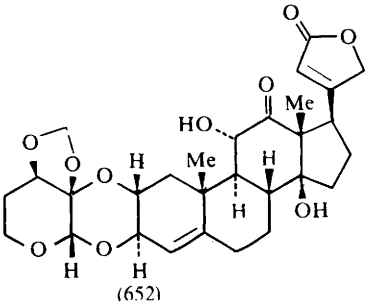
Free Sugars and Simple Derivatives Thereof.—β-L-Arabinopyranose (n.d.),[1000] 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranose,[1001] 1,2,3,4-tetra-*O*-benzoyl-β-D-xylopyranose,[1002] 1,2,3,4,6-penta-*O*-acetyl-α-D-gulopyranose,[1003] α-D-talopyranose (two independent determinations),[1004] α-DL-fucopyranose,[1005] β-D-fructopyranose (by *X*-ray[1006] and n.d.[1007]), β-maltose (n.d.),[1008] β-cellotriose undeca-acetate,[1009] and a 3:2-mixture of laminarabiose and 3-*O*-α-D-glucopyranosyl-β-D-glucopyranose.[1010]

Glycosides and their Derivatives.—Methyl β-D-xylopyranoside (n.d.),[1000] methyl β-D-galactopyranoside,[1011] methyl α-D-glucopyranoside (n.d.),[1012] methyl α-D-mannopyranoside (n.d.),[1012] methyl 2,3:4,5-di-*O*-isopropylidene-α-D-glucoseptanoside (n.d.),[1013] methyl β-D-glucopyranoside,[1014] methyl 3,4-dideoxy-3-(salicylidene)amino-α-D-*erythro*-pentopyranoside,[1015] methyl 3,4-di-*O*-acetyl-6-deoxy-6-iodo-2-*O*-toluene-*p*-sulphonyl-α-D-mannopyranoside,[1016] mascaroside (651), a new bitter constituent from coffee beans,[1017] D-glucosylphytosphingosine hydrochloride,[1018] and elaeodendroside A (652), a novel cytotoxic cardiac glycoside containing a doubly-linked sugar substituent.[1019]

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(651)



(652)

Amino- and Anhydro-sugar Derivatives.—3-Amino-1,6-anhydro-3-deoxy-β-D-glucopyranose (n.d.)[1020] and its hydrochloride,[1021] methyl 3,4-di-*O*-acetyl-2,6-anhydro-β-D-talopyranoside,[1022] and methyl 3-acetamido-2,3,6-trideoxy-3-*C*,4-*O*-dimethyl-β-L-*arabino*-hexopyranoside (383).[545]

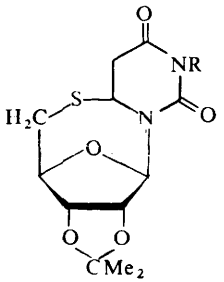
Acid Derivatives.—2,2[1]:5,6-Di-*O*-isopropylidene-2-*C*-hydroxymethyl-L-gulono-1,4-lactone,[1023] *N*-(2-chloroethyl)-D-gluconamide (n.d.),[1024] 5-azido-5-deoxy-*NN*-diethyl-1,2-*O*-isopropylidene-3-*O*-methanesulphonyl-β-L-iodofuranuronamide,[1025] 3,6-anhydro-7-(4-bromobenzoyl)-2-deoxy-4,5-*O*-isopropylidene-D-*altro*-heptononitrile,[1026] sodium D-isoascorbate,[1027] and reductic acid (2,3-dihydroxycyclopent-2-enone).[1028].

Cyclitols, Alditols, and other Acyclic Derivatives.—*epi*-Inositol.$SrCl_2$,[1029] DL-mannitol,[1030] 1,3,4,6-tetra-*O*-acetyl-2,5-*O*-methylene-D-mannitol,[1031] D-mannitol 1,2:3,4:5,6-tris(phenylboronate),[1032] 1-(1-*S*-ethyl-1-thio-D-arabinitol-1-yl)-5-fluorouracil and its 2′,3′,4′,5′-tetra-acetate,[1033] 4,5,6-tri-*O*-benzoyl-2,3-di-*S*-ethyl-

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2,3-dithio-D-allose diethyl dithioacetal,[1034] 6-chloro-9-(1-*S*-ethyl-1-thio-D-glucitol-1-yl)purine,[1035] 2,5-di-*O*-acetyl-1,4:3,6-dianhydro-1,6-dithio-D-iditol (*R*,*R*)-disulphoxide,[1036] and 4-*O*-β-D-galactopyranosyl-L-rhamnitol.[1037]

Nucleosides, Nucleotides, and their Derivatives.—3′,5′-Di-*O*-acetyluridine,[1038] 5-*O*-acetyl-1-(2-deoxy-α-D-*erythro*-pentofuranosyl)uracil,[1039] 2-thiouridine,[1040] 4-deoxy-3-deazauridine,[1041] 5-nitrouridine,[1042] 2′,3′-*O*-isopropylideneuridine,[1043] 2′,3′-*O*-(*S*)-methoxymethyleneuridine,[1044] the cyclonucleosides (653; R = H and Me),[1043] 2′-*O*-acetyluridine 3′,5′-(benzyl phosphate),[1045, 1045a] (uridine 5′-phosphate)(2,2′-dipyridylamine)copper(II).[1046]



(653) R = H or Me

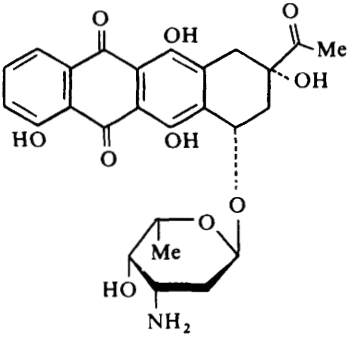
2′-*O*-Methylcytidine,[1047] 3-deazacytidine,[1048] 1-β-D-arabinofuranosylcytosine 2′,5′-phosphate,[1049] cytidine 5′-diphosphocholine(sodium salt),[1050] thymidine 3′,5′-(dimethylphosphoramidate),[1051] 4-α-D-erythrofuranosyl-1-(4-tolyl)imidazoline-2-thione,[1052] and 2,2′-anhydro-1-β-D-arabinofuranosyl-2-hydroxy-4(1*H*)-pyridinone.[1053]

8-Aza-adenosine,[1054] 8-bromo-2′,3′-*O*-isopropylideneadenosine,[1055] a [bis-

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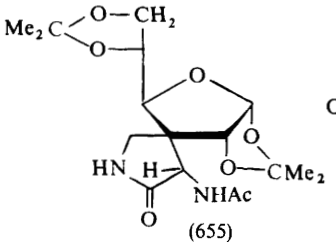
(acetylacetonato)(2′-deoxyadenosine)cobalt(III)] complex,[1056] a 2:2 intercalation [(adenosine 5′-phosphate)(chloroterpyridyl)platinum(II)] complex,[1056a] 2′-deoxy-6-thioguanosine,[1057] [(inosine 5′-phosphate)(2,2′-dipyridyl)copper(II)] nitrate,[1058] and 6,7-dimethyl-1-β-D-ribofuranosyl-lumazine.[1059]

Antibiotics.—Formycin B hydrochloride,[1060] carminomycin (654),[1061] the polyether antibiotic carriomycin,[1062] and antibiotic W-7783 (80) from *Polyangium cellulosum* var. *fulvum*.[230]

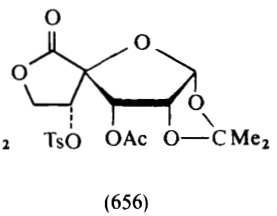


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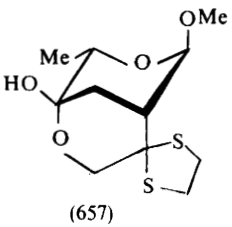
Other Derivatives.—3,4,6-Tri-*O*-acetyl-2-(*N*-acetylacetamido)-D-allal and -gulal,[1063] 2,3,4,6-tetra-*O*-benzoyl-2-chloro-α-D-mannopyranosyl chloride,[347] the 1-tosylate of (436),[609] spiro-3,4′-(*R*)-(3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranose)-3′-(*R*)-acetamido-2′-pyrrolidinone (655) and its 3′-(*S*)-diastereomer (see p. 91),[1064] the spiro-lactone (656),[1065] and methyl 2,3,6-trideoxy-2-*C*-[2-hydroxy-1,1-(ethylenedithio)ethyl]-α-L-*threo*-hexopyranosid-4-ulo-2[2],4-pyranose (657).[1065a]



(655)



(656)



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E.S.R. and Mössbauer Spectroscopy

E.s.r. spectroscopy has been used to study the interactions of Cu^{2+} cations with nucleosides in aqueous DMSO solutions at different pH values[620] (see p. 142). The ^{119}Sn Mössbauer spectra of several *O*-stannyl derivatives of mono-, di-, and oligosaccharides have been examined in connection with the impregnation of cellulosic materials with dilute solutions of the fungicide $(Bu_3Sn)_2O$.[614]

Polarimetry and Circular Dichroism

The application of g.l.c. and optical rotatory techniques enabled the values of $-106°$ ($\pm 10\%$) and $+64°$ ($\pm 10\%$) to be calculated for the probable specific rotations of α- and β-D-fructofuranose, respectively, in water.[54]

A symmetry rule has been derived relating the sign of the n–π* band of the carbonyl form of aldoses and ketoses to the absolute configuration of the sugar.[53] The sign of this c.d. band is positive when the adjacent chiral centre has the (*S*) configuration, whereas it is negative for the (*R*) configuration. The rule also holds for *aldehydo*- and *keto*-sugar peracetates and for 2-alkoxy-γ-lactones. The relationship between the proportion of the carbonyl form in aqueous solution and the structure and configuration of an aldose or a ketose was discussed. The mutarotation of D-glucose, D-fructose, and D-ribose has been studied by use of their c.d.[44] These kinetic studies support the view that a sugar mutarotates through *pseudo*-acyclic intermediates, which then give rise to either the acyclic form or another ring form.

Fragment spectra that may be summed algebraically to predict the vacuum- u.v. c.d. spectra of certain pyranoid monosaccharides (*e.g.* α- and β-L-arabinopyranose and methyl α-L-arabinopyranoside) have been presented.[1066] A limited number of these fragment spectra were used to calculate a large number of c.d. spectra that had been measured previously and to predict the c.d. spectra of other monosaccharides whose spectra have yet to be measured. The c.d. spectra of acyclic ketoses (*e.g.* D-*threo*- and D-*erythro*-pentulose) show two overlapping bands of opposite sign at *ca.* 310 and 270 nm which can be related to the configuration at chiral centres.[1067]

The c.d. spectra of 1-thio-α- and -β-D-glycofuranosides have been measured down to *ca.* 200 nm, and Drude calculations (based on o.r.d. measurements) were then used to obtain the sign, strength, and λ_{max} of c.d. bands below 200 nm.[1068] A tentative assignment of the second carbon–ring-oxygen band (λ_{max} 140—170 nm) associated the α-anomers with a positive c.d. band (or the β-anomers with a negative c.d. band) in this region. Studies of the chiroptical properties of 1-thio-D-glycopyranosides gave fundamentally the same result, and a ring-oxygen helicity rule was proposed to express the relationship between the sign of the c.d. band at λ_{max} 140–180 nm and the anomeric configuration.[1069]

El Khadem has developed a generalized c.d. rule that predicts the configuration at C-1 of alditol-1-yl derivatives of nitrogen heterocycles from the sign of the

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Cotton effect.[1070,1070a] The sign of the first band (*ca.* 270 nm) in the c.d. spectra of D-pentose phenylhydrazones is determined by the configuration at C-2.[452] The chiroptical properties of common nucleosides that had been oxidized with periodate and then reduced[1071] and those of cAMP and some 8-substituted adenosine derivatives[1072] have been examined. The c.d. spectra of such sugar–metal complexes as (444)[619] and (445)[621] have been measured.

The c.d. spectra of 2-acetamido-2-deoxyaldopyranos(id)es in 1,1,1,3,3,3-hexafluoropropan-2-ol solutions showed a positive band in the n–π* region (209 nm) in contrast to a negative c.d. band in aqueous solution.[1073] This difference was ascribed to a change in the average orientation of the hydroxy-group(s) adjacent to the amide group, and the c.d. spectra of methyl 2-acetamido-2-deoxy-3-*O*-methylaldopyranosides confirmed this interpretation. The c.d. spectrum of a disaccharide such as di-*N*-acetylchitobiose in 1,1,1,3,3,3-hexafluoropropan-2-ol solution reflects strongly the position, and sometimes the configuration, of the inter-sugar linkage. Far-u.v. c.d. spectra have been reported for 2-acetamido-2-deoxy-D-glucose and D-gluconic acid in aqueous solutions and for hyaluronic acid in aqueous solutions and in films.[1074] The features observed in the c.d. spectra were assigned to n–π* and π–π* transitions of the amide and carboxylic acid chromophores. The large negative rotational strength of the amide π–π* transition in films of sodium hyaluronate was attributed to decreased rotational freedom of the acetamido-groups when they participate in intramolecular hydrogen-bonding in the helical structure.

The absolute configurations of chiral 1-*O*-arylglycerols and related compounds have been assigned on the basis of their chiroptical properties in Cupra A solution,[1075] and chiroptical data have been reported for a series of 2-phenyl-1,3-dioxolan, -dioxan, and -dioxepan (*i.e.* *O*-benzylidene) derivatives of glycerol and D-mannitol.[1076] In the latter study, the signs of the Cotton effects of ^1L_b and ^1L_a transitions of the phenyl chromophore were rationalized in terms of recently proposed sector rules for these transitions.

The o.r.d. spectra of 2,3-*O*-acyl-D-tartaric acids and the corresponding tartaric anhydrides have been measured and assigned.[1077]

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Separatory and Analytical Methods

Chromatographic Methods

Gas–Liquid Chromatography.—Nanogram to picogram amounts of aldoses, ketoses, and deoxy-sugars, as their per-*O*-trifluoroacetates, can be analysed on glass-capillary columns using an electron-capture detector.[1078] D-Ribose can be freed from D(or L)-arabinose, ribitol, and other impurities by preparative g.l.c. of the TMS derivatives.[1079] Neutral sugars, alditols, and hexosamines in hydrolysates of reduced oligo- and poly-saccharides can be determined simultaneously, after conversion of the hexosamines into 2,5-anhydroaldoses with nitrous acid, by g.l.c. of suitable derivatives.[1080] This procedure was used to determine the DP of oligo- and poly-saccharides. G.l.c. data have been reported for methyl *O*-acetyl-*O*-methyl-α-D-mannofuranosides, *O*-acetyl-*O*-methyl-D-mannitols,[1081] the TMS derivatives of methyl glycosides formed during the methanolysis of glycoproteins,[1082] *O*-acetyl-*O*-methyl-D-gluconononitriles,[1083] acetylated menthyl esters of enantiomeric lactic and glyceric acids,[1084] and the TMS derivatives of muramic acid,[1085] aldosulose and dialdose methyl oximes,[990] sucrose (on an open tubular column),[1086] and several disaccharide oximes.[1087] Whereas the TMS derivatives of some disaccharide oximes (*e.g.* maltose and lactose oximes) gave a single main peak, those of others (*e.g.* cellobiose oxime) gave two or three peaks, presumably due to the presence of isomeric forms of the oximes.

Ion-exchange Chromatography.—Nanomole to micromole amounts of neutral mono- and oligo-saccharides (DP $\leqslant$ 15) can be separated as borate complexes of the sugars on basic ion-exchange resins.[1088, 1089] Mixtures of sugars and alditols (obtained by simultaneous isomerization and hydrogenation of D-glucose) have also been analysed by anion-exchange chromatography using an eluant containing boric acid, sodium borate, sodium chloride, and acetic acid.[1090, 1091] The presence

[1078] G. Eklund, B. Josefsson, and C. Roos, *J. Chromatog.*, 1977, **142**, 575.
[1079] V. M. Svetlaeva, L. M. Kirshchenya, and M. Ts. Yanotovskii, *Khim.-Farm. Zhur.*, 1977, **11**, 132 (*Chem. Abs.*, 1977, **86**, 190 368x).
[1080] R. Varma and R. S. Varma, *J. Chromatog.*, 1977, **139**, 303.
[1081] G. R. Woolard and E. B. Rathbone, *S. African J. Chem.*, 1977, **30**, 69 (*Chem. Abs.*, 1977, **87**, 202 008r).
[1082] D. G. Pritchard and C. W. Todd, *J. Chromatog.*, 1977, **133**, 133.
[1083] D. Anderle and P. Kovàč, *Chem. Zvesti*, 1976, **30**, 355 (*Chem. Abs.*, 1977, **87**, 136 225f).
[1084] J. P. Kamerling, G. J. Gerwig, J. F. G. Vliegenthart, M. Duran, D. Ketting, and S. K. Wadman, *J. Chromatog.*, 1977, **143**, 117.
[1085] D. J. Casagrande and K. Park, *J. Chromatog.*, 1977, **135**, 208.
[1086] D. Nurok and T. J. Reardon, *Carbohydrate Res.*, 1977, **56**, 165.
[1087] T. Toba and S. Adachi, *J. Chromatog.*, 1977, **135**, 411.
[1088] H. Bauer and W. Voelter, *Chromatographia*, 1976, **9**, 433 (*Chem. Abs.*, 1977, **86**, 30 016c).
[1089] J. F. Kennedy and J. E. Fox, *Carbohydrate Res.*, 1977, **54**, 13.
[1090] L. A. Th. Verhaar and J. M. H. Dirkx, *Carbohydrate Res.*, 1977, **59**, 1.
[1091] L. A. Th. Verhaar and J. M. H. Dirkx, *Carbohydrate Res.*, 1977, **53**, 247.

of acetic acid influenced the order in which the components were eluted, while the presence of sodium chloride decreased the elution time.

A review of ligand-exchange chromatography—where sorption takes place through the formation of co-ordination bonds—has discussed its use in the separation of amino-sugars on Cu^{2+} forms of cation-exchange resins.[1092] Amino-acid analysers have been employed in separations of amino-acids, hexosamines, and hexosaminitols (obtained by hydrolysis of borohydride-treated peptidoglycans),[1093] of hexosaminitols derived from oligosaccharides containing *N*-acetylhexosamines,[1094] and of anomeric 2-amino-2-deoxy-D-glyco-furanosides and -pyranosides (using cation-exchange chromatography).[1095] Ion-exchange chromatography has also been used in separations of oligosaccharides and oligosaccharides containing a terminal aldonic acid residue,[1096] of naturally occurring adenine nucleosides and nucleotides,[1097] of purines and pyrimidines and corresponding nucleosides and nucleotides,[1098] and of the products obtained on reduction of the antibiotic spectinomycin.[1099]

Thin-layer Chromatography.—Reducing sugars, particularly aldoses, reacted with dansyl hydrazine to give fluorescent hydrazones that can be separated by t.l.c. and, after elution, determined by spectrofluorometry.[1100] Vanadium pentoxide in dilute sulphuric acid has been recommended as a non-specific reagent for the detection of sugars on t.l.c. plates (or paper chromatograms).[1101] *In situ* acid and enzymic hydrolyses on thin layers of silica gel have been used in determining the compositions and structures of plant oligosaccharides,[1102] and the solvent system ethyl acetate–pyridine–water–acetic acid–propionic acid (50:50:10:5:5 v/v) gave quite speedy separations of mono- and oligo-saccharides on thin layers of cellulose.[1103] Mixtures of (2,4-di- and 2,4,5-tri-chloro)phenoxyacetic esters of sugars have been separated by t.l.c. on silica gel; the isomeric monoesters can be separated on thin layers of silica gel impregnated with boric acid.[1104] Chromatography on thin layers of cellulose gave good separations of the disaccharides released on digestion of various chondroitin sulphates with chondroitinases,[1105] and malto-oligosaccharides (DP $\leqslant$ 10) have been separated on thin layers of silica gel over a temperature range 22—70 °C using mixtures of acetone, ethanol, propan-2-ol, and water as the eluant.[1106] The [^{14}C]dansylated derivative of adenosine 3′,5′-phosphate can be determined by scintillation counting after separation by t.l.c. on silica gel,[1107] and nucleosides and their 5′-phosphates

[1092] V. A. Davankov and A. Y. Semechkin, *J. Chromatog.*, 1977, **141**, 313.
[1093] O. Hadžija and D. Keglević, *J. Chromatog.*, 1977, **138**, 458.
[1094] A. S. R. Donald, *J. Chromatog.*, 1977, **134**, 199.
[1095] S. Hirano and M. Ishigami, *Carbohydrate Res.*, 1977, **54**, 139.
[1096] K. Larsson and O. Samuelson, *J. Chromatog.*, 1977, **134**, 195.
[1097] J. Arendes, R. K. Zahn, and W. E. G. Müller, *J. Chromatog.*, 1977, **140**, 118.
[1098] K. Szyfter and J. Chelkowski, *Chem. Anal. (Poland)*, 1976, **21**, 1267 (*Chem. Abs.*, 1977, **87**, 136 229k).
[1099] J. C. Knight, *J. Chromatog.*, 1977, **136**, 432.
[1100] G. Avigad, *J. Chromatog.*, 1977, **139**, 343.
[1101] K. M. Haldorsen, *J. Chromatog.*, 1977, **134**, 467.
[1102] A. Lombard, M. L. Tourn, and M. Buffa, *J. Chromatog.*, 1977, **134**, 242.
[1103] J. W. Walkley and J. Tillman, *J. Chromatog.*, 1977, **132**, 172.
[1104] J. Arct and Z. Eckstein, *Chem. Anal. (Poland)*, 1976, **21**, 1297 (*Chem. Abs.*, 1977, **87**, 136 196x).
[1105] L. Wasserman, A. Ber, and D. Allalouf, *J. Chromatog.*, 1977, **136**, 342.
[1106] D. Nurok and A. Zlatkis, *J. Chromatog.*, 1977, **142**, 449.
[1107] N. N. Osborne and V. Neuhoff, *J. Chromatog.*, 1977, **134**, 489.

were revealed as dark-blue spots on silica-gel plates following chlorination of the heterocyclic bases and reaction of the chlorinated products with *o*-toluidine.[1108]

T.l.c. has been used to assay cladinose and methyl cladinoside in the presence of other acid-degradation products of erythromycin oxime or of erythromycylamine,[1109] to separate various acylated and other derivatives of erythromycin,[1110, 1111] and, in combination with bioautography, to classify antibiotics displaying antitumour activity.[1112]

High-performance Liquid Chromatography.—A differential u.v.-detector operating at 192 nm was appreciably more sensitive than a differential refractometer for detecting monosaccharides during h.p.l.c., although its sensitivity decreases for di- and tri-saccharides, *etc.*[1113] H.p.l.c. has been used in separations of mixtures of D-fructose, D-glucose, sucrose, maltose, and lactose (*i.e.* foodstuff components),[1114] alditol and mono-, di-, and tri-saccharide 4-nitrobenzoates,[1115, 1116] iridoid and secoiridoid D-glucopyranosides,[1117] flavonoid *O*- and *C*-glycosides,[1118] and oestrogen D-glucuronides and their sulphates and phosphates.[1119]

Among other uses reported for h.p.l.c. were the following: the separation of ribo- and 2′-deoxyribo-nucleosides,[1120] the selective assay of adenosine in the presence of other nucleic-acid components,[1121] and the separation of heterocyclic bases, nucleosides, and nucleoside mono- and poly-phosphates.[1122–1124] H.p.l.c. has also been used to monitor nucleotide pools at the nanogram level during the biosynthesis of polysaccharides by four representative species of Ascomycetes.[1125]

Electrophoresis

Thin-layer electrophoresis on silica-gel plates in the presence of borate–phosphate buffers has provided an efficient means for separating adenosine 3′,5′-phosphate and its metabolites prior to their assay.[1126]

Other Analytical Methods

A modification, utilizing mutarotase, of an enzymic colorimetric system for determining D-glucose with D-glucose oxidase, peroxidase, and 2,2′-diazobis-(3-

[1108] H. J. Issaq and E. W. Barr, *J. Chromatog.*, 1977, **132**, 121.
[1109] T. Lazarevski, Z. Tamburašev, and S. Djokić, *J. Chromatog.*, 1977, **132**, 309.
[1110] G. Kobrehel, Z. Tamburašev, and S. Djokic, *J. Chromatog.*, 1977, **133**, 415.
[1111] A. Vilim, M. J. LeBelle, W. L. Wilson, and K. C. Graham, *J. Chromatog.*, 1977, **133**, 239.
[1112] H. J. Issaq, E. W. Barr, T. Wei, C. Meyers, and A. Aszalos, *J. Chromatog.*, 1977, **133**, 291.
[1113] J. Hettinger and R. E. Majors, *Varian Instrum. Appl.*, 1976, **10**, 6 (*Chem. Abs.*, 1977, **86**, 182 647x).
[1114] A. D. Jones, I. W. Burns, S. G. Sellings, and J. A. Cox, *J. Chromatog.*, 1977, **144**, 169.
[1115] R. Schwarzenbach, *J. Chromatog.*, 1977, **140**, 304.
[1116] F. Nachtmann and K. W. Budna, *J. Chromatog.*, 1977, **136**, 279.
[1117] B. Meier and O. Stichter, *J. Chromatog.*, 1977, **138**, 453.
[1118] H. Becker and G. Wilking, *J. Chromatog.*, 1977, **136**, 174.
[1119] Sj. van der Wal and J. F. K. Huber, *J. Chromatog.*, 1977, **135**, 305.
[1120] H.-J. Breter, G. Seibert, and R. K. Zahn, *J. Chromatog.*, 1977, **140**, 251.
[1121] R. A. Hartwick and P. R. Brown, *J. Chromatog.*, 1977, **143**, 383.
[1122] A. Floridi, C. A. Palmerini, and C. Fini, *J. Chromatog.*, 1977, **138**, 203.
[1123] Y. Kato, T. Seita, T. Hashimoto, and A. Shimizu, *J. Chromatog.*, 1977, **134**, 204.
[1123a] H. G. Schneider and A. J. Glazko, *J. Chromatog.*, 1977, **139**, 370.
[1124] J. Borák and M. Smrž, *J. Chromatog.*, 1977, **133**, 127.
[1125] C. Y. Ko, H. M. McNair, and J. R. Vercellotti, *Carbohydrate Res.*, 1977, **58**, 453.
[1126] A. C. Fisher and V. Stefanovich, *J. Chromatog.*, 1977, **137**, 240.

ethylbenzothiazoline-6-sulphonic acid) was satisfactory for the assay of the anomers of D-glucopyranose released by enzymic hydrolysis of D-glucopyranosides.[1102] D-Glucose oxidase, catalase, and a polarographic oxygen electrode have been used for the assay of D-glucose in mixtures containing D-fructose and sucrose.[1127] Once the D-glucose content of the mixture is known, the amounts of D-fructose and sucrose can be determined from the total reducing-sugar content and by measuring the amount of D-glucose liberated by invertase, respectively.

The proportion of D-galactose in mixtures containing the L-enantiomer can be determined by ^{1}H n.m.r. spectroscopy following the conversion of D-galactose into *meso-galacto*-hexodialdose with D-galactose oxidase in the presence of oxygen.[1128] The magnitude of the aldehydrol-proton signals of the oxidized product relative to those of the anomeric-proton signals provided the fractional content of D-galactose in the mixture.

A comparison of colorimetric, enzymic, and paper-chromatographic techniques used for assaying the carbohydrate contents of natural waters indicated that enzymic techniques are superior when specific enzymes are available.[1129] Aldoses can be determined spectrophotometrically following their reaction with hydrazinium sulphate and treatment of the resulting hydrazones with fluorescamine,[1130] or following their reactions with diazotized sulphanilic acid,[1131] 4-aminobenzoic acid–thiourea,[1132] 2,3,5-triphenyltetrazolium bromide,[1133] and 2,6-dimethylaniline (pentoses and hexuronic acids, unlike hexoses and alditols, gave pink chromophores having λ_{max} 520 nm).[1134, 1135]

Oxidation of nanomole amounts of *O*-substituted 2-acetamido-2-deoxy-D-galactitol and -glucitol, reduction of the resulting aldehydes with sodium borotritiide, and hydrolysis with acid afforded 2-amino-2-deoxy[ω-^{3}H]alditols, which can be separated by t.l.c. and detected by autoradiography.[639] Identification of the 2-amino-2-deoxy[ω-^{3}H]alditols determined the positions of substitution of the 2-acetamido-2-deoxyhexitols. A simple, rapid, and sensitive method for the simultaneous assay of free and ketosidically bonded sialic acids is noted[413] in Chapter 8.

[1127] H.-Y. Sung and J.-C. Su, *Chung-Kuo Nung Yeh Hua Hsueh Hui Chih*, 1977, **15**, 1 (*Chem. Abs.*, 1977, **87**, 168 302v).

[1128] J. N. C. Whyte and J. R. Englar, *Carbohydrate Res.*, 1977, **57**, 273.

[1129] A. Geller, *Arch. Hydrobiol., Suppl.*, 1975, **47**, 295 (*Chem. Abs.*, 1977, **86**, 145 477w).

[1130] G. Avigad, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 307.

[1131] K. N. Bagdasarov, Z. P. Nebovidova, and V. D. Ponomarev, *Fiz.-khim. Metody Anal. Kontrolya Proizvod.*, 1975, 96 (*Chem. Abs.*, 1977, **87**, 126 664j).

[1132] A. Nanba and Y. Matsuo, *Hiroshima Daigaku Kogakubu Kenkyu Hokoku*, 1977, **25**, 47 (*Chem. Abs.*, 1977, **87**, 184 808v).

[1133] S. G. Verenikina, A. S. Guseva, L. G. Kuznetsova, V. M. Svetlaeva, and M. Ts. Yanotovskii, *Khim. Farm Zhur.*, 1977, **11**, 135 (*Chem. Abs.*, 1977, **87**, 157 251b).

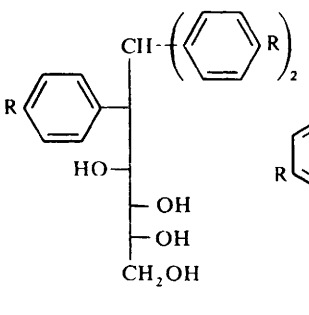
[1134] A. M. Desmaison and J. L. Beneytout, *Bull. Soc. Pharm. (France)*, 1976, **115**, 104 (*Chem. Abs.*, 1977, **87**, 201 927c).

[1135] A. M. Desmaison and J. L. Beneytout, *Bull. Soc. Pharm. (France)*, 1976, **115**, 69 (*Chem. Abs.*, 1977, **87**, 33 285w).

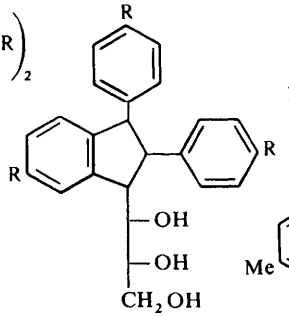
23

The Synthesis of Optically Active Non-carbohydrate Compounds

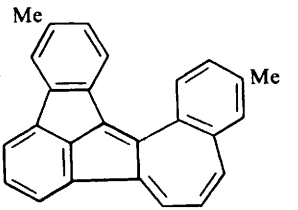
A brief summary has appeared on the use of carbohydrate precursors for the synthesis of chiral acyclic, heterocyclic, and carbocyclic compounds.[1136] The reaction of monosaccharides (*e.g.* D-mannose) with benzene or toluene in liquid hydrogen fluoride yielded 1,1,2-triaryl-1,2-dideoxyalditols [*e.g.* (658)] that were further transformed into condensed aromatic hydrocarbons [*e.g.* (659) and purpuricene (660)].[1137]



(658) R = H or Me



(659) R = H or Me

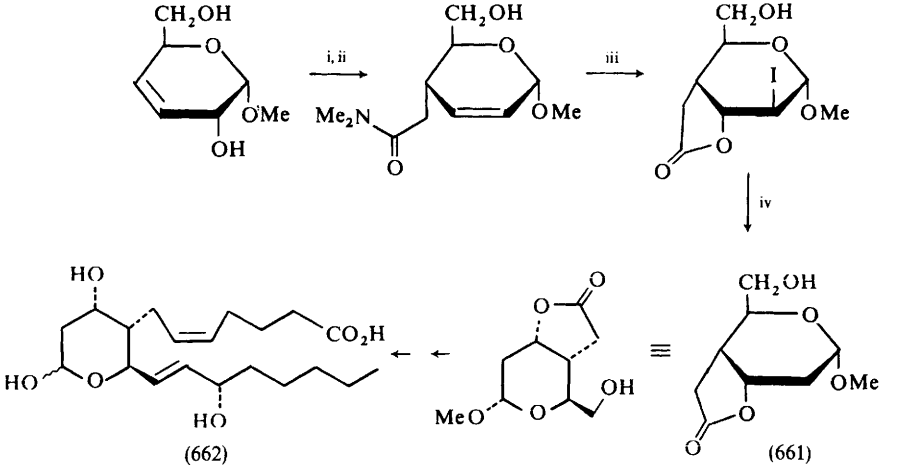


(660)

Two groups have used D-glucose for the stereocontrolled synthesis of the hydroxylactone (661)[1138] or its 6-*O*-(t-butyldiphenylsilyl) derivative (663),[1139] which can be converted into thromboxane B_2 (662) by procedures already described. The later stages of syntheses of (661) and (663) are outlined in Schemes 99 and 100. (−)-Isoavenaciolide (664), an antifungal metabolite, has been synthesized by the route illustrated in Scheme 101.[1140]

A biomimetic synthesis of (+)-biotin methyl ester (665), involving stereospecific ring-closure to a tetrahydrothiophen system during the final stage, is shown in

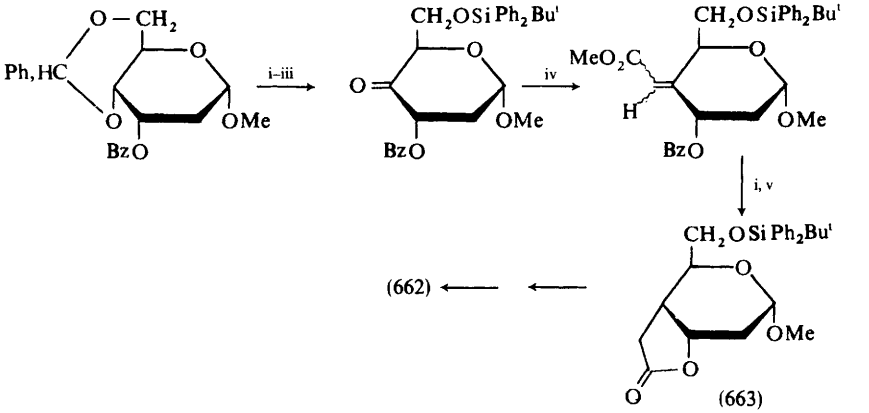
[1136] S. Rakhit, *Chem. Canad.*, 1977, **29**, 8 (*Chem. Abs.*, 1977, **87**, 184 781f).
[1137] G. Louis, H. Knauel, F. Micheel, and M. Pesenacker, *Amer. Chem. Soc. Symposium Ser.*, 1976, **39** (Synthetic Methods for Carbohydrates Symposium), 227 (*Chem. Abs.*, 1977, **87**, 68 541d).
[1138] E. J. Corey, M. Shibasaki, and J. Knolle, *Tetrahedron Letters*, 1977, 1625.
[1139] S. Hanessian and P. Lavallee, *Canad. J. Chem.*, 1977, **55**, 562.
[1140] R. C. Anderson and B. Fraser-Reid, *Tetrahedron Letters*, 1977, 2865.



Reagents: i, $MeC(OMe)_2NMe_2$–diglyme–heat; ii, K_2CO_3–MeOH; iii, I_2–THF–H_2O; iv, Bu_3SnH

Scheme 99

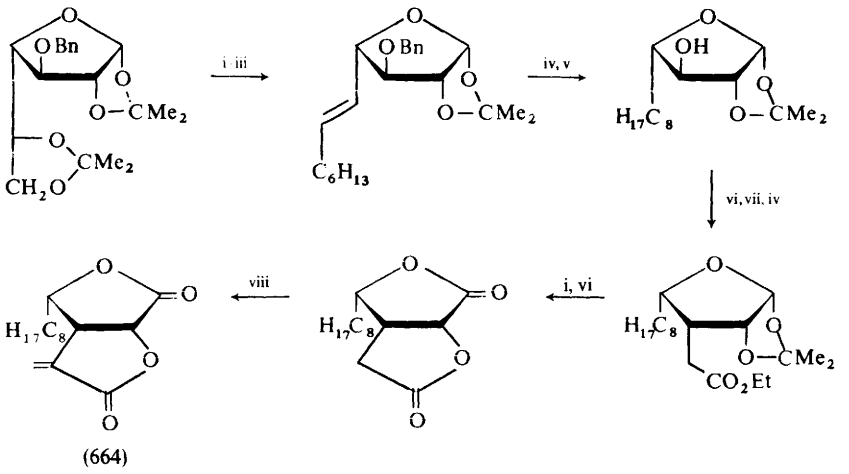
Scheme 102[1141] (see also Vol. 9, p. 201). The pheromones (−)-frontalin (666) and (−)-*exo*-brevicomine (667) of species of bark and pine beetles have been synthesized from D-glucose, as shown in Scheme 103,[1142] or from diethyl (2*R*,3*R*)-tartrate,[1143] respectively.



Reagents: i, $Pd(OH)_2$–C–H_2; ii, Bu^tPh_2SiCl–py; iii, DMSO–$Me_2N(CH_2)_3N{=}C{=}NEt$–$pyH^+CF_3CO_2^-$; iv, $Me_3P{=}CHCO_2Me$; v, K_2CO_3–MeOH

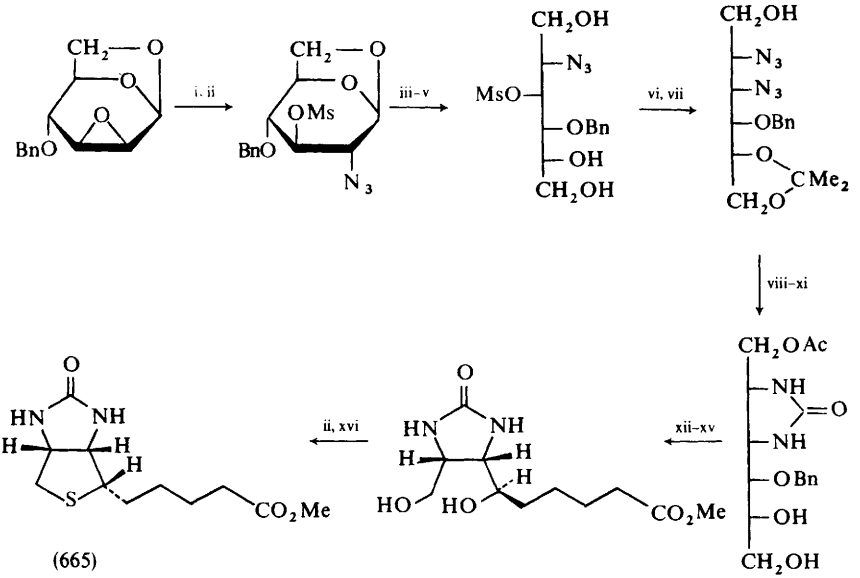
Scheme 100

[1141] T. Ogawa, T. Kawano, and M. Matsui, *Carbohydrate Res.*, 1977, **57**, C31.
[1142] H. Ohrui and S. Emoto, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 2267.
[1143] H. H. Meyer, *Annalen*, 1977, 732.



Reagents: i, H_3O^+; ii, $NaIO_4$; iii, $Ph_3P{=}CHC_6H_{13}$; iv, H_2–Ni; v, H_2–Pd; vi, CrO_3–Me_2CO; vii, $Ph_3P{=}CHCO_2Et$; viii, Vol. 9, p. 201.

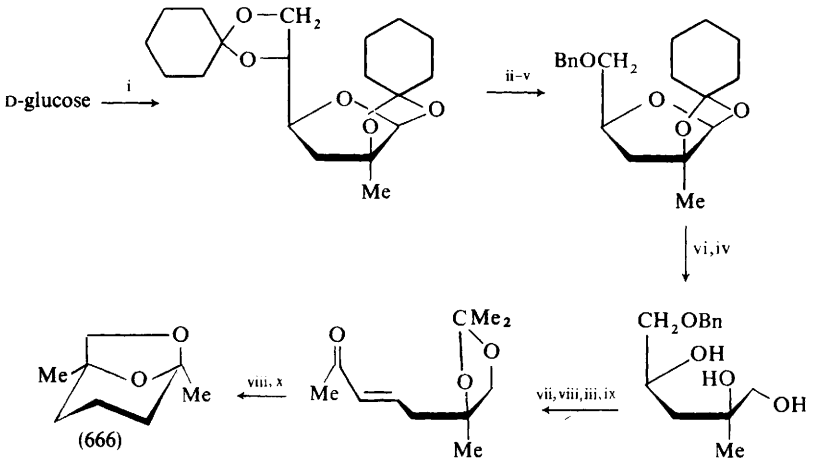
Scheme 101



Reagents: i, NaN_3–NH_4Cl; ii, MsCl–py; iii, Ac_2O–$BF_3 \cdot Et_2O$; iv, MeOH–HCl; v, $NaBH_4$; vi, $Me_2C(OMe)_2$–DMF–H^+; vii, LiN_3–DMF; viii, H_2–Lindlar catalyst; ix, $COCl_2$–CCl_4; x, Ac_2O–py; xi, 80% AcOH; xii, $NaIO_4$; xiii, $Ph_3P{=}CHCH{=}CHCO_2Me$–CH_2Cl_2; xiv, H_2–Pd–C; xv, MeONa; xvi, Na_2S–DMF.

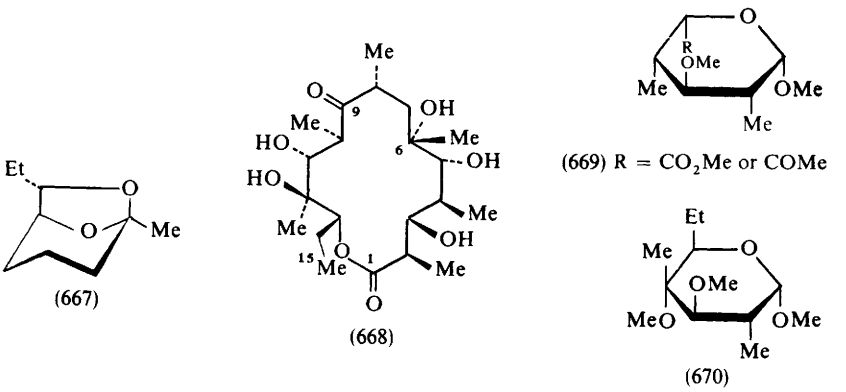
Scheme 102

Recognition that the chiral centres at 1–6 and 9–15 of the macrolide erythronolide A (668) have the L-*ido* and D-*gluco* configurations, respectively, has prompted the synthesis of the branched-chain sugars (669) and (670), which between them contain eight of the ten chiral centres present in erythronolide A.[1144] Both (669) and (670) were derived from D-glucose using essentially standard procedures for the systematic and stereocontrolled introduction of the various functional groups.



Reagents: i, Vol. 9, p. 50; ii, AcOH; iii, $NaIO_4$; iv, $NaBH_4$; v, BnCl–KOH; vi, H_3O^+; vii, Me_2CO–H^+; viii, H_2–Pd–C; ix, $Ph_3P{=}CHCOMe$; x, TsOH–H_2O–Et_2O

Scheme 103



[1144] S. Hanessian and G. Rancourt, *Canad. J. Chem.*, 1977, **55**, 1111.

Part II

MACROMOLECULES

1
Introduction

The objectives and the format of Part II remain the same as those of previous Reports.

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2
General Methods

BY R. J. STURGEON

Analysis

Carbohydrate components of cells have been visualized for light or electron microscopy by means of a lectin–peroxidase affinity technique.[1] Electron-dense markers of a size suitable for transmission or scanning electron microscopy have been prepared using gold granules labelled with a monolayer of specific macromolecules.[2] This technique was used to locate the polysaccharides and glycoproteins in yeast cell walls. Histochemical methodology for the oxidation of polysaccharides with periodic acid in anhydrous DMSO and in aqueous DMSO has been reported.[3] Periodic acid in anhydrous DMSO readily induced a strong Schiff's reaction with a variety of polysaccharide-containing structures, but not with glycogen. Glycogen was oxidized only when water was added to the DMSO, but the addition of water did not impair the selective location of other polysaccharides.

Trimethylsilylation of glass-fibre filter paper has overcome the problem of endo-osmotic flow in the electrophoresis of pectins, lignin–carbohydrate complexes, and other macromolecules from plant cell walls.[4]

4-Hydrazinoacridine has been used as a fluorescent stain for the detection of proteins and glycoproteins on electrophoretograms.[5] The yellow fluorescence of glycoproteins distinguished them from other proteins, which fluoresce less intensely at a different wavelength. The migration of glycopeptides of molecular weight 1.9—3.3 $\times$ 10^3 on thin-layer gel filtration using Sephadex G50 in 6M-guanidine did not differ significantly from that of peptides in the same molecular-weight range, indicating that peptides can be used as calibration standards in the estimation of the molecular weights of glycoproteins in this molecular-weight range.[6]

Molecular species of higher glycolipids have been separated by h.p.l.c. of their *O*-acetyl-*N*-(4-nitrobenzoyl) derivatives on a reversed-phase column.[7] The advantages of using *O*-acetyl-*N*-(4-nitrobenzoyl) derivatives, rather than benzoyl derivatives, were discussed. Gangliosides and neutral glycosphingolipids have been separated by h.p.l.c. using silica gel as the stationary phase.[8] Derivatization of the glyco-

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[2] M. Horisberger and J. Rosset, *J. Histochem. Cytochem.*, 1977, **25**, 295.
[3] G. I. Malinin, *J. Histochem. Cytochem.*, 1977, **25**, 188.
[4] M. C. Jarvis, D. R. Threlfall, and J. Friend, *Phytochemistry*, 1977, **16**, 849.
[5] S. D. Carson, *Analyt. Biochem.*, 1977, **78**, 428.
[6] C. H. Hung, D. K. Strickland, and B. G. Hudson, *Analyt. Biochem.*, 1977, **80**, 91.
[7] A. Suzuki, S. Handa, and T. Yamakawa, *J. Biochem. (Japan)*, 1977, **82**, 1185.
[8] U. R. Tjaden, J. H. Krol, R. P. van Hoeven, E. P. M. Oomen-Meulemans, and P. Emmelhot, *J. Chromatog.*, 1977, **136**, 233.

lipids is unnecessary if a universal detector is used. Gangliosides have been separated by high-pressure thin-layer chromatography (h.p.t.l.c.) using selected solvents of high resolving-power.[9] An improved method for separating the principal phospholipids and glycolipids from plant extracts has been reported.[10]

Malto-oligosaccharides have been separated by h.p.t.l.c. at temperatures of 22—70 °C.[11] Cello-oligosaccharides (up to cellohexaose) have been separated by two h.p.l.c. systems, which also separated them from their borohydride-reduced derivatives.[12] Numerous partially and fully substituted carbohydrates have been separated by h.p.l.c. on two types of gel-permeation columns.[13] The relative retention times depend on the relative polarities and molecular weights of the carbohydrates. The separation by h.p.l.c. and the determination of the 4-nitrobenzoates of some mono-, di-, and tri-saccharides have been reported.[14] These relatively apolar derivatives are rapidly separated on such polar absorbents as silica gel and alumina. Careful control of the support loading has been found to improve the separation of some oligo- and mono-saccharides by h.p.l.c.[15] Neutral oligosaccharides and oligosaccharides containing a terminal aldonic acid residue have been separated simultaneously by anion-exchange chromatography.[16] Relative g.l.c. retention times have been recorded for the TMS derivatives of ten disaccharide oximes.[17] Maltose, gentiobiose, turanose, and lactose oximes gave single peaks, but the other disaccharide oximes exhibited multiple peaks owing to the presence of isomeric forms of the oximes. Commercially available t.l.c. plates coated with cellulose, which required no pre-treatment, have been used in separations of mono-, di-, and tri-saccharides.[18] A fully automated ion-exchange and gel-permeation chromatographic system has been devised for the routine identification and determination of neutral mono-, di-, and oligo-saccharides.[19] The preparation [using *N*-methylbis(trifluoroacetamide)] and g.l.c. of highly volatile trifluoroacetylated saccharides have been described.[20] Mono-, di-, tri-, and tetra-saccharides could be analysed in a single run using chromatographic conditions less severe than those of other methods.

Amperometric measurement, with an oxygen electrode, of the rate of depletion of oxygen has been used in enzyme-linked determinations of lactose[21] and maltose.[22] Following enzymic hydrolysis of the disaccharide with the appropriate glycosidase, the liberated D-glucose was oxidized to D-gluconic acid with D-glucose oxidase in the presence of molecular oxygen.

A new colorimetric reaction for the determination of reducing sugars is based on

[9] J. P. Zanetta, F. Vitiello, and J. Robert, *J. Chromatog.*, 1977, **137**, 481.
[10] M. U. Khan and J. P. Williams, *J. Chromatog.*, 1977, **140**, 179.
[11] D. Nurok and A. Zlatkis, *J. Chromatog.*, 1977, **142**, 449.
[12] E. K. Gum and R. D. Brown, *Analyt. Biochem.*, 1977, **82**, 372.
[13] G. D. McGinnis and P. E. Fang, *J. Chromatog.*, 1977, **130**, 181.
[14] F. Nachtmann and K. W. Budna, *J. Chromatog.*, 1977, **136**, 279.
[15] A. D. Jones, I. W. Burns, S. G. Sellings, and J. A. Cox, *J. Chromatog.*, 1977, **144**, 169.
[16] K. Larsson and O. Samuelson, *J. Chromatog.*, 1977, **134**, 195.
[17] T. Toba and S. Adachi, *J. Chromatog.*, 1977, **135**, 411.
[18] J. W. Walkley and J. Tillman, *J. Chromatog.*, 1977, **132**, 172.
[19] J. F. Kennedy and J. E. Fox, *Carbohydrate Res.*, 1977, **54**, 13.
[20] J. E. Sullivan and L. R. Schewe, *J. Chromatog. Sci.*, 1977, **15**, 196.
[21] F. S. Cheng and G. D. Christian, *Analyst*, 1977, **102**, 124.
[22] L. B. Marshall, G. D. Christian, and A. Kumar, *Analyst*, 1977, **102**, 424.

measurement of the chromogen 'Acid Blue', which is produced when alkaline solutions of 1,5-dihydroxy-4,8-dinitroanthraquinone-2,6-disulphonic acid are heated with D-glucose, D-fructose, or D-galactose.[23] Addition of sodium bismuth tartrate to the alkaline solution of 4-hydroxybenzoic acid hydrazide used in the determination of reducing sugars (see Vol. 10, p. 211) enabled the reaction to be carried out more rapidly at a lower temperature and with greater sensitivity.[24] Almost quantitative yields of the coloured products were obtained, and the method was adapted for use in continuous-flow analysis. Aldoses (*e.g.* D-glucose) have been converted into the corresponding hydrazones, which gave quantitative yields of fluorescent products on treatment with fluorescamine [4-phenyl-spiro-furan-2-(3*H*),1′-phthalan-3,3′-dione].[25] This method was used in the assay of aldoses in glycoside hydrolysates, but it cannot be applied to hydrolysates containing primary amines or proteins, which react rapidly with fluorescamine. Reducing sugars, particularly aldoses, react with dansylhydrazine to give fluorescent hydrazones, which can be separated by t.l.c. and then determined spectrofluorometrically after elution from the chromatogram.[26] A continuous-flow system has been devised to improve the separation of common sugars on plates coated with silica gel.[27] A modified method has been described for the detection of urinary aldoses on paper chromatograms with aniline citrate.[28] The reaction between sodium periodate and several mono- and di-saccharides has been monitored using a perchlorate-selective electrode as a periodate-sensor.[29] The relative oxidation rate constant of each saccharide compared with D-glucose is introduced as a new characteristic constant, the 'periodate index', which could be related to the molecular configuration. Since appreciable differences are found between the values of the 'periodate index' for various saccharides, this constant can be used for the identification of 20 mg amounts of single, pure compounds. The monosaccharide compositions of various types of macromolecule isolated from cultured animal cells have been analysed by radiochromatographic techniques following either enzymic or acidic hydrolysis and reduction of the monosaccharides with sodium borotritide.[30] The method was used in the compositional analysis of mucopolysaccharides, glycoproteins, and glycolipids.

Rapid and complete separation of mixtures of D-glucose, D-fructose, and D-mannose has been achieved by ion-exchange chromatography.[31]

Analytical procedures used in the routine analysis of glycoproteins often involve methanolysis and subsequent *N*-acetylation of the liberated amino-sugars. However, it has been demonstrated that partial *O*-acetylation of alditols (commonly employed as internal standards) accompanies the *N*-acetylation of amino-sugars.[32] It was also shown that a hot neutral solution of methyl chloride in methanol releases L-fucose, sialic acid, and some D-galactose residues from α_1-acid

[23] R. Soloniewicz and M. Teodorczyk, *Z. analyt. Chem.*, 1977, **283**, 304.
[24] M. Lever, *Analyt. Biochem.*, 1977, **81**, 21.
[25] G. Avigad, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 307.
[26] G. Avigad, *J. Chromatog.*, 1977, **139**, 343.
[27] D. S. Bailey, *J. Chromatog.*, 1977, **130**, 431.
[28] V. Vitek and K. Vitek, *J. Chromatog.*, 1977, **143**, 65.
[29] C. E. Efstathiou and T. P. Hadjiioannou, *Analyt. Chim. Acta*, 1977, **89**, 56.
[30] D. E. Roll and H. E. Conrad, *Analyt. Biochem.*, 1977, **77**, 397.
[31] L. A. T. Verhaar and J. M. H. Dirkx, *Carbohydrate Res.*, 1977, **53**, 247.
[32] D. G. Pritchard and C. W. Todd, *J. Chromatog.*, 1977, **133**, 133.

glycoprotein. Neutral sugars and amino-sugars have been released from glycoproteins and glycosaminoglycans by resin-catalysed hydrolysis.[33,34] Deamination of the resin-bound amino-sugars with nitrous acid gave 1,5-anhydroaldoses, which, together with other neutral sugars, were determined by g.l.c. of the aldononitrile acetates. Trifluoroacetylated monosaccharides have been separated by g.l.c. on glass capillary columns.[35] Methyl β-D-glucopyranoside has been used as an internal standard in the g.l.c. of monosaccharide alditol acetates.[36]

Interest has continued in the modification of specific enzymic methods for the determination of D-glucose. The deliberations of a workshop conference on the methodology and clinical applications of continuous *in vivo* analysis of D-glucose have been published.[37] Serum D-glucose has been measured by the hexokinase and colorimetric procedures and, in conjunction with oxygen-electrode techniques, by D-glucose oxidase.[38] Evaluation of these methods showed that each is accurate and suitable for routine use in clinical laboratories. D-Glucose oxidase physically entrapped between membranes has been used in the construction of an enzyme-membrane sandwich reactor for the assay of D-glucose with an autoanalyser.[39] The levels of D-glucose in sera have been determined with a D-glucose oxidase–peroxidase system in which 4-aminoantipyrine and *NN*-dimethylaniline were used to produce the chromogen.[40] Reproducible and more sensitive values were obtained with this method than with the *o*-dianisidine method. An electrochemical enzyme sensor has been developed for the determination of D-glucose, which is converted into D-gluconic acid with D-glucose oxidase in the presence of hexacyanoferrate(III) as an electron acceptor.[41] The hexacyanoferrate(II) formed is re-oxidized at a platinum electrode and this reaction is monitored amperometrically. The influence of different sample characteristics on the measurement was investigated and optimized so that the method could be used for the determination of D-glucose in microbiological media. D-Glucose in polysaccharide hydrolysates has been measured following its oxidation to D-gluconic acid with immobilized D-glucose oxidase and peroxidase.[42] Hydrogen peroxide was removed by oxidation of I^- ions to I_3^- ions. A system for measuring the anomeric forms of D-glucose uses D-glucose oxidase, peroxidase, mutarotase, and 2,2′-diazobis-(3-ethylbenzothiazoline-6-sulphonic acid).[43] Significant differences in the levels of D-glucose in whole blood or plasma have been noted when the *o*-toluidine procedure was used in the presence, or in the absence, of protein.[44] D-Glucose oxidase attached to the inner wall of autoanalyser tubing has been used in the analysis of D-glucose with a continuous-flow system operating at high sampling-rates.[45]

Manual and mechanized versions of several types of analyser for the kinetic

[33] R. Varma and R. S. Varma, *J. Chromatog.*, 1976, **128**, 45.
[34] R. Varma and R. S. Varma, *J. Chromatog.*, 1977, **139**, 303.
[35] G. Eklund, B. Josefsson, and C. Roos, *J. Chromatog.*, 1977, **142**, 575.
[36] I. Kusakabe, Y. Kamiyama, and T. Yasui, *J. Agric. Chem. Soc. Japan*, 1977, **51**, 167.
[37] C. Trendelenburg, *J. Clin. Chem. Clin. Biochem.*, 1977, **15**, 31.
[38] T. R. Koch and H. C. Nipper, *Clinica Chim. Acta*, 1977, **78**, 315.
[39] J. Campbell, A. S. Chawla, and T. M. S. Chang, *Analyt. Biochem.*, 1977, **83**, 330.
[40] M. Sugiura and K. Hirano, *Clinica Chim. Acta*, 1977, **75**, 387.
[41] J. R. Mor and R. Guarnaccia, *Analyt. Biochem.*, 1977, **79**, 319.
[42] P. Cremonesi, R. Bovara, B. Focher, and L. d'Angiuro, *Cellulose Chem. Technol.*, 1977, **11**, 341.
[43] J. Okuda, I. Miwa, K. Maeda, and K. Tokui, *Carbohydrate Res.*, 1977, **58**, 267.
[44] E. S. C. Quek and J. E. Buttery, *Clinica Chim. Acta*, 1977, **75**, 497.
[45] L. P. Léon, M. Sansur, L. R. Snyder, and C. Horvath, *Clinical Chem.*, 1977, **23**, 1556.

determination of D-glucose with D-glucose dehydrogenase have been described.[46] The results obtained with deproteinized samples did not differ significantly from those obtained with samples that were not deproteinized. The results were also correlated with those obtained with the hexokinase method. The D-glucose dehydrogenase method has been modified for use with an autoanalyser, permitting the quantities of the enzyme and the coenzyme to be reduced without affecting linearity.[47] The levels of D-glucose in plasma have been measured using D-glucose dehydrogenase.[48] The sensitivity of the method was improved by using an immobilized enzyme, and the limits of detection of D-glucose were lowered as interference from the fluorescent background—originating from the presence of enzymic protein in the reaction mixture—was completely abolished. The consumption of ATP in the hexokinase determination of D-glucose has been measured using the luciferin–luciferase assay system.[49]

Hydrogen peroxide formed during the oxidation of D-galactose with D-galactose oxidase reacted with homovanillic acid in the presence of a peroxidase to give a highly fluorescent product.[50] Measurement of D-galactose in blood by this fluorometric method avoids the non-specific fluorescence caused by endogenous NADH that is encountered with methods using D-galactose dehydrogenase. The ^{1}H n.m.r. spectrum (in deuterium oxide) of *meso-galacto*-hexo-1,5-dialdose, a sugar produced on oxidation of D-galactose with D-galactose oxidase in the presence of oxygen, has been used to determine D-galactose in a DL-mixture.[51] The magnitude of the aldehydrol-proton signals (which are obtained only from the oxidized D-enantiomer) relative to those of the anomeric protons gives the proportion of the D-enantiomer. In a coulometric determination of serum D-galactose, hydrogen peroxide formed during the oxidation of D-galactose with D-galactose oxidase reacts with iodide ion in the presence of a molybdenum catalyst to produce iodine, which is determined with thiosulphate.[52]

Ketohexoses (1–50mM) have been determined by measurement of the stable chromogens obtained when they react with urea and perchloric acid.[53] This method is highly specific for ketohexoses and such carbonyl compounds as pyruvic acid do not interfere. The levels of D-fructose in vesicular secretions, seminal plasma, and blood plasma have been measured by means of a fluorometric method that uses zirconyl chloride.[54]

L-Fucose liberated from oligosaccharides with α-L-fucosidase has been determined by measurement of the NADH formed following treatment with L-fucose dehydrogenase.[55]

D-Xylose ($\leqslant$0.4mM) has been measured by an enzymic method based on a

[46] H. Rindfrey, R. Helger, and H. Lang, *J. Clin. Chem. Clin. Biochem.*, 1977, **15**, 217.
[47] K. J. Kornmüller and O. Müller-Plathe, *J. Clin. Chem. Clin. Biochem.*, 1977, **15**, 603.
[48] J. W. Kuan, S. S. Kuan, and G. G. Guilbault, *Clinical Chem.*, 1977, **23**, 1058.
[49] B. Z. Cavari and G. Phelps, *Appl. Environ. Microbiol.*, 1977, **33**, 1237.
[50] G. A. Mason, G. K. Summer, H. H. Dutton, and R. C. Schwaner, *Clinical Chem.*, 1977, **23**, 971.
[51] J. N. C. Whyte and J. R. Englar, *Carbohydrate Res.*, 1977, **57**, 273.
[52] C. D. McGlothlin and W. C. Purdy, *Analyt. Chim. Acta*, 1976, **88**, 33.
[53] K. Tsutsui, T. Tanaka, and T. Oda, *Analyt. Biochem.*, 1977, **79**, 349.
[54] J. Schlegelová and K. J. Hruška, *Analyt. Biochem.*, 1977, **79**, 583.
[55] G. C. Tsay and G. Dawson, *Analyt. Biochem.*, 1977, **78**, 423.

coupled reaction of D-xylose isomerase with D-glucitol dehydrogenase (Scheme 1).[56] The method was also used in a kinetic study of β-D-xylosidase using D-xylobiose as a substrate.

$$\alpha\text{-D-xylose} \xrightarrow[\text{isomerase}]{\text{D-xylose}} \alpha\text{-D-xylulose}$$

$$\alpha\text{-D-xylulose} + NADH + H^+ \xrightarrow[\text{dehydrogenase}]{\text{D-glucitol}} \text{xylitol} + NAD^+$$

Scheme 1

The nature of the *N*-acyl group has been shown to affect the determination of 2-acylamino-2-deoxy-D-hexoses by the Reissig modification of the Morgan–Elson reaction.[57] Whereas colour formation by amino-sugars *N*-acylated with either benzoic acid or fatty acids containing 3–8 carbon atoms is identical to that obtained with 2-acetamido-2-deoxy-D-glucose, electronegative substituents on the aromatic nucleus inhibit colour formation to various extents. 2-Acetamido-2,3-dideoxy-D-*erythro*-hex-2-enofuranose has been synthesized and shown to be identical to Chromogen I produced from 2-acetamido-2-deoxy-D-glucose in the Morgan–Elson reaction.[58] Mercaptoethanol and other sulphydryl compounds inhibited the formation of chromogens from 2-acetamido-2-deoxy-D-glucose, but this inhibition is overcome by alkylation (with iodoacetamide) of the thiols.[59]

2-Amino-2-deoxyhexoses, 2-amino-2-deoxyhexitols, and di- and tri-saccharides containing 2-amino-2-deoxyhexose residues have been separated by cation-exchange chromatography using an amino-acid analyser.[60] Amino-sugars and neutral hexoses released from glycoproteins with trifluoroacetic acid, as well as the corresponding alditols, have been determined as the trifluoroacetyl derivatives.[61] Two new procedures based on the Elson–Morgan reaction have been used for the quantitative determination of 2-amino-2-deoxy-D-glucose and -galactose.[62] One of them was used to estimate the total amino-sugar content, since chromogens of equal intensity were formed with equal concentrations of 2-amino-2-deoxy-D-glucose and -galactose, while the other allowed 2-amino-2-deoxy-D-galactose to be determined without interference from 2-amino-2-deoxy-D-glucose. Cation-exchange chromatography has been used in the separation of anomeric 2-amino-2-deoxy-D-aldo-furanosides and -pyranosides.[63] Muramic acid has been determined as its TMS ether by g.l.c.[64]

[56] H. Kersters-Hilderson, E. van Doorslaer, C. K. de Bruyne, and K. Yamanaka, *Analyt. Biochem.*, 1977, **80**, 41.
[57] L. I. Glebko, G. Y. Ilchenko, M. G. Vafina, and N. V. Molodtsov, *Analyt. Biochem.*, 1977, **80**, 322.
[58] J. M. Beau, P. Rollin, and P. Sinaÿ, *Carbohydrate Res.*, 1977, **53**, 187.
[59] K. Zwierz, J. Bialobrzeska, and S. Kreczko, *Analyt. Biochem.*, 1977, **77**, 574.
[60] A. S. R. Donald, *J. Chromatog.*, 1977, **134**, 199.
[61] Y. Arakawa, T. Imanari, and Z. Tamura, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 2032.
[62] N. Blumenkrantz and G. Absoe-Hansen, *Clinical Biochem.*, 1976, **9**, 269.
[63] S. Hirano and M. Ishigami, *Carbohydrate Res.*, 1977, **54**, 139.
[64] D. J. Casagrande and K. Park, *J. Chromatog.*, 1977, **135**, 208.

The chemistry and analysis of sialic acids have been reviewed.[65,66] Picomole quantities of sialic acids have been measured by the fluorescence produced by the periodate-oxidized acid in the thiobarbituric acid reaction.[67] Contamination of the sialic acids with 2-deoxy-D-*erythro*-pentose (derived from cellular material) could be detected by a downfield shift of the excitation maximum. Fluorescent derivatives are also produced when free sialic acids react with pyridoxamine, a procedure that compares favourably with the thiobarbituric acid reaction for determining sialic acids.[68] Keto-acids (*e.g.* pyruvic acid) interfere with the determination, but 2-deoxy-D-*arabino*-hexose and 2-deoxy-D-*erythro*-pentose do not. A non-fading chromophore is produced when DMSO is used instead of n-butanol in the thiobarbituric acid assay for sialic acids.[69] A new histochemical method for the visualization and identification of unmodified or *O*-acylated sialic acids has been reported.[70]

Automated anion-exchange chromatography has been applied to the analysis of hexuronic acids.[71] Stable baselines were achieved by using a single eluting buffer, and the same analytical system could be used in determinations of neutral sugars. Anion-exchange chromatography has been used to separate D-glucuronic and D-gluconic acids, which were then determined by a spectrophotometric technique based on measurement of the hydroxamic acids formed on condensation of the acids with hydroxylamine in the presence of DCC.[72] The structures of the isomeric products obtained on trimethylsilylation of naturally occurring hexuronic acids and their sodium salts and lactones have been established by the application of ^{1}H n.m.r. spectroscopy (for the anomeric configuration) and m.s. (for the ring size).[73] The proportions of furanoid forms in the tautomeric equilibria were affected both by DMSO and hexamethyldisilazane.

Trioses and tetroses have been separated as their trimethylsilylated oximes by g.l.c.; mass-spectral data for these derivatives were also recorded.[74] Mixtures of xylitol, arabinitol, mannitol, glucitol, and maltitol have been separated efficiently by h.p.l.c. of the corresponding 4-nitrobenzoates.[75] Alditols have been determined in the presence of neutral sugars by oxidation with acidified sodium periodate and colorimetric measurement of the formaldehyde released.[76] G.l.c. retention data have been reported for the TMS derivatives of a large number of aldonic acids and their lactones and aldaric acids.[77]

Complex formation with Toluidine Blue 'O' has been used to determine the

[65] R. W. Ledeen and R. K. Yu, in 'Biological Roles of Sialic Acid', ed. A. Rosenberg and C. L. Schengrund, Plenum Press, New York, 1976, p. 1.
[66] R. Schauer, *Seikagaku*, 1977, **49**, 1120.
[67] K. S. Hammond and D. S. Papermaster, *Analyt. Biochem.*, 1976, **74**, 292.
[68] J. F. Murayama, M. Tomita, A. Tsuji, and A. Hamada, *Analyt. Biochem.*, 1976, **73**, 535.
[69] L. Skoza and S. Mohos, *Biochem. J.*, 1976, **159**, 457.
[70] C. F. A. Culling, P. E. Reid, and W. L. Dunn, *J. Histochem. Cytochem.*, 1976, **24**, 1225.
[71] M. J. Spiro, *Analyt. Biochem.*, 1977, **82**, 348.
[72] Y. Kasai, T. Tanimura, Z. Tamura, and Y. Ozawa, *Analyt. Chem.*, 1977, **49**, 655.
[73] J. F. Kennedy, S. M. Robertson, and M. Stacey, *Carbohydrate Res.*, 1977, **57**, 205.
[74] D. Anderle, J. Königstein, and V. Kováčik, *Analyt. Chem.*, 1977, **49**, 137.
[75] R. Schwarzenbach, *J. Chromatog.*, 1977, **140**, 304.
[76] S. H. Bok and A. L. Demain, *Analyt. Biochem.*, 1977, **81**, 18.
[77] G. Petersson, *J. Chromatog. Sci.*, 1977, **15**, 245.

sulphate released from mucopolysaccharides in the presence of phosphate and citrate ions.[78] Methods have been developed for the analysis of nucleotides, *etc.*, as an aid to studies of the biosynthesis of polysaccharides in fungi using h.p.l.c.[79]

The kinetics of the reactions of periodate ions with a number of vicinal glycols have been studied using a perchlorate ion-selective electrode as the periodate-ion sensor.[80] Periodate ions can be determined in the presence of iodate and other anions by reduction to iodate ions with leucothionine, which is generated *in situ* by a photochemical reaction between H_4edta and blue thionine.[81] Biamperometric curves, representing the time for reduction of the periodate ion *versus* the current generated in oxidizing the excess of leucothionine after the end-point, were used in the determination. A simple spectrofluorometric technique has been developed for the detection and measurement of hydroxy-radicals generated during the oxidation of carbohydrates with aqueous periodate solutions.[82]

Structural Methods

Polysaccharide methodology, with particular emphasis on such analytical techniques as g.l.c., m.s., and ^{1}H and ^{13}C n.m.r. spectroscopy and on fragmentation procedures, has been reviewed.[83]

G.l.c. of the corresponding peracetylated aldononitriles has permitted 2,3,4- and 2,3,6-tri-*O*-methyl-D-glucose to be separated for the first time.[84] Chemical-ionization mass-spectral data for most of the possible methylated hexitol acetates that can be derived from D-glucose, D-galactose, and D-mannose have been recorded.[85] Many arrangements of the *O*-methyl and *O*-acetyl groups can be distinguished on the basis of the chemical-ionization mass spectra.

Fully methylated aldobiouronic and pseudoaldobiouronic acids containing all possible types of linkage have been studied by chemical-ionization m.s. (using ammonia as the reagent gas).[86] Although the molecular weights and individual cycle masses of the fully methylated acids can be deduced, the spectra did not yield any information on the type of linkage between the monomers. A procedure that is particularly suitable for g.l.c.–m.s. of the uronic acid portion [*i.e.* methyl (methyl *O*-methylpyranosid)uronates] of methanolysates of methylated acidic polysaccharides has been reported.[87] The production of ions resulting from secondary and further processes is greatly diminished at 12 eV (*cf.* 70 eV) and the spectra are simpler and easier to interpret. A modified procedure has been reported for determination of the site(s) of attachment of hexuronic acid residues in polysaccharides.[88] In a single operation, the methylated polysaccharide is degraded with base (1M-sodium methylsulphinylmethanide in DMSO) and then, without work-up,

[78] H. B. Nader and C. P. Dietrich, *Analyt. Biochem.*, 1977, **78**, 112.
[79] C. Y. Ko, H. M. McNair, and J. R. Vercellotti, *Carbohydrate Res.*, 1977, **58**, 453.
[80] C. E. Efstathiou, T. P. Hadjiioannou, and E. McNelis, *Analyt. Chem.*, 1977, **49**, 410.
[81] F. Sierra, G. Sanchez-Pedreno, T. P. Ruiz, and C. Martinez Lozano, *Analyt. Chim. Acta*, 1977, **94**, 129.
[82] J. E. Scott and D. P. P. Thomas, *Carbohydrate Res.*, 1976, **52**, 214.
[83] G. O. Aspinall, in 'Carbohydrates', ed. G. O. Aspinall, MTP International Review of Science, Organic Chemistry, Series Two, Vol. 7, Butterworths, London, 1976, p. 201.
[84] F. R. Seymour, M. E. Slodki, R. D. Plattner, and A. Jeanes, *Carbohydrate Res.*, 1977, **53**, 153.
[85] M. McNeil and P. Albersheim, *Carbohydrate Res.*, 1977, **56**, 239.
[86] V. Kováčik, V. Mihálov, T. Higuchi, J. Hirsh, and P. Kováč, *J. Carbohydrates, Nucleosides, Nucleotides*, 1977, **4**, 223.
[87] V. Kováčik, V. Mihálov, and P. Kováč, *Carbohydrate Res.*, 1977, **54**, 23.
[88] G. O. Aspinall and K. G. Rosell, *Carbohydrate Res.*, 1977, **57**, C23.

alkylated with trideuteriomethyl or ethyl iodide to label the original site(s) of attachment of the uronic acid residues. The procedure avoids the isolation of the degraded polysaccharide and possible loss of acid-labile glycosyl substituents during subsequent selective acidic hydrolysis of unsaturated glycosyluronic acid residues. The modified polysaccharide is then hydrolysed with acid and the resulting alkylated sugars are analysed by g.l.c.–m.s. of the corresponding alditol acetates.

Linkage analysis of neuraminic acid residues in gangliosides has been achieved by g.l.c.–m.s. of the methylated methyl glycoside methyl esters[89] or the partially methylated TMS derivatives.[90] G.l.c. retention times and mass-spectral fragmentation patterns have been obtained for the TMS derivatives of 2-acetamido-2-deoxy-D-glucose, -galactose, and -mannose and various methyl glyco-pyranosides and -furanosides derived from them.[91] The mass spectra of the TMS derivatives of free and partially methylated hexosamines could be used to distinguish the sites of methylation and the size of the sugar ring. Monosaccharide sulphates have been converted into the corresponding acetylated methyl sulphates and chlorosulphates, which are sufficiently volatile for m.s.[92]

An established microchemical procedure for determining the DP of neutral oligo- and poly-saccharides has been used in studies on D-glucans.[93] The site of attachment of the rest of the chain to the reducing-end residue of an oligosaccharide or a polysaccharide (*e.g.* amylose or laminarin) can be established using the sequence of reduction, methylation (or ethylation), acidic hydrolysis, and g.l.c.–m.s. of the resulting alkylated alditol.[94]

The application of ^{13}C n.m.r. spectroscopy in structural studies of oligo- and poly-saccharides has been reviewed.[95,96] The products obtained on enzymic hydrolysis of agarose have been examined by ^{13}C n.m.r. spectroscopy, which enabled the average chain-length of the agaro-oligosaccharides to be estimated.[97] Four peracetylated disaccharides selectively enriched with ^{13}C at the C-1′ position and having α-D-(1′ → 3)-, β-D-(1′ → 3)-, α-D-(1′ → 4)-, and β-D-(1′ → 4)-linkages have been studied on the basis of their $^{3}J_{^{13}C,\,^{1}H}$ coupling constants (through the glycosidic bond) in relation to the conformations of the oligosaccharides in solution (as models for the corresponding polymer).[98] Values obtained for the torsional angle ψ were compared with *X*-ray crystallographic data for similar compounds. ^{1}H N.m.r. measurements have shown that $Eu(fod)_3$ binds preferentially to two neighbouring MeO-oxygens having an *ax-eq* relationship in permethylated disaccharides consisting of D-glucopyranosyl and D-galactopyranosyl residues in (1→2)-, (1→4)-, or (1→6)-linkage, despite the large number of possible binding sites.[99] The signals

[89] H. Rauvala and J. Kärkkäinen, *Carbohydrate Res.*, 1977, **56**, 1.
[90] J. Haverkamp, J. P. Kamerling, J. F. G. Vliegenthart, R. W. Veh, and R. Schauer, *F.E.B.S. Letters*, 1977, **73**, 215.
[91] P. L. Coduti and C. A. Bush, *Analyt. Biochem.*, 1977, **78**, 21.
[92] A. I. Kalenovsky, N. I. Belogortseva, S. V. Moroz, A. F. Pavlenko, A. K. Dzizenko, and Y. S. Ovodov, *Carbohydrate Res.*, 1977, **58**, 473.
[93] H. Yamaguchi and K. Makino, *J. Biochem. (Japan)*, 1977, **81**, 563.
[94] H. Yamaguchi and K. Okamoto, *J. Biochem. (Japan)*, 1977, **82**, 571.
[95] A. S. Shashkov and O S. Chizhov, *Bioorg. Khim.*, 1976, **2**, 437.
[96] A. S. Perlin, in ref. 83, p. 1.
[97] G. K. Hamer, S. S. Bhattacharjee, and W. Yaphe, *Carbohydrate Res.*, 1977, **54**, C7.
[98] E. Excoffier, D. Y. Gagnaire, and F. R. Taravel, *Carbohydrate Res.*,1977, **56**, 229.
[99] D. G. Streefkerk and A. M. Stephen, *Carbohydrate Res.*, 1977, **57**, 25.

for most of the methoxy-groups of the disaccharide derivatives could be assigned on the basis of $Eu(fod)_3$ effects and from comparisons of the chemical shifts of the corresponding groups in the permethylated mono- and di-saccharides.

C.d. spectra have been recorded for 2-acetamido-2-deoxy-D-hexoses and some of their *O*-methylated derivatives, various disaccharides containing 2-acetamido-2-deoxy-D-glucopyranose, chito-oligosaccharides, and 2-acetamido-1-*N*-β-L-aspartyl-2-deoxy-β-D-glucopyranose.[100] The spectra revealed that, in contrast to the $n \rightarrow \pi^*$ band, the $\pi \rightarrow \pi^*$ band reflects changes in the anomeric configurations and structures of the di- and oligo-saccharides. In particular, substitution at O-3 of an amino-sugar produced a negative c.d. at 190 nm, whereas substitution at O-4 produced a positive c.d. in this region. Differences between the c.d. spectra of disaccharides containing 2-acetamido-2-deoxy-D-hexosyl residues in water (negative band in the $n \rightarrow \pi^*$ region) and in 1,1,1,3,3,3-hexafluoropropan-2-ol (positive band in the $n \rightarrow \pi^*$ region) have been ascribed to a change in the average orientation of the hydroxy-groups adjacent to the amide group.[101]

The possible existence of multiple helices in various homopolysaccharides has been predicted by the calculation of conformational-energy contour maps: included among the structures studied were homopolymers of D-xylose, D-glucose, D-mannose, and D-galactose containing α- and β-(1→2)-, -(1→3)-, and -(1→4)-linkages.[102] A number of double parallel-stranded, double antiparallel-stranded, and triple parallel-stranded helices were predicted, all of them stabilized by inter-strand hydrogen-bonding. At least three of the predicted multiple helices are known to exist.

[100] P. L. Coduti, E. C. Gordon, and C. A. Bush, *Analyt. Biochem.*, 1977, **78**, 9.
[101] H. R. Dickinson, P. L. Coduti, and C. A. Bush, *Carbohydrate Res.*, 1977, **56**, 249.
[102] T. L. Bluhm and A. Sarko, *Carbohydrate Res.*, 1977, **54**, 125.

3

Plant and Algal Polysaccharides

BY R. J. STURGEON

Starch

Aspects of the structures and of the modes of biosynthesis of starch and glycogen have been reviewed.[1,2]

Evidence for a new unit cell of crystalline A-amylose, based on well-oriented *X*-ray fibre-diagrams, favours a double-helical arrangement of amylose chains.[3] A new method based on refinement of the structure with respect to either stereochemical constraints or *X*-ray diffraction intensities has been used in predicting and solving the crystal structures of such linear homopolysaccharides as V-amylose.[4] It appears that, contrary to six-fold molecular symmetry in the $P2_12_12_1$ space group suggested previously, the V-amylose molecule exhibits only two-fold symmetry with the asymmetric unit containing three D-glucopyranosyl residues in one half-turn of the helix. These residues are non-equivalent, owing principally to unequal rotational positions of the hydroxymethyl groups. The crystal structure of V-amylose predicted from stereochemical refinement is identical to that obtained on refinement of the *X*-ray data.

A Naegeli-type amylodextrin of DP 21 and containing one branch-point in each molecule has been prepared from waxy corn starch.[5] The adsorption of monobasic fatty acids from n-hexane on to dry amylose has been found to follow Langmuir's adsorption isotherm.[6] Changes in the physical properties of a potato starch with a high content of phosphate in the presence of cations have been described.[7] Alkali-metal cations produced an increase in the viscosity of solutions of this potato starch, whereas alkaline-earth-metal cations initiated a two-step swelling process and lowered the viscosity.

D-Glucose and malto-oligosaccharides (DP $\leqslant$ 9) in starch hydrolysates have been separated by h.p.l.c.[8] Oxidized (sodium hypochlorite) starch has been degraded by the joint actions of amyloglucosidase and α- and β-amylases.[9] An unidentified, modified trisaccharide, which resisted further enzymic degradation, was isolated.

Maltotriose, maltotetraose, and higher starch oligosaccharides are taken up by

[1] W. J. Whelan, *T.I.B.S.*, 1976, **1**, 292.
[2] W. J. Whelan, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 101.
[3] H. C. H. Wu and A. Sarko, *Carbohydrate Res.*, 1977, **54**, C3.
[4] P. Zugenmaier and A. Sarko, *Biopolymers*, 1976, **15**, 2121.
[5] S. Hizukuri, M. Kobayashi, and Y. Takeda, *J. Agric. Chem. Soc. Japan*, 1976, **50**, 573.
[6] H. Suzuki and K. Hasue, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 131.
[7] K. Kainuma, S. Miyamoto, S. Yoshioka, and S. Suzuki, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 59.
[8] K. Richter and H. U. Woelk, *Stärke*, 1977, **29**, 273.
[9] S. K. Fischer and F. Piller, *Stärke*, 1977, **29**, 262.

the gel phase of native starch granules in amounts in excess of those expected on the basis of their molecular size because they bind specifically to molecular chains of starch in the gel phase.[10] These chains constitute either inter-branch regions of branched starch molecules or segments of linear molecules in either the gel or amorphous phase, the segments being of sufficient length to form either a double helix or other association with the linear oligosaccharides.

Agarose-gel filtration has been used to estimate the molecular-weight distribution of native wheat starch and some oxidation products thereof.[11] Cations bound to the phosphate groups of potato starch have been extracted with mineral acids and then determined by atomic absorption spectroscopy.[12]

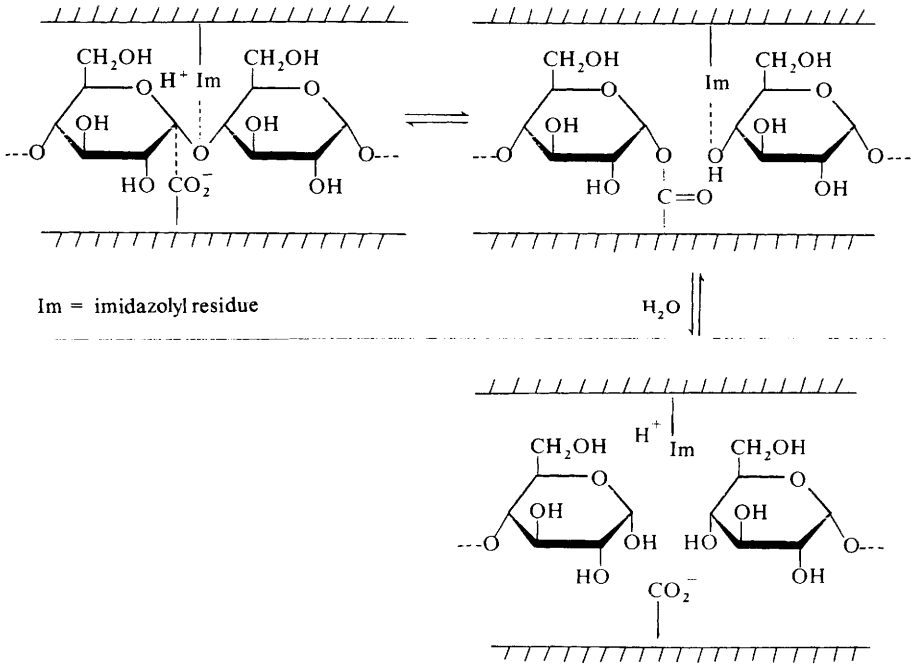
Soluble starch has been hydrolysed by passage in turn through columns of immobilized α-amylase and glucoamylase: the treatment with α-amylase is required to produce an effective increase in the concentration of the substrate for glucoamylase.[13] The DP of soluble starch acted on by immobilized amyloglucosidase has been shown to affect the rate of hydrolysis to D-glucose.[14] Differences exist between immobilized and soluble amyloglucosidases with regard to the dependence of the rate of hydrolysis on the DP of starch. The hydrolysis of starch in the presence of a co-polymer of vinyl alcohol and vinyl sulphonic acid has been shown to follow Michaelis–Menten kinetics.[15] Poly(vinyl alcohol) acted as a competitive inhibitor of the hydrolysis.

Dextrins obtained on γ-irradiation of starch have been found to contain appreciable proportions of unidentified carbonyl residues.[16] The properties of starch for starch-gel electrophoresis were improved by γ-irradiation.[17] The decreases in viscosity and iodine-absorption values, as well as the increased reducing properties, indicated that amylose and amylopectin are degraded by γ-irradiation in the same way as native starches.[18] Although each of the amylose and amylopectin components of amylomaize starch in γ-irradiated and untreated samples was hydrolysed by α-amylase to the same extent, γ-irradiated amylose had a low β-amylolysis limit. γ-Irradiated amylomaize and corn starches were hydrolysed to greater extents by α-amylase as the radiation dose was increased,[19] and differences were detected in the products of enzymic degradation of amylomaize and corn starches following γ-radiolysis.[20] The water-soluble dextrins formed on γ-irradiation of maize starch have been examined.[21,22]

Theoretical models for the action of α-amylase on amylose have indicated that multiple attack is of little or no significance in the action patterns of α-amylases, with the exception of those from the pancreas.[23] Six α-limit dextrins containing a

[10] S. A. Brown and D. French, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 215.
[11] H. Bruun and H. Henriksnäs, *Stärke*, 1977, **29**, 122.
[12] S. Miyamoto, C. Tsutsumi, and K. Kainuma, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 91.
[13] T. Handa, S. Goto, T. Akino, T. Mizukura, and Y. Ayukawa, *J. Jap. Soc. Starch Sci.*, 1977, **24**, 1.
[14] J. Kučera, *Coll. Czech. Chem. Comm.*, 1976, **41**, 2978.
[15] K. Arai, Y. Ogiwara, and N. Ise, *Makromol. Chem.*, 1975, **176**, 2871.
[16] J. P. Michel, M. Rigouard, G. Berger, and L. Saint-Lèbe, *Stärke*, 1977, **29**, 254.
[17] R. M. A. El Saadany, F. M. El Saadany, and Y. H. Foda, *Stärke*, 1977, **29**, 157.
[18] Y. Watanabe, Y. Ayano, and T. Obara, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 77.
[19] Y. Watanabe, K. Tanaka, F. Ohta, Y. Ayano, and T. Obara, *J. Jap. Soc. Starch Sci.*, 1977, **24**, 9.
[20] Y. Watanabe, Y. Ayano, and T. Obara, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 137.
[21] G. Berger, J. P. Agnel, and L. Saint-Lèbe, *Stärke*, 1977, **29**, 40.
[22] G. Berger, J. F. Dauphin, J. P. Michel, G. Enrico, J. P. Agnel, F. Seguin, and L. Saint Lèbe, *Stärke*, 1977, **29**, 80.

single (1 → 6)-α-D-glucosidic linkage, in addition to (1 → 4)-α-D-glucosidic linkages, were isolated from the products of the so-called 'second stage' of the hydrolysis of amylopectin by salivary α-amylase.[24] The structures of the limit dextrins furnished information about those (1 → 4)-α-D-glucosidic linkages of amylopectin that are cleaved by salivary α-amylase and those that are not. A mechanism (Scheme 1)



Scheme 1

proposed for the action of amylases on the α-D-glucosidic linkages of starch is based on the identification of carboxylate and imidazole groups as the functional groups at the active sites of *Asperigillus awamori* glucoamylase and α-amylase, *A. oryzae* α-amylase, and malted barley α- and β-amylases.[25] In this mechanism, the inductive effects of the oxygen atoms of the (1 → 4)-α-D-glucosidic linkage and the pyranose ring are considered to promote nucleophilic attack at the C-1—O linkage by the carboxylate–imidazole system.

Earlier reports indicating that starch within intact spinach chloroplasts is degraded both by the Embden–Meyerhof pathway and by a pathway involving amylolytic cleavage have been confirmed.[26]

The number and length of the outer chains of amylopectin have been altered by partial debranching with pullulanase and re-synthesis with potato phosphorylase.[27]

[23] W. Banks and C. T. Greenwood, *Carbohydrate Res.*, 1977, **57**, 301.
[24] M. Abdullah, W. J. Whelan, and B. J. Catley, *Carbohydrate Res.*, 1977, **57**, 281.
[25] N. A. Zherebtsov, L. F. Zabelina, and A. I. Éktona, *Biochemistry (U.S.S.R.)*, 1976, **41**, 1723.
[26] D. G. Peavey, M. Steup, and M. Gibbs, *Plant Physiol.*, 1977, **60**, 305.
[27] B. Pfannemüller and C. Potratz, *Stärke*, 1977, **29**, 73.

Star-shaped molecules possessing similar properties have also been obtained by the action of muscle phosphorylase on intact amylopectin. Both the synthesis and the degradation of starch are regulated by the level of inorganic phosphate.[28] The highest rates of starch synthesis by spinach chloroplasts in the light occurred at < 0.25 mmole of phosphate, whereas a ten-fold higher concentration of phosphate is required for the breakdown of starch in the dark.

Cellulose

The proceedings of a conference on the chemistry and biochemistry of cellulose have been published.[29] The changes in linen and cotton celluloses induced by γ-radiation have been studied by e.s.r. spectroscopy.[30] The number of carboxy- and carbonyl-groups introduced into these celluloses increased with increasing dosage, and their chains were cleaved to the same extent. The photochemical degradation of cellulose has also been studied by e.s.r. spectroscopy, and mechanisms were advanced to account for each type of intermediate radical observed.[31] Localization of the energy absorbed by cellulose is considered to produce free radicals by chain-scission, dehydrogenation, dehydroxylation, and dehydroxymethylation. Related studies have been carried out with wood, rayon, and amorphous celluloses and absorbent cotton.[32] The line-shapes and intensities of the e.s.r. signals from photodegraded cellulose depend largely on the degree of crystallinity, the type of lattice, and the arrangement of the molecules. The effects of the degree of crystallinity and the amount of sensitizer (Fe^{3+} ions) absorbed indicated that photochemical reactions occur exclusively in the amorphous regions of cellulose.

DMSO containing paraformaldehyde is an excellent non-degrading solvent for celluloses of DP16–8000, which can be regenerated from the solution by the addition of either water or methanol.[33] The formation of methylolcellulose (a hemiacetal derivative) is crucial for the dissolution of cellulose in this solvent system, which ^{1}H n.m.r. measurements showed to contain several oxymethylene derivatives, principally the dimer and the trimer, and significant amounts of methylene glycol and formaldehyde. Some properties of methylolcellulose in DMSO have been reported, and the solutions appear to be suitable for light-scattering, sedimentation, and osmometric measurements.[34]

Quantitative birefringence measurements on wood-pulp fibres have indicated that lignin is randomly oriented in the wood and the wood-pulp, whereas cellulose is highly oriented, and that the hemicellulose chains lie parallel to the cellulose fibres.[35]

Delignification of *Eucalyptus saligna* and *E. globolus* wood meals, without severe depolymerization of cellulose, has been achieved with oxygenated alkali in the presence of low concentrations of sodium hydrogencarbonate.[36] Manganese

[28] M. Steup, D. G. Peavey, and M. Gibbs, *Biochem. Biophys. Res. Comm.*, 1976, **72**, 1554.
[29] Proceedings of the 8th Cellulose Conference, Applied Polymer Symposia, ed. T. E. Timell, 1976, Vol. 28.
[30] W. M. Dziedziela and J. Rosiak, *Cellulose Chem. Technol.*, 1977, **11**, 261.
[31] N. S. Hon, *J. Polymer Sci., Polymer Chem.*, 1976, **14**, 2497.
[32] N. S. Hon, *J. Polymer Sci., Polymer Chem.*, 1976, **14**, 2513.
[33] D. C. Johnson, M. D. Nicholson, and F. C. Haigh, in ref. 29, p. 931.
[34] H. A. Swenson, in ref. 29, p. 945.
[35] D. H. Page, F. El-Hosseiny, M. L. Bidmade, and R. Binet, in ref. 29, p. 923.
[36] D. Pal and O. Samuelson, *Svensk Papperstidn.*, 1976, **79**, 531.

salts and traces of copper salts retarded the depolymerization of cellulose in this delignification system.[37] Changes in the DP of cellulose in DMF brought about by nitrogen tetroxide have been studied.[38]

Freeze etching of stelar tissue in *Zea mays* roots has demonstrated the association of globular complexes with the ends of nascent microfibrils.[39] The results support a model for the synthesis of cellulose in which an enzyme complex in the granule adds D-glucopyranosyl residues to the developing end of a microfibril. The ultrastructure of the cellulosic network of the primary cell wall of pine wood and its development during the formation of tracheids have been studied by electron microscopy.[40]

Fractionation of the cellulose from the alga *Valonia* afforded a component of weight-average DP 4.4×10^4, which is thought to determine the size of the template required for the synthesis of the polysaccharide.[41] The synthesis of cellulose II growing at right-angles to the axis of the microfibril. An alkali-resistant chain structure. Cellulose I crystallizes after it is detached from the site of synthesis.

Cellulose possessing a 'shishkebab' microfibrillar morphology has been isolated following slow deacetylation of cellulose acetate of low DP in the presence of *Valonia* cellulose microfibrils.[42] Each 'shishkebab' microfibril consists of a central filament of cellulose I (corresponding to the original microfibril) with crystalline cellulose II growing at right angles to the axis of the microfibril. An alkali-resistant material from the cyst walls of *Acetabularia mediterranea* has been identified (by *X*-ray diffraction and electron microscopy) as cellulose I in the form of ribbon-shaped fibrils of high crystallinity.[43] Electron microscopy of the unicellular green alga *Oocystis* has indicated that the synthesis and orientation of the cellulose microfibrils involve a linear enzyme complex, which is embedded in the B face of the plasma membrane.[44] Granular bands on the A face and complementary ridges on the B face are thought to assist in orienting the newly synthesized microfibrils. Changes in the chemical composition of cotton-fibre cell walls during development have been measured.[45]

Gums and Mucilages

Structural features of the gum exudates from some *Acacia* species of the series *Phyllodineae* and *Botryocephalae* have been investigated.[46] Five polydisperse gums of relatively low molecular weights and containing residues of L-arabinose, L-rhamnose, and D-galactose were converted into monodisperse polysaccharides after a single Smith degradation. The molecular weights of the Smith-degraded polysaccharides are close to the values expected if the respective gum polysaccharides, on losing periodate-vulnerable peripheral residues, were also cleaved at regular intervals between the otherwise (1→3)-linked chains of D-galactopyranosyl

[37] C. Glassell, O. Samuelson, and B. Wennergren, *Svensk Papperstidn.*, 1977, **80**, 171.
[38] D. Mislovičová and M. Pašteka, *Cellulose Chem. Technol.*, 1977, **11**, 115.
[39] S. C. Mueller, R. M. Brown, and T. K. Scott, *Science*, 1976, **194**, 949.
[40] A. Y. Kalninsh and Y. Y. Katkevich, *Cellulose Chem. Technol.*, 1976, **10**, 645.
[41] A. Palma, G. Büldt, and S. M. Jovanović, *Makromol. Chem.*, 1976, **177**, 1063.
[42] A. Buleon, H. Chanzy, and E. Roche, *J. Polymer Sci., Polymer Letters*, 1977, **15**, 265.
[43] W. Herth, A. Kuppel, and W. Franke, *J. Ultrastructure Res.*, 1975, **50**, 289.
[44] R. M. Brown and D. Montezinos, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 143.
[45] M. C. Meinert and D. P. Delmer, *Plant Physiol.*, 1977, **59**, 1088.
[46] S. C. Churms, E. H. Merrifield, and A. M. Stephen, *Carbohydrate Res.*, 1977, **55**, 3.

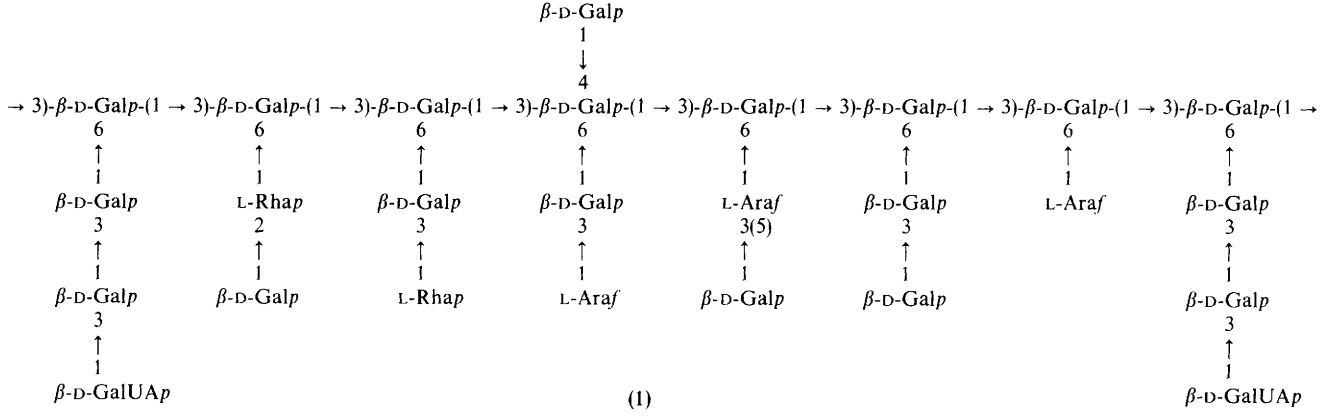
residues. A component of low molecular weight (6×10^3) has been obtained from whole *A. pycnantha* gum by fractional precipitation with aqueous ethanol.[47] Structural investigations suggested that the polysaccharide components of *A. pycnantha* gum are composed of repeating blocks of sugars, which, with minor variations, have the same structure as, and whose molecular weights are, in general, simple multiples of, that of the component of low molecular weight.

The structure of a purified bael (*Aegle marmelos*) gum has been examined.[48] Although the data did not permit the correct sequence of branches on the main chain of the molecule to be assigned, the average repeating-unit of the polysaccharide is represented by (1).

The gum exudates from some *Combretum* species (*C. collinum*, *C. erythrophyllum*, *C. nigricans*, *C. fragrans*, and *C. gelatinosum*) are composed of residues of D-glucuronic acid, D-galacturonic acid, 4-*O*-methyl-D-glucuronic acid, D-galactose, L-arabinose, and L-rhamnose.[49] In comparison with the genus *Acacia*, both the botanical classification and synonomy and the chemistry are more complex. As a group, the genus *Combretum* produces gum exudates that are more viscous, of higher molecular weight, and more acidic than those of the *Acacia* group. A one-step purification of arabinogalactans containing (1 → 3)- and (1 → 6)-linked β-D-galactopyranosyl residues (*e.g.* gum arabic) has been achieved[50] by affinity chromatography on Sepharose to which was attached murine myeloma protein J539, which is known[51] to interact with (1 → 6)-linked β-D-galactans.

A mucilage (mol. wt. 3.4×10^4) containing residues of L-rhamnose, D-galactose, D-galacturonic acid, and D-glucuronic acid (molar proportions 3:2:3:3) has been purified to homogeneity.[52] The polysaccharide has a backbone of L-rhamnopyranosyl and D-galactopyranosyluronic acid residues, all of the D-galactopyranosyluronic acid residues forming branch points with single D-glucopyranosyluronic acid residues and one-third of the L-rhamnopyranosyl residues forming branch-points with side-chains consisting of two (1 → 4)-linked D-galactopyranosyl residues. A mucilage (mol. wt. 1.1×10^5) obtained from the inner bark of *Hydrangea paniculata* was shown to be homogeneous on examination by ultracentrifugation and electrophoresis; it contains residues of L-rhamnose, D-galactose, 4-*O*-methyl-D-glucuronic acid, and D-galacturonic acid.[53] Preliminary structural studies, based on oxidation with periodate, indicated that all of the 4-*O*-methyl-D-glucopyranosyluronic acid residues and 75% of the D-galactopyranosyluronic acid residues in this highly branched polysaccharide are at non-reducing, terminal positions. A glucomannan (mol. wt. 3.9×10^5) containing D-glucose and D-mannose in the molar proportions 2:1 has been isolated from the bulbs of *Lilium speciosum*.[54] Methylation analysis, oxidation with periodate, and partial acetolysis showed that this glucomannan is composed of a (1 → 4)-linked backbone of D-mannopyranosyl and

[47] J. W. H. Adam, S. C. Churms, A. M. Stephen, D. G. Streefkerk, and E. H. Williams, *Carbohydrate Res.*, 1977, **54**, 304.
[48] A. Roy, A. K. Mukherjee, and C. V. N. Rao, *Carbohydrate Res.*, 1977, **54**, 115.
[49] D. M. W. Anderson and P. C. Bell, *Carbohydrate Res.*, 1977, **57**, 215.
[50] I. G. Andrew and B. A. Stone, *Proc. Austral. Biochem. Soc.*, 1977, **10**, 28.
[51] M. Potter and C. P. J. Glaudemans, *Methods Enzymol.*, 1973, **28**, 388.
[52] M. Tomoda, S. Kaneko, M. Ebashi, and T. Nagakura, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 1357.
[53] M. Tomoda and N. Satoh, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 230.
[54] M. Tomoda and S. Kaneko, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 2157.


β-D-Galp
1
↓
4
→ 3)-β-D-Galp-(1 → 3)-β-D-Galp-(1 → 3)-β-D-Galp-(1 → 3)-β-D-Galp-(1 → 3)-β-D-Galp-(1 → 3)-β-D-Galp-(1 → 3)-β-D-Galp-(1 → 3)-β-D-Galp-(1 →
6 6 6 6 6 6 6 6
↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑
1 1 1 1 1 1 1 1
β-D-Galp L-Rhap β-D-Galp β-D-Galp L-Araf β-D-Galp L-Araf β-D-Galp
3 2 3 3 3(5) 3 3
↑ ↑ ↑ ↑ ↑ ↑ ↑
1 1 1 1 1 1 1
β-D-Galp β-D-Galp L-Rhap L-Araf β-D-Galp β-D-Galp β-D-Galp
3 3 3
↑ ↑ ↑
1 1 1
β-D-GalUAp β-D-GalUAp

(1)

D-glucopyranosyl residues, some of which have non-reducing, terminal D-mannopyranosyl residues at O-3. Fractionation of the mucilage from the leaves of *Portulaca oleracea* yielded an acidic component and a neutral component.[55] The acidic component (mol. wt. 1.5×10^4) contains D-galactopyranosyluronic acid residues, 60% of which are present as the calcium salt, joined by α-(1 → 4)-linkages. The neutral fraction (mol. wt. 2×10^3) contains 41% of L-arabinose, 43% of D-galactose, and traces of L-rhamnose. A highly viscous, acidic polysaccharide (mol. wt. $> 2 \times 10^6$) secreted by the leaves of *Drosera capensis* is composed of residues of D-xylose, D-mannose, D-galactose, D-glucuronic acid, and sulphate (molar proportions 1:6:6:6:1).[56] Its high viscocity suggested that the polysaccharide molecules interact strongly with each other and are highly hydrated in aqueous solution; the viscosity of the solutions decreased under physical forces that break hydrogen bonds within and between macromolecules. A mucilagous polysaccharide from the epidermal strips of *Allium cepa* is composed of residues of D-xylose, L-arabinose, D-glucose, D-galactose, and D-galacturonic acid.[57]

Pectins

Pectins differing in their monosaccharide compositions have been isolated from the leaves of *Aster tripolium* by varying the concentration of sodium chloride used in the extraction procedure.[58] Changes in the polysaccharides and oligosaccharides in lupin cotyledons during germination have been examined.[59] In the late phase of cell-wall expansion, the arabinogalactan side-chains of the pectic polysaccharides appear to be selectively hydrolysed, leaving a primary cell wall with a high content of hexuronic acid. During growth, the leafy liverwort *Lophocolea heterophylla* produces sporophytes with seta cells that elongate 50-fold in 3—4 days.[60] The pectic fraction of the seta cells contains more D-mannose, L-fucose, and L-rhamnose, but less L-arabinose and D-xylose, than are usually found in the cells of vascular plants. The proportion of hexuronic acid residues increases during elongation. Light-scattering measurements have been used to study the molecular weights and radii of gyration of pectins, which were shown to have random-coil conformations.[61] A pectic fraction from the inner cell wall (intine) of mature pollen (*Cryptomeria japonica*) grains is composed of residues of D-galactose, L-rhamnose, L-arabinose, D-xylose, and partly esterified hexuronic acid.[62]

Changes in the levels of the enzymes responsible for the interconversion of sugars during cell growth have been measured during differentiation of cambium cells to xylem cells in angiosperms (sycamore and poplar)[63] and gymnosperms (pine and fir),[64] and during extension of the cell walls of pea seedlings.[65] At the final stage of differentiation of cambium cells to xylem cells in sycamore and poplar

[55] E. S. Amin and S. M. El-Deeb, *Carbohydrate Res.*, 1977, **56**, 123.
[56] K. Rost and R. Schauer, *Phytochemistry*, 1977, **16**, 1365.
[57] H. Schnabl, *Planta*, 1977, **135**, 307.
[58] A. Perrelle, *Compt. rend.*, 1977, **284**, *D*, 1401.
[59] N. K. Matheson and H. S. Saini, *Phytochemistry*, 1977, **16**, 59.
[60] R. J. Thomas, *Plant Physiol.*, 1977, **59**, 337.
[61] A. Kawabata and S. Sanayama, *J. Agric. Chem. Soc. Japan*, 1977, **51**, 15.
[62] A. Hara, H. Yamashita, and A. Kobayashi, *Plant and Cell Physiol.*, 1977, **18**, 381.
[63] G. Dalessandro and D. H. Northcote, *Biochem. J.*, 1977, **162**, 267.
[64] G. Dalessandro and D. H. Northcote, *Biochem. J.*, 1977, **162**, 281.
[65] G. Dalessandro and D. H. Northcote, *Planta*, 1977, **134**, 39.

trees, there is a decrease in the activities of UDP-D-galactose 4-epimerase and UDP-L-arabinose 4-epimerase, which are involved in producing soluble precursors of pectin, and an increase in those of UDP-D-glucose dehydrogenase and UDP-D-glucuronate decarboxylase, which produce the precursors of hemicellulose. Although the changes in enzymic activities correlate with changes in the chemical composition of the cell wall during differentiation, the epimerases do not, in the main, control pectin synthesis, since they are still active at stages of growth when no pectin is formed. Changes in the polysaccharide composition, which is a feature of cell-wall differentiation, are therefore controlled primarily at another stage, probably by the transglycosylases of the synthase reactions. The data obtained for gymnosperms suggest that some modulation of polysaccharide synthesis is exerted by controlling the synthesis of precursors of pectin and hemicellulose. Changes were also found in the levels of UDP-D-glucose 4-epimerase, UDP-D-glucuronate 4-epimerase, and UDP-D-xylose 4-epimerase during the elongation of pea seedlings. These changes also correlate with variations in the composition and physical properties of the pectins formed during cell-wall extension. However, the high levels of epimerases in completely elongated cells indicate that the synthesis of pectin is again controlled primarily by the synthase reactions.

Hemicelluloses

The carbohydrate composition of mesophyll cell walls prepared from perennial rye-grass leaves has been investigated.[66] The proportion of D-glucose in the polysaccharides of these cell walls is similar to that in the leaf cell-wall fraction as a whole, but the proportions of L-arabinose, D-galactose, and hexuronic acid are higher, and that of D-xylose is lower. The mesophyll cell walls were readily digested by a fungal cellulase preparation, by snail digestive juices, and by sheep rumen. Treatment of a crude cell-wall preparation of *Lolium perenne* (rye-grass) with sodium methoxide in methanol has been used to hydrolyse certain ester linkages (possibly involving phenolic acids) which hold the cell wall together.[67] The rupture of these linkages allows reagents that are much milder than alkali to be used to extract part of the hemicellulose complex. In this case, a fairly pure galactoarabinoxylan could be obtained by treatment with acid. Phenolic acids or lignin-like materials were still closely associated with all of the fractions, implying that lignin is linked to the cell-wall polysaccharides. The combined actions of cellulase and hemicellulase on a lignin–hemicellulose complex from Norwegian spruce (*Picea abies*) yielded a lignin preparation containing about 20% of carbohydrate.[68] Degradative studies on this preparation indicated that lignin is linked to L-arabinose side-chains in a D-xylan and to D-galactose side-chains in a galactoglucomannan. A similar study on a purified lignin–hemicellulose fraction from *Pinus densiflora* showed that lignin is linked glycosidically to residues of D-galactose, D-xylose, and L-arabinose in the hemicellulose, with two sugar residues linking a single lignin fragment.[69] The stabilities of model cyclic acetals of carbohydrates

[66] A. H. Gordon, A. J. Hay, D. Dinsdale, and J. S. D. Bacon, *Carbohydrate Res.*, 1977, **57**, 235.
[67] I. M. Morrison, *Carbohydrate Res.*, 1977, **57**, C4.
[68] O. Eriksson and B. O. Lindgren, *Svensk Papperstidn.*, 1977, **80**, 59.
[69] T. Koshijima, F. Yaku, and B. Tanaka, in ref. 29, p. 1025.

towards acid and alkali have been used to assess the nature of the linkage between lignin and hemicelluloses.[70] The relative stability of lignin–hemicellulose fractions from acetylated beechwood towards hydrolysis suggested that there is a glycosidic linkage between the polysaccharide and an aromatic residue in the lignin portion. The acid and alkaline hydrolyses of aryl β-D-glucopyranosides containing various substituents on the aromatic ring were therefore examined as representative of those of the glycosidic linkages in lignin–hemicellulose complexes.[71] A linear correlation between the logarithm of the first-order rate constant and the Hammett acidity function was found for the acidic hydrolysis of each of the glycopyranosides, and the entropy of activation indicated that the hydrolysis is a unimolecular process.

Hemicelluloses from the tropical wood species *Triplochiton scleroxylon*, *Terminalia ivorensis*, and *Pterygota macrocarpa* contain high proportions of pentoses (L-arabinose and D-xylose) and low proportions of hexoses, but they do not contain polyuronides.[72] The compositions of the hemicelluloses from the redwood *Sequoia sempervirens* are similar to those of polysaccharides from other conifers, although the D-xylan and glucogalactomannan fractions contain a relatively high proportion of hexuronic acid residues.[73] In this respect, the non-cellulosic polysaccharides resemble those of the Western red cedar (*Thuja plicata*). Specific enzymic degradation of the polysaccharides in delignified spruce and beech woods has been achieved using fungal preparations containing an *endo*-β-1,4-D-mannanase, *endo*-β-1,4-D-xylanases, cellulases, and a β-D-glucosidase.[74] Analysis of the products of enzymic degradation of the holocellulose, in conjunction with electron microscopy of the degraded material, indicated that the D-xylan is selectively hydrolysed by the *endo*-β-1,4-D-xylanases, whereas the mannan is not degraded by the *endo*-β-1,4-D-mannanase, and that cellulose is attacked only after the D-xylan is partly removed. This evidence supports the view that the D-xylan is deposited between cellulose fibrils.

Enzymic analyses of aqueous extracts of barley obtained at 25—100°C showed that there is an exponential relationship between the β-D-glucan content and the extraction temperature, which could, in part, reflect the increased solubility at higher temperatures of larger molecules containing more sequences of (1 → 3)-β-D-glucopyranosyl residues.[75] A homogeneous D-glucan of DP 23 has been isolated from the endosperm of sorghum grain.[76] Methylation analysis and oxidation with periodate indicated that the D-glucan contains both (1 → 4)- and (1 → 6)-linkages (molar ratio 2:1) with, on average, branches on three of the D-glucose residues. ^{1}H N.m.r. spectroscopy showed the presence of α- and β-D-glucosidic linkages in the molar ratio 4:1.

The rate of synthesis of a β-D-glucan from UDP-D-glucose in slices of pea-epicotyl tissue was increased by preliminary, short-term treatment of the tissue with the cellulases obtained from auxin-treated peas.[77] The enzymic treatment

[70] D. Joniak and B. Košiková, *Cellulose Chem. Technol.*, 1976, **10**, 691.
[71] B. Košiková and D. Joniak, *Cellulose Chem. Technol.*, 1976, **10**, 695.
[72] A. K. Lal, R. Butnaru, and C. Simionescu, *Cellulose Chem. Technol.*, 1977, **11**, 59.
[73] G. G. S. Dutton and J. P. Joseleau, *Cellulose Chem. Technol.*, 1977, **11**, 313.
[74] M. Sinner, N. Parameswaran, N. Yamazki, W. Liese, and H. H. Dietrichs, in ref. 29, p. 993.
[75] M. Fleming and K. Kawakami, *Carbohydrate Res.*, 1977, **57**, 15.
[76] G. R. Woolard, E.B. Rathbone, L. Novellie, and J. T. Ohlsson, *Carbohydrate Res.*, 1977, **53**, 95.
[77] Y. S. Wong, G. B. Fincher, and G. A. MacLachlan, *Science*, 1977, **195**, 679.

is considered to introduce chain-ends, which act as primers for elongation of the chains, in accessible regions of the cellulose microfibrils. Detached cotton fibres and a particulate fibre fraction containing a D-glucan synthase incorporated D-glucose from UDP-D-[^{14}C]glucose into polysaccharides that were insoluble both in hot water and in chloroform–methanol.[78] The polysaccharide obtained both at high and at low substrate concentrations with the detached fibres and at a high substrate concentration with the particulate fraction was identified as a linear (1 → 3)-β-D-glucan.[79]

β-D-Glucan synthase activity has been found in a Golgi vesicle fraction from the pollen tubes of *Petunia hybrida*, although, unexpectedly, the enzyme was able to use UDP-D-[^{14}C]glucose, but not GDP-D-[^{14}C]glucose, in the synthesis of an alkali-insoluble D-glucan that contained both β-(1 → 3)- and β-(1 → 4)-linkages.[80] The activities and stabilities of β-1,3-D-glucanases involved in the synthesis of polysaccharides in Citrus plants have been reported.[81]

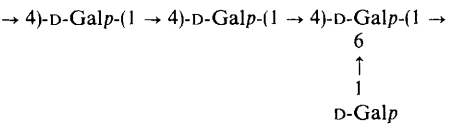
The ^{13}C n.m.r. spectra of lichenin [a D-glucan having regular sequences of two (1 → 4)- and one (1 → 3)-β-D-glucopyranosyl residues], cellulose, and laminarin have been reported.[82] The ^{13}C n.m.r. spectrum of lichenin showed fifteen distinct signals, three of them of double intensity, corresponding to the eighteen theoretically possible signals. The resonances in the ^{13}C spectrum of lichenin were assigned by comparison with those in the ^{13}C spectra of the other polysaccharides.

The reserve polysaccharide in the endosperm of *Carum carvi* has been characterized as a (1 → 4)-β-D-mannan on the basis of partial hydrolysis with acid, enzymic hydrolysis, and acetolysis.[83] Gel chromatography has indicated that mannan A from ivory nuts has an average chain-length of 15.[84] Acetolysis of the polysaccharide has been optimized to produce mannobiose and higher homologues in gram quantities. An acidic lipid fraction from pig liver stimulated the incorporation of D-mannose from GDP-D-[^{14}C]mannose into lipid-linked oligosaccharides by a particulate enzyme fraction from maturing cotton fibres.[85] The oligosaccharides contained 6—20 residues of D-mannose. The endogenous acceptor of D-mannose and 2-acetamido-2-deoxy-D-glucose in pea seedlings has been tentatively identified as dolichyl phosphate, which is the acceptor of these sugars in mammalian systems.[86] Membrane fractions prepared from cultures of sycamore (*Acer pseudoplatanus*) cells catalysed the incorporation of D-mannose from GDP-D-mannose into a D-mannan, a D-mannosyl-lipid, and lipid-linked D-manno-oligosaccharides.[87] The D-mannan consists of (1→4)-linked β-D-mannopyranosyl residues, with few branch points, and it does not appear to be covalently linked to proteins or peptides. The D-mannosyl-lipid was identified as a D-mannopyranosyl polyprenyl phosphate. At least two oligosaccharyl-lipid complexes were separated on the basis of their molecular weights; one of them (mol. wt. 1.7×10^3) contains two residues of

[78] D. P. Delmer, U. Heiniger, and C. Kulow, *Plant Physiol.*, 1977, **59**, 713.
[79] U. Heiniger and D. P. Delmer, *Plant Physiol.*, 1977, **59**, 719.
[80] J. P. F. G. Helsper, H. H. Veerkamp, and M. M. A. Sassen, *Planta*, 1977, **133**, 303.
[81] J. Carbonell, J. P. Beltran, and V. Conejero, *Phytochemistry*, 1977, **16**, 809.
[82] D. Gagnaire and M. Vincendon, *Bull. Soc. chim. France*, 1977, 479.
[83] H. Hopf and O. Kandler, *Phytochemistry*, 1977, **16**, 1715.
[84] J. Thiem, A. Sievers, and H. Karl, *J. Chromatog.*, 1977, **130**, 305.
[85] W. T. Forsee and A. D. Elbein, *J. Biol. Chem.*, 1977, **252**, 2444.
[86] C. T. Brett and L. F. Leloir, *Biochem. J.*, 1977, **161**, 93.
[87] M. M. Smith, M. Axelos, and C. Peaud-Lenoël, *Biochimie*, 1976, **58**, 1195.

D-mannose linked to unidentified hexose and 2-acetamido-2-deoxyhexose residues.

Extraction of garlic (*Allium sativum*) bulbs with hot water afforded fractions containing D-galactan, D-galacturonan, L-arabinan, D-glucan, and D-fructan.[88] Methylation analysis, oxidation with periodate, and Smith degradation of the D-galactan identified (2) as the average repeating-unit.



(2)

The binding of four murine myeloma immunoglobulins with inulin and with a series of inulin-derived oligosaccharides has been examined.[89] The binding sites on two of the immunoglobulins showed highest affinity for a sequence of three D-fructofuranosyl residues, whereas it was highest with a sequence of four D-fructofuranosyl residues for the others.

An enzyme (transfructosylase) preparation from the stems of *Agave vera cruz* synthesized 1-kestose [β-D-Fru-(2 → 1)-β-D-Fru-(2 → 1)-D-Glc] from sucrose.[90,91] The enzyme also acted on 1-kestose to form two series of homologous oligosaccharides (DP ⩽ 8) with structures based on 1-kestose or neokestose, and on other oligosaccharides (*e.g.* nystose) from the stem juices of *Agave vera cruz* to give a series of higher oligosaccharides (DP 8—10), The synthesis of these oligosaccharides appears to be enhanced significantly under conditions that promote the synthesis of D-fructans.

Hydrolysis of lily glucomannan with a crude cellulase preparation from *Trichophyton viride* released the trisaccharides 4′-*O*-β-D-mannopyranosylcellobiose and *O*-β-D-mannopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 4)-D-mannopyranose, indicating the presence of both isolated and contiguous D-glucopyranosyl residues in the structure.[92] Partial hydrolysis of the galactomannan from *Cassia fistula* seeds with acid afforded 4-*O*-β-D-mannopyranosyl-D-mannopyranose, the homologous tetra-, penta-, and hexa-saccharides, 6-*O*-α-D-galactopyranosyl-D-mannopyranose, and *O*-α-D-galactopyranosyl-(1 → 6)-*O*-β-D-mannopyranosyl-(1 → 4)-D-mannopyranose.[93] These findings support earlier structural work which indicated that the galactomannan consists of a backbone of about 25 (1 → 4)-linked β-D-mannopyranosyl residues, to which eight α-D-galactopyranosyl side-chains are attached by (1 → 6)-linkages. A microbial *endo*-β-1,4-D-mannanase has been shown to require at least two contiguous and unsubstituted D-mannopyranosyl residues in the D-mannan backbone of galactomannans in order to manifest its activity.[94] It follows from studies with this enzyme that the D-galactopyranosyl residues in carob galactomannan are randomly distributed along the D-mannan

[88] N. N. Das, A. Das, and A. K. Mukherjee, *Carbohydrate Res.*, 1977, **56**, 337.
[89] D. G. Streefkerk and C. P. J. Glaudemans, *Biochemistry*, 1977, **16**, 3760.
[90] M. N. Satyanarayana, *Indian J. Biochem. Biophys.*, 1976, **13**, 398.
[91] M. N. Satyanarayana, *Indian J. Biochem. Biophys.*, 1976, **13**, 408.
[92] K. Kato, A. Takigawa, Y. Yamaguchi, and Y. Ueno, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 2495.
[93] J. Lal and P. C. Gupta, *Planta Medica*, 1976, **30**, 378.
[94] B. V. Macleary, *Proc. Austral. Biochem. Soc.*, 1977, **10**, 29.

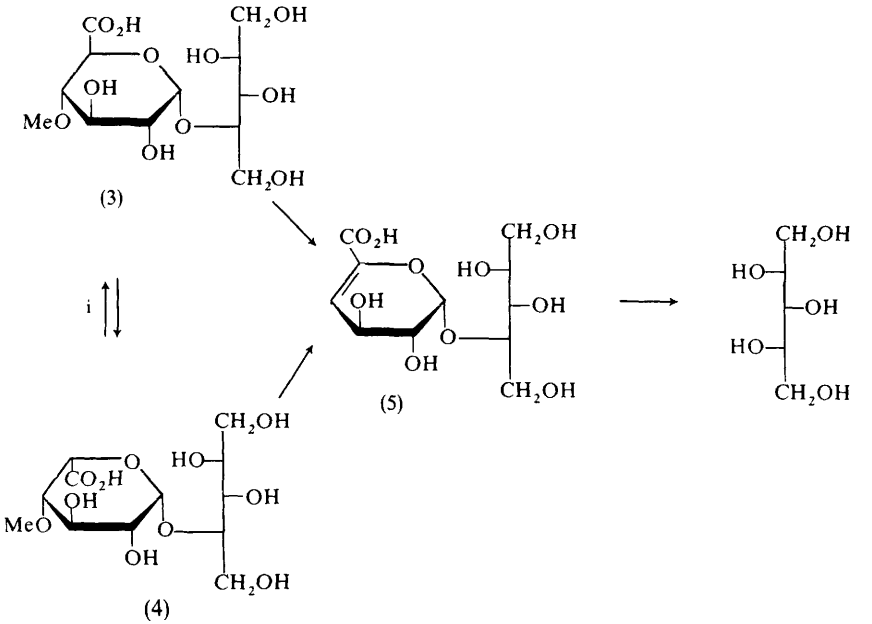
backbone, rather than being uniformly distributed or in blocks. Ion-exchange chromatography of an acidic polysaccharide complex from suspension-cultured cells of *Rosa glauca* yielded a neutral xyloglucan consisting of (1 → 4)-linked β-D-glucopyranosyl residues, to which numerous short side-chains containing one or two D-xylopyranosyl residues are attached at O-6.[95] The structure of this xyloglucan is similar to those of the polysaccharides synthesized by *Acer* and *Phaseolus* cells *in vitro*.

A glucuronomannan has been isolated from the extracellular polysaccharides synthesized by suspension-cultured tobacco cells.[96] Methylation analysis and partial hydrolysis with acid showed that the interior chain of this glucuronomannan is similar to those of the plant gums leiocarpan A and gum ghatti—each of which contains a disaccharide repeating-unit composed of 2-*O*-D-mannopyranosyl and 4-*O*-D-glucopyranosyluronic acid residues—and that many of the D-mannopyranosyl residues are substituted at O-3 with a single L-arabinofuranosyl residue. Ion-exchange chromatography of alkali-soluble material from the mid-rib of *Nicotiana tabacum* has yielded a 4-*O*-methylglucuronoxylan.[97] Methylation analysis, partial hydrolysis with acid, and Smith degradation showed that this polysaccharide is composed of a linear chain of (1 → 4)-linked β-D-xylopyranosyl residues, with a single (1 → 2)-linked residue of 4-*O*-methyl-α-D-glucopyranosyluronic acid attached to roughly every ninth residue. This polysaccharide differs from the D-xylan previously isolated[98] from tobacco stalk, and provides another example of how the structures of the D-xylans in the same plant can vary. The structures of the acidic D-xylans from rapeseed and tobacco stalk appear to be almost identical.[99]

A significant proportion of the hexuronic acid residues comprising the 4-*O*-methylglucuronoxylans of wood are cleaved during treatment with alkali. In order to obtain information on the cleavage reaction, model experiments have been conducted with 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylitol (3), which, on treatment with 1 M-sodium hydroxide at 150 °C, gave initially the epimeric 2-*O*-(4-*O*-methyl-β-L-idopyranosyluronic acid)-D-xylitol (4); on further reaction, both (3) and (4) gave 2-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronic acid)-D-xylitol (5) and, ultimately, xylitol (Scheme 2).[100] An analogous reaction probably removes the 4-*O*-methylhexuronic acid residues from D-xylans during alkaline pulping at high temperature. The kinetics and mechanisms of the oxidation of glucuronoxylan and D-xylose with sodium hypochlorite have been investigated over a wide range of pH values.[101] Oxidation at pH 6—12 is assumed to proceed by a free-radical mechanism, the main reacting species being HOCl and ClO^-, possibly in the form of Cl_2OOH, whereas oxidation at pH 2—4 probably takes place by an ionic mechanism involving hydride or proton transfer.

Attempts have been made to characterize the rice-straw hemicelluloses that may act as binding constituents in the manufacture of paper.[102]

[95] F. Barnoud, A. Mollard, and G. G. S. Dutton, *Physiol. Veg.*, 1977, **15**, 153.
[96] K. Kato, F. Watanabe, and S. Eda, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 539.
[97] S. Eda, F. Watanabe, and K. Kato, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 429.
[98] S. Eda, A. Ohnishi, and K. Kato, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 359.
[99] I. R. Siddiqui and P. J. Wood, *Carbohydrate Res.*, 1977, **54**, 231.
[100] M. H. Johansson and O. Samuelson, *Carbohydrate Res.*, 1977, **54**, 295.
[101] D. K. Jain, S. V. Singh, and S. R. D. Guha, *Cellulose Chem. Technol.*, 1976, **10**, 703.
[102] F. Mobarak, A. E. El-Ashmawy, and H. Augustin, *Cellulose Chem. Technol.*, 1977, **11**, 109.



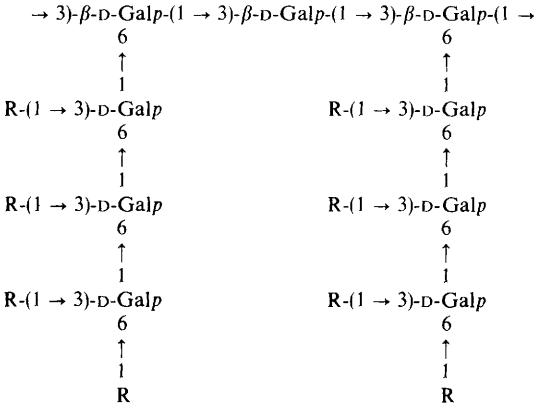
Reagent: i, 1M-NaOH at 150°C

Scheme 2

Cell walls obtained from the etiolated hypocotyls of *Glycine max* and *Vigna sesquipedalis* contain xyloglucans that have a number of structural features in common with those from *Phaseolus aureus*.[103] Hydrolysis of the cell-wall polysaccharides with cellulase showed that the proportions of the two principal oligosaccharide fragments—one composed of residues of D-glucose, D-xylose, D-galactose, and L-fucose and the other of residues of D-xylose and D-glucose—are similar, regardless of their origin. A highly branched, alkali-soluble fucoamyloid from rapeseed (*Brassica campestris*) is composed of residues of D-galactose, D-glucose, D-xylose, and L-fucose (molar proportions 3:14:10:2), [104] while a fucogalactoxyloglucan from rapeseed hulls is composed of residues of L-arabinose, L-fucose, D-xylose, D-galactose, and D-glucose (molar proportions 2:8:25:13:52).[105] The products of acetolysis of the fucogalactoxyloglucan included cellobiose, 6-*O*-α-D-xylopyranosyl-D-glucopyranose, and 2-*O*-β-D-galactopyranosyl-D-xylopyranose, which, together with other evidence, showed that the polysaccharide is a member of the xyloglucan group in which additional residues of L-fucose and D-galactose terminate some of the side-chains. Features of the structures of structurally related xyloglucans from nasturtium seeds and suspension-cultured sycamore cells were also re-examined. Little change in the compositions of the cell-wall polysaccharides of suspension-cultured tobacco cells was observed with ageing of the

[103] Y. Kato, N. Asano, and K. Matsuda, *Plant and Cell Physiol.*, 1977, **18**, 821.
[104] I. R. Siddiqui and P. J. Wood, *Carbohydrate Res.*, 1977, **53**, 85.
[105] G. O. Aspinall, T. N. Krishnamurthy, and K. G. Rosell, *Carbohydrate Res.*, 1977, **55**, 11.

cultures.[106] L-Arabinosyl and D-galactosyl residues were detected in the pectinic acid fraction, suggesting that an arabinogalactan may be glycosidically linked to the rhamnogalacturonan. The structure of a neutral polysaccharide containing residues of L-arabinose, D-galactose, and L-rhamnose (molar proportions 47:45:8) from suspension-cultured tobacco cells has been examined.[107] The results indicated that the polysaccharide is composed of a main chain of (1 → 3)-β-D-galactopyranosyl residues, roughly half of which are substituted at O-6, and that most of the side-chains consist of three (1 → 6)-linked D-galactopyranosyl residues, to which L-arabinosyl residues are attached at O-3. L-Arabinofuranosyl, L-arabinopyranosyl, and, less frequently, L-rhamnopyranosyl residues are present as non-reducing end-groups on the side-chains. The structure (6) of this arabinogalactan is similar to those from coniferous woods and that of the extracellular polysaccharide from cultured sycamore cells.



(6) R = L-Araf, β-L-Arap-(1 → 5)-L-Araf, or L-Rhap

The molecular structures of water-soluble and -insoluble hemicelluloses from bamboo leaves, and of a hemicellulosic D-xylan of the former, have been examined.[108] Attached to the main D-xylan chain of all the polysaccharides are the side-chains (7)—(10) and single residues of L-arabinofuranose and D-glucopyranosyluronic acid and its 4-*O*-methyl ether. The polysaccharides differ in the degree of substitution of the main D-xylan chain by these mono-, di-, and tri-saccharides.

A D-apiosyltransferase that is involved in the synthesis of the apiobiosyl side-chains of apiogalacturonans has been characterized.[109] The enzyme transfers D-apiosyl residues from UDP-D-apiose to an unknown acceptor present in cell-

[106] K. Kato and M. Noguchi, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1923.
[107] K. Kato, F. Watanabe, and S. Eda, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 533.
[108] K. C. B. Wilkie and S. L. Woo, *Carbohydrate Res.*, 1977, **57**, 145.
[109] Y. T. Pan and P. K. Kindel, *Arch. Biochem. Biophys.*, 1977, **183**, 131.

D-Gal*p*-(1 → 5)-L-Ara*f*
(7)

D-Gal*p*-(1 → 4)-D-Xyl*p*-(1 → 2)-L-Ara*f*
(8)

D-Xyl*p*-(1 → 2)-L-Ara*f*-(1 → 2)-L-Ara*f*
(9)

D-GlcUA*p*-(1 → 4)-D-Xyl*p*-(1 → 4)-D-Gal*p*
(10)

free extracts of duckweed (*Lemna minor*). The products of this enzymic reaction were separated into five apiogalacturonan fractions, each of which could be degraded with a fungal pectinase and from which the apiobiosyl side-chains could be removed by hydrolysis at pH 4.[110] Since none of the polysaccharides could be obtained completely free from traces of D-xylose, it is possible that D-xylose is a constituent of the apiogalacturonans of *L. minor*.

High-resolution electron microscopy and autoradiography have been used to locate the pentosans in the cell wall of aspen (*Populus tremuloides*), following growth of the tissue in the presence of L-[1-^{3}H]arabinose.[111] Relatively large amounts of labelled pentosans were found in the parenchyma cell walls. Computer-assisted analyses of the grain-density distributions in electron micrographs of the cell walls of individual fibres were used to localize the labelled wall components after various periods of incubation. The analyses indicated that pentosans are added to the secondary cell wall of developing fibres by an appositional (*i.e.* layer upon layer) mechanism.

D-Xylans in the ultrastructure of rapidly growing tissues of the reed *Arundo donax* have been located following partial hydrolysis with an *endo*-xylanase and staining; the results were then compared with those from control sections.[112] Electron microscopy of the enzyme-treated tissues showed that D-xylans are present in all the primary cell walls of immature internodes of *A. donax*.[113] Fibrillar elements separate during lysis of the D-xylan matrix, thereby confirming the adhesive properties of D-xylans in the growing cell. Changes in the polysaccharide composition of the stem cell walls of *A. donax* during maturation have also been examined.[114] The structure of the principal hemicellulose (an arabinoglucurono-xylan) remains the same, regardless of the age of the tissues, although the average DP greatly increases during maturation. The number of *O*-acetyl groups on the D-xylan backbone and of 4-*O*-methyl groups on the D-glucuronic acid side-chains also increases during maturation.

Pyrolysis of D-xylan, xylobiose, xylotriose, and methyl α- and β-D-xylopyranosides produced, in addition to 2-furaldehyde, 3-hydroxypent-2-eno-1,5-lactone from the non-reducing D-xylopyranosyl residues.[115] Methylation analysis and

[110] L. J. Mascaro and P. K. Kindel, *Arch. Biochem. Biophys.*, 1977, **183**, 139.
[111] R. H. Mullis, N. S. Thompson, and R. A. Parham, *Planta*, 1976, **132**, 241.
[112] K. Ruel, J. P. Joseleau, J. Comtat, and F. Barnoud, in ref. 29, p. 971.
[113] K. Ruel, J. Comtat, and F. Barnoud, *Compt. rend.*, 1977, **284**, *D*, 1421.
[114] J. P. Joseleau and F. Barnoud, in ref. 29, p. 983.
[115] A. Ohnishi, K. Kato, and E. Takagi, *Carbohydrate Res.*, 1977, **58**, 387.

oxidation with periodate of two water-soluble L-arabinans from the inner bark of *Rosa glauca* stems revealed that, although they have different DPs, they have similar highly branched structures containing (1 → 2)-, (1 → 3)-, and (1 → 5)-linked L-arabinofuranosyl residues.[116] A pentosan composed of residues of L-arabinose and D-galactose and protein has been extracted from wheat flour with aqueous trichloroacetic acid.[117]

The role of plant and algal polysaccharides as non-cytotoxic antitumour substances has been discussed.[118]

Algal Polysaccharides

Alginic Acids.—The secondary and tertiary structures of alginic acid, carrageenans, and agarose have been discussed.[119] Natural-abundance ^{13}C n.m.r. spectroscopy has been used for the first time to determine the C-1 doublet and C-5 triplet frequencies in alginates—which contain an unbranched chain composed of blocks of (1 → 4)-linked β-D-mannuronate and α-L-guluronate residues.[120] The triplet frequencies provide information on the monomer sequences in alginates. An alginate lyase, which is associated with bacteriophage infection by *Azotobacter vinelandii*, degraded sodium alginate to a series of oligouronides, each of which contains a terminal 4-deoxy-α-L-*erythro*-hex-4-enopyranosyluronic acid residue.[121] This enzyme appears to attack only those regions of sodium alginate that contain D-mannopyranosyluronic acid residues.

The time-dependence of the modulus of elasticity of alginate gels at different temperatures has been investigated.[122]

Miscellaneous Algal Polysaccharides.—^{13}C N.m.r. spectroscopy has been used to analyse the products of enzymic hydrolysis of agarose.[123] The spectral data obtained with purified neoagaro- and agaro-oligosaccharides were used to estimate the number of disaccharide repeating-units in each of the oligosaccharides. Following extraction of the blue-green alga *Anabaena flos-aquae* with aqueous phenol, a D-glucan was isolated from the aqueous phase and a polysaccharide containing D-mannose and D-glucose (molar proportions 5:1) from the phenolic phase.[124] Carrageenan is thought to be the main contributor to the birefringence of the cell walls of *Chondrus crispus*, where it may surround a cellulosic core in the microfibrils,[125] since, after extraction of *C. crispus* with hot water, little sulphate or birefringence remained.[126]

Two types of binding site for Ca^{2+} ions were recognized in an acidic poly-

[116] J. P. Joseleau, G. Chambat, M. Vignon, and F. Barnoud, *Carbohydrate Res.*, 1977, **58**, 165.
[117] A. H. Tran and P. Nordin, *Stärke*, 1977, **29**, 153.
[118] R. L. Whistler, A. A. Bushway, P. P. Singh, W. Nakahara, and R. Tokuzen, *Adv. Carbohydrate Chem. Biochem.*, 1976, **32**, 235.
[119] D. A. Rees and E. J. Welsh, *Angew. Chem. Internat. Edn.*, 1977, **16**, 214.
[120] H. Grasdalen, B. Larsen, and O. Smidsrød, *Carbohydrate Res.*, 1977, **56**, C11.
[121] I. W. Davidson, C. J. Lawson, and I. W. Sutherland, *J. Gen. Microbiol.*, 1977, **98**, 223.
[122] I.-L. Andresen and O. Smidsrød, *Carbohydrate Res.*, 1977, **58**, 271.
[123] G. K. Hamer, S. S. Bhattacharjee, and W. Yaphe, *Carbohydrate Res.*, 1977, **54**, C7.
[124] A. W. Wang and A. Hill, *J. Bacteriol.*, 1977, **130**, 558.
[125] E. M. Gordon-Mills and E. L. McCandless, *Phycologia*, 1977, **16**, 169.
[126] E. L. McCandless, W. T. Okada, J. N. A. Lott, C. M. Vollmer, and E. M. Gordon-Mills, *Canad. J. Bot.*, 1977, **55**, 2053.

saccharide from the coccoliths of *Emiliania huxleyi*.[127] One of them has a low affinity for Ca^{2+} ions and is possibly associated with the hexuronic acid residues, whereas the other unidentified site exhibits a high affinity for Ca^{2+} ions.

An L-fucosyltransferase (GDP-L-fucose:L-fucose α-fucosyltransferase) that catalyses the transfer of L-fucose from GDP-L-fucose to either L-fucose or fucoidin has been found in the Golgi apparatus of *Fucus serratus*.[128]

The storage D-glucan from *Glaucocystis nostochinearum* forms an iodine complex that has a similar absorption spectrum to those obtained with D-glucans from other green algae, but which differs from those of D-glucans from blue-green algae.[129] A D-glucosyltransferase that is able to insert (1 → 6)-α-D-glucopyranosyl branch-points into amyloses, but which is unable to insert additional (1 → 6)-α-D-glucopyranosyl residues into moderately branched amylopectins, was isolated from *G. nostochinearum*. Chromatography on DEAE-cellulose–molybdate has been used to isolate three D-glucans of low molecular weights from the seaweed *Pelvetia canaliculata*.[130] Each polysaccharide is composed of mainly (1 → 3)-linked β-D-glucopyranosyl residues and a small proportion of (1 → 6)-linked β-D-glucopyranosyl residues. Immunoelectrophoresis of the three types of D-glucosyltransferases (*i.e.* phosphorylases, synthases, and branching enzymes) from *Oscillatoria princeps* suggested that they may be derived from a common protein precursor.[131] Autoradiography of the fronds and spores of *Padina santae-crucis* Borg growing in the presence of $^{35}SO_4^{2-}$ showed that fucoidin accumulates in the rhizoid of the developing spore and may be incorporated into the new cell wall.[132]

The extracellular polysaccharide from *Porphyridium cruentum* contains residues of D- and L-galactose, D-xylose, D-glucose, D-glucuronic acid, 2-*O*-methyl-D-glucuronic acid, and sulphate.[133] Structural studies indicated that all of the D-glucuronic acid can be accounted for in the products of partial acidic hydrolysis, which were identified as 3-*O*-(α-D-glucopyranosyluronic acid)-L-galactose,3-*O*-(2-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose, and 3-*O*-(2-*O*-methyl-α-D-glucopyranosyluronic acid)-D-glucose. Oxidation with periodate suggested that the hexose and hexuronic acid residues are linked glycosidically to O-3 of other residues.

A convenient and simple ^1H n.m.r. assay of D-galactose in a DL-mixture has been applied to the hydrolysate of a galactan from *Rhodymenia pertusa*.[134] After oxidation of D-galactose to *meso-galacto*-hexo-1,5-dialdose with D-galactose oxidase, the magnitude of the aldehydrol signals (derived solely from the oxidized D-enantiomer) relative to those of the anomeric signals furnishes the fractional content of the D-enantiomer.

Rhamnose and 3-*O*-methylxylose have been identified (by g.l.c. and g.l.c.–m.s., respectively) as components of the extracellular mucilage from the red alga *Rhodella maculata*.[135] The principal polysaccharide extracted from both *Urospora*

[127] E. W. de Jong, L. Bosch, and P. Westbroek, *European J. Biochem.*, 1976, **70**, 611.
[128] S. Coughlan, *F.E.B.S. Letters*, 1977, **81**, 33.
[129] J. F. Fredrick, *Phytochemistry*, 1977, **16**, 1489.
[130] J. R. Stark, *Carbohydrate Res.*, 1977, **57**, C11.
[131] J. F. Fredrick, *Phytochemistry*, 1977, **16**, 55.
[132] K. R. Rodriguez and L. B. Liddle, *Phytochemistry*, 1977, **16**, 132.
[133] J. Heaney-Kieras and D. J. Chapman, *Carbohydrate Res.*, 1976, **52**, 169.
[134] J. N. C. Whyte and J. R. Englar, *Carbohydrate Res.*, 1977, **57**, 273.
[135] V. S. Fareed and E. Percival, *Carbohydrate Res.*, 1977, **53**, 276.

wormskioldii and *Codiolum pusillum*—which are different life-forms of an arctic alga—is a polydisperse, sulphated glucuronoxylorhamnan, although polysaccharides containing (1 → 3)-, (1 → 4)-, and triply linked D-glucopyranosyl residues and amylopectin-type polysaccharides can also be isolated from both forms of the alga.[136] *Codiolum pusillum*, unlike *Urospora wormskioldii*, contains a sulphated (1 → 4)-linked β-D-mannan that is branched at O-6 and sulphated at O-2.

[136] G. E. Carlberg and E. Percival, *Carbohydrate Res.*, 1977, **57**, 223.

4
Microbial Polysaccharides

BY R. J. STURGEON

Bacterial Cell Walls

Teichoic Acids.—Recent reviews have referred to the occurrence and functions of membrane teichoic acids,[1] the biosynthesis of bacterial cell walls[2] and of teichoic acids in bacterial cell walls and membranes,[3] and the surface carbohydrates of prokaryotic cells.[4]

The ribitol teichoic acids in whole cells and isolated cell walls of *Bacillus subtilis* W23 have been determined by g.l.c., which was used to measure ribitol and 2-*O*-β-D-glucopyranosyl-L-ribitol (as the TMS derivatives) following degradation of the teichoic acids with 60% hydrogen fluoride.[5]

Since lipoteichoic acids are known to influence biological processes occurring in the mouth, the accumulation of extracellular lipoteichoic acid has been examined in cultures of *Streptococcus mutans*, which proliferates at a rate comparable to those of oral bacteria.[6] The amounts of lipoteichoic acids in cells growing under different conditions remained fairly constant, but the highest amount of extracellular lipoteichoic acid was obtained with the slowest-growing culture. Lipoteichoic acid, as well as the antigenic M protein, has been located on the fimbriae of group A Streptococci; the lipoteichoic acid, rather than the M protein, is considered to be responsible for the binding of epithelial cells.[7]

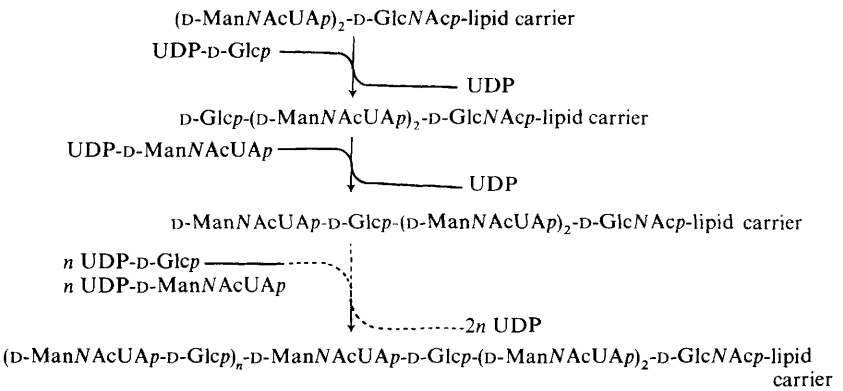
Studies of the effects of thermal stress on three strains of *Staphylococcus aureus* have indicated that the teichoic acid in the cell walls aids the survival of the cells by maintaining the accessible pool of Mg^{2+} ions on the cell surface.[8] A spore-forming Gram-negative bacterium with properties closely similar to those of *Bacillus circulans*, and which hydrolyses the 2-acetamido-2-deoxy-D-glucosylribitol teichoic acid from *Staph. aureus*, has been isolated from soil.[9] The immunogenicities of teichoic and lipoteichoic acids from the cell walls and plasma membranes of several oral bacteria that produce lactic acid have been reported.[10]

[1] P. A. Lambert, I. C. Hancock, and J. Baddiley, *Biochim. Biophys. Acta*, 1977, **472**, 1.
[2] G. Cerny and E. Laver, *Naturwiss.*, 1977, **64**, 440.
[3] M. Duckworth, in 'Surface Carbohydrates of the Prokaryotic Cell', ed. I. W. Sutherland, Academic Press, New York and London, 1977, p. 177.
[4] A. A. Lindberg, in ref. 3, p. 289.
[5] A. J. Anderson, R. S. Green, and A. R. Archibald, *Carbohydrate Res.*, 1977, **57**, C7.
[6] N. A. Jacques, K. W. Knox, and A. J. Wicken, *Proc. Austral. Biochem. Soc.*, 1977, **10**, 95.
[7] E. H. Beachey and I. Ofek, *J. Exp. Med.*, 1976, **143**, 759.
[8] D. G. Hoover and R. J. H. Gray, *J. Bacteriol.*, 1977, **131**, 477.
[9] S. Iwata, K. Tochikubo, K. Kato, T. Hirata, S. Kotani, and K. Yagi, *Japan J. Microbiol.*, 1976, **20**, 123.
[10] A. J. Wicken and K. W. Knox, *J. Dental Res.*, 1976, **55**, C34.

A survey of the constituents of the cell walls of several strains of *B. megaterium* and *B. cereus* revealed that the cell walls of only one strain contain teichoic acid and that the cell walls of a number of strains contain neither teichoic acid nor teichuronic acid.[11]

Digestion of cell-wall sacculi from *B. megaterium* with lysozyme, followed by ion-exchange chromatography, yielded a teichuronic acid composed of residues of D-glucuronic acid, D-glucose, and L-rhamnose in the molar proportions 1:1.2:2.1.[12] Although complete linkage analysis of this teichuronic acid was not attempted, partial hydrolysis with acid and other studies suggested that it is composed of repeating-units of D-glucopyranosyluronic acid → L-rhamnopyranose → D-glucopyranose. The cell walls of a novobiocin-resistant mutant (*nov*-12) of *B. licheniformis* do not contain a teichuronic acid composed of repeating-units of D-glucose and 2-acetamido-2-deoxy-D-mannuronic acid, which is essential for the binding and activity of *N*-acetylmuramyl-L-alanine amidase, the principal autolytic enzyme of the organism.[13]

The *in vitro* biosynthesis of the teichuronic acid in the cell walls of *Micrococcus lysodeikticus* occurs in two distinct stages.[14,15] The initial stage, which involves UDP-2-acetamido-2-deoxy-D-glucose, UDP-2-acetamido-2-deoxy-D-mannuronic acid, and the associated glycosyltransferases, results in the formation of the intermediates 2-acetamido-2-deoxy-D-glucopyranosyl, 2-acetamido-(2-acetamido-2-deoxy-D-mannopyranosyluronic acid)-2-deoxy-D-glucopyranosyl, and 2-acetamido-(2-acetamido-2-deoxy-D-mannopyranosyluronic acid)$_2$-2-deoxy-D-glucopyranosyl undecaprenyl phosphates. The lipid carrier was identified as undecaprenyl phosphate by chromatography and from inhibition studies with bacitracin, and from the observation that concomitant synthesis of peptidoglycan or membrane D-mannan inhibited the synthesis of the teichoic acid intermediates.[14] In the second stage (Scheme 1), the transfer of residues of D-glucopyranose and 2-acetamido-2-



Scheme 1

[11] P. J. White, *J. Gen. Microbiol.*, 1977, **102**, 435.
[12] P. J. White and C. Gilvarg, *Biochemistry*, 1977, **16**, 2428.
[13] R. L. Robson and J. Baddiley, *J. Bacteriol.*, 1977, **129**, 1051.
[14] T. E. Rohr, G. N. Levy, N. J. Stark, and J. S. Anderson, *J. Biol. Chem.*, 1977, **252**, 3460.

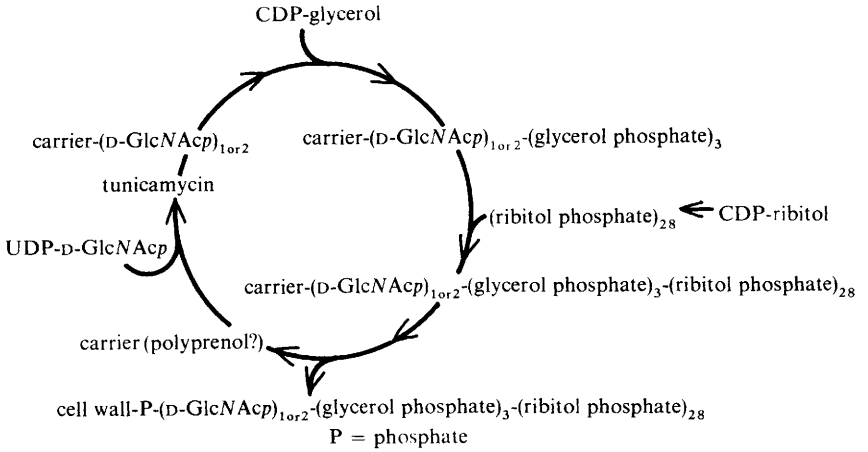
deoxy-D-mannopyranosyluronic acid alternately to the trisaccharyl undecaprenyl phosphate results in elongation of the chain.[15] The biosynthesis of the teichuronic acid linked to the cell walls of *M. luteus* has also been examined.[16] It was concluded that a residue of 2-acetamido-2-deoxy-D-glucose forms a bridge between the teichuronic acid and the peptidoglycan in the cell wall. Enzymic degradation of the cell walls of *M. lysodeikticus* released a teichuronic acid composed of residues of D-glucose and 2-acetamido-2-deoxy-D-mannopyranosyluronic acid, as well as a small proportion of peptidoglycan.[17] Partial hydrolysis with acid and treatments with phosphomonoesterase and alkaline sodium borohydride indicated that the teichuronic acid is linked to O-3 of 2-acetamido-2-deoxy-D-glucopyranose, which is attached by a phosphoric diester linkage to O-6 of a muramic acid residue in the peptidoglycan.

Teichoic and teichuronic acids from *B. subtilis* have been found to bind different amounts of Mg^{2+} ions.[18] When these polysaccharides are incorporated into cell walls, the differences are no longer apparent and the cells exhibit closely similar binding and surface-charge properties. The similarity in cation affinities of cell walls containing the two polysaccharides demonstrates the ability of teichuronic acid to replace teichoic acid in binding cations when the organism grows in the presence of a limited supply of phosphate.

Changes in the cell walls of *B. subtilis* induced by a change of temperature have been shown to alter the proportions of teichoic acid and peptidoglycan.[19]

A membrane cell-wall preparation from *B. subtilis* has been used to study the *in vitro* synthesis of the peptidoglycan and teichoic acid and their linkage to the pre-existing cell walls.[20] Synthesis of the teichoic acid showed an ordered requirement for the incorporation of 2-acetamido-2-deoxy-D-glucose from UDP-2-acetamido-2-deoxy-D-glucose, which was followed by the addition, in turn, of glycerol phosphate (from CDP-glycerol) and poly(ribitol phosphate) (Scheme 2). The preparation can also synthesize peptidoglycan and a cell-wall-linked teichoic acid from UDP-2-acetamido-2-deoxy-D-glucose. The completed teichoic acid is covalently attached to the pre-existing cell wall by a linkage between a 2-acetamido-2-deoxy-D-glucopyranosyl residue in the teichoic acid and an unidentified component in the cell wall. Other schemes for the *in vitro* synthesis of cell-wall-linked teichoic acid have proposed that poly(ribitol phosphate) is transferred from a lipoteichoic acid carrier either to a nascent peptidoglycan that already contains residues of glycerol phosphate and 2-acetamido-2-deoxy-D-glucopyranose[21] or to a glycerol phosphate trimer that is attached to either the growing glycan chain or an intermediate 2-acetamido-2-deoxy-D-glucopyranosylpentapeptidyl-*N*-acetylmuramyl polyprenol.[22] Muramic acid phosphate has been isolated from a membrane cell-wall preparation of *B. licheniformis* in which *in vitro* synthesis of covalently linked teichoic acid and peptidoglycan had taken

[15] N. J. Stark, G. N. Levy, T. E. Rohr, and J. S. Anderson, *J. Biol. Chem.*, 1977, **252**, 3466.
[16] A. Weston and H. R. Perkins, *F.E.B.S. Letters*, 1977, **76**, 195.
[17] S. Hase and Y. Matsushima, *J. Biochem. (Japan)*, 1977, **81**, 1181.
[18] J. E. Heckels, P. A. Lambert, and J. Baddiley, *Biochem. J.*, 1977, **162**, 359.
[19] M. A. Shiflett, D. Brooks, and F. E. Young, *J. Bacteriol.*, 1977, **132**, 681.
[20] A. W. Wyke and J. B. Ward, *J. Bacteriol.*, 1977, **130**, 1055.
[21] R. Bracha and L. Glaser, *J. Bacteriol.*, 1976, **125**, 872.
[22] I. Hancock and J. Baddiley, *J. Bacteriol.*, 1976, **125**, 880.



Scheme 2

place.[23] The finding that the phosphate group of muramic acid phosphate originates from UDP-2-acetamido-2-deoxy-D-glucose, rather than from CDP-glycerol, suggested that the glycerol phosphate unit of the teichoic acid is attached to muramic acid by way of one or more residues of 2-acetamido-2-deoxy-D-glucopyranose 1-phosphate, rather than by a phosphoric diester linkage between glycerol and muramic acid. Indeed, chemical evidence has shown that a residue of 2-acetamido-2-deoxy-α-D-glucopyranose 1-phosphate is involved in linking the teichoic acid to the peptidoglycan in the cell walls of a *Staph. aureus* mutant.[24] The ribitol teichoic acid is considered to be attached to the cell-wall peptidoglycan by way of an oligomer of glycerol phosphate and a residue of 2-acetamido-2-deoxy-α-D-glucopyranose 1-phosphate, as shown in (1). Phosphoric diester linkages between glycerol and O-4 of 2-acetamido-2-deoxy-D-glucopyranose are known to be particularly labile to alkali, so that the structure (1) readily accounts for the lability of the linkage unit to alkali and to acid.

Exhaustive digestion of *B. megaterium* cells with lysozyme yielded a teichuronic acid containing a small amount of peptidoglycan.[25] Degradative studies indicated that the teichuronic acid is glycosidically linked to a tetrasaccharide unit of the peptidoglycan; the tetrasaccharide unit does not contain cross-linked peptide chains.

Peptidoglycans.—The biosynthesis of the peptidoglycans of bacterial cell walls and the action of penicillin on the biosynthesis of peptidoglycans have been reviewed.[26] Other reviews have discussed the effects of endogenous and exogenous factors on the primary structures of bacterial peptidoglycans[27] and the com-

[23] A. W. Wyke and J. B. Ward, *F.E.B.S. Letters*, 1977, **73**, 159.
[24] J. Coley, A. R. Archibald, and J. Baddiley, *F.E.B.S. Letters*, 1977, **80**, 405.
[25] R. J. Ivatt and C. Gilvarg, *Biochemistry*, 1977, **16**, 2436.
[26] J. M. Ghuysen, *J. Gen. Microbiol.*, 1977, **101**, 13.
[27] K. H. Schleifer, W. P. Hammes, and O. Kandler, *Adv. Microbial Physiol.*, 1976, **13**, 246.

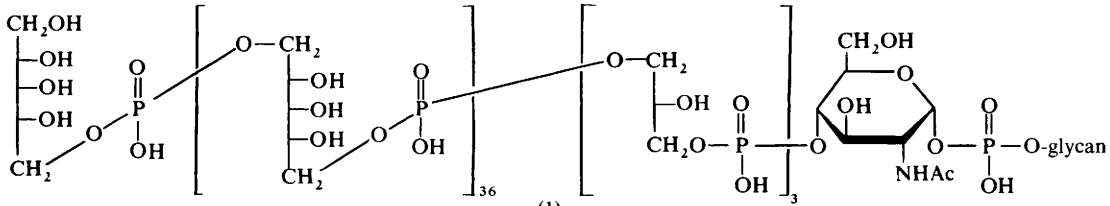
positions of the cell walls of mycobacteria.[28] The biological activities of peptidoglycans and their subunits in bacterial cell walls, particularly the immuno-adjuvant activities, have been discussed.[29]

The multi-layered cell wall of the cyanobacterium *Anacystis nidulans* has been studied by freeze etching and by polyacrylamide gel electrophoresis of solubilized portions of the cell wall.[30] Chemical analysis of the sacculi revealed the presence of a high proportion of 2-amino-2-deoxy-D-mannose, as well as components (diaminopimelic acid, muramic acid, 2-acetamido-2-deoxy-D-glucose, glutamic acid, and alanine) typically found in peptidoglycans. *Arthrobacter globiformis* growing under different conditions in continuous and batch cultures produced rods or cocci or cystites.[31] All the morphological forms were resistant to the action of lysozyme and the cell-wall peptidoglycans contained similar molar proportions of alanine, glutamic acid, threonine, and lysine. The synthesis of polymers by a number of bacteria was inhibited by tunicamycin, which inhibits the membrane-bound phospho-*N*-acetylmuramylpentapeptide translocase that participates in the initial reaction leading to the synthesis of the cell-wall peptidoglycan.[32,33] The oligosaccharides (2)—(4) were isolated and characterized following digestion of a partially *N*-acetylated peptidoglycan from *B. cereus* with lysozyme.[34] The hexasaccharide (2) inhibited the action of lysozyme on the cell-wall peptidoglycan, whereas the hexasaccharide (3) was hydrolysed by lysozyme to the tetrasaccharide (4) and 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-acetylmuramic acid. The results demonstrate that the lysozyme-catalysed hydrolysis of the peptidoglycan requires a 2-acetamido-2-deoxy-β-D-glucopyranosyl residue to be located at subsite C of the enzyme–substrate complex.

An enzyme preparation from the cells of *B. cereus* was able to incorporate [^{3}H]diaminopimelic acid into a polymer resembling the peptidoglycan from the spore cortex.[35] A study of the biosynthesis of peptidoglycans *in vivo* has been undertaken in order to decide whether monomeric precursors are incorporated directly into the cell wall or whether they are first incorporated into oligomeric intermediates that are subsequently transferred to the sacculus.[36] All the peptidoglycan precursors synthesized by a double auxotroph of *B. megaterium*, which required both diaminopimelic acid and lysine for growth, were specifically labelled with radioactive diaminopimelic acid. An oligomeric intermediate in the biosynthetic pathway was isolated and identified as a disaccharyl(pentapeptidyl)$_{12}$ undecaprenyl pyrophosphate.

Inhibition of the synthesis of peptidoglycans by *B. megaterium* with amphomycin resulted in an accumulation of UDP-*N*-acetylmuramylpeptides.[37]

[28] C. Ratledge, *Adv. Microbial Physiol.*, 1976, **13**, 116.
[29] S. Kotani, *Seikagaku*, 1976, **48**, 1081.
[30] J. R. Golecki, *Arch. Mikrobiol.*, 1977, **114**, 35.
[31] T. Duxbury, T. R. G. Gray, and G. P. Sharples, *J. Gen. Microbiol.*, 1977, **103**, 91.
[32] G. Tamura, T. Saski, M. Matsuhashi, A. Takatsuki, and M. Yamasaki, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 447.
[33] J. B. Ward, *F.E.B.S. Letters*, 1977, **78**, 151.
[34] K. Amano, H. Hayashi, Y. Araki, and E. Ito, *European J. Biochem.*, 1977, **76**, 299.
[35] S. M. Pearce, *Canad. J. Microbiol.*, 1977, **23**, 964.
[36] E. Fuchs-Cleveland and C. Gilvarg, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 4200.
[37] H. Tanaka, Y. Iwai, R. Oiwa, S. Shinohara, S. Shimizu, T. Oka, and S. Omura, *Biochim. Biophys. Acta*, 1977, **497**, 633.



(1)

β-D-GlcNH_2*p*-(1 → 4)-β-Mur*N*Ac*p*-(1 → 4)-β-D-GlcNH_2*p*-(1 → 4)-β-Mur*N*Ac*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 4)-Mur*N*Ac*p*

(2)

β-D-Glc*N*Ac*p*-(1 → 4)-β-Mur*N*Ac*p*-(1 → 4)-β-D-GlcNH_2*p*-(1 → 4)-β-Mur*N*Ac*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 4)-Mur*N*Ac*p*

(3)

β-D-GlcNH_2*p*-(1 → 4)-β-Mur*N*Ac*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 4)-Mur*N*Ac*p*

(4)

Amphomycin apparently inhibits the formation of *N*-acetylmuramylpentapeptidyl undecaprenyl pyrophosphate from undecaprenyl phosphate and *N*-acetylmuramyl-pentapeptide. Membrane preparations from *B. subtilis* transferred 2-acetamido-2-deoxy-D-glucose from the nucleotide precursor to undecaprenyl phosphate by transphosphorylation and subsequent transglycosylation of as many as six residues of 2-acetamido-2-deoxy-D-glucopyranose.[38] The kinetics of cell-wall turnover in *B. subtilis* have been examined by pulse-labelling of the peptidoglycan with 2-acetamido-2-deoxy-D-glucose.[39] The results suggest that the turnover of the cell wall is related directly to the growth and elongation of the cells. Several possible mechanisms for controlling the autolytic enzymes in the cell walls were discussed in the light of this observation. Factors that cause the normally straight-rod cells of *B. subtilis* to assume a helical shape have been reported and discussed.[40]

A peptidoglycan from *Caulobacter crescentus* has been found to contain residues of diaminopimelic acid, alanine, and glutamic acid in the molar proportions 1:2:1,[41] although previous reports have suggested that there is no diaminopimelic acid present.[42] Proteins have been found in association with the peptidoglycans from various strains of *Escherichia coli* and other Enterobacteriaceae.[43] These complexes appear to form, or are part of, hydrophobic channels passing through the outer membrane. Staining techniques have revealed appreciable differences in the distribution of polysaccharides in the cell envelopes of *Brucella abortus* and *E. coli*.[44] The outer membrane of *B. abortus* contains less polysaccharide than that of *E. coli*, which seems to have an irregular distribution of polysaccharides.

Cephalexin (a β-lactam antibiotic) and nalidixic acid (an inhibitor of DNA synthesis) have been used to induce the formation of filaments by *E. coli*.[45] Differences were found in the extent of cross-linking of the peptide side-chains in the peptidoglycans (mureins) from filamentous and rod-shaped (normally dividing) cells. The formation of new cell-ends (septa) appears to depend on a proper balance between the activities of the D-alanine carboxypeptidase, which regulates the availability of cell-wall precursors, and the transpeptidase, which catalyses the cross-linking of the newly-synthesized peptidoglycan and its attachment to the cell wall. Intact minicells of *E. coli*, which are anucleate, are able to synthesize peptidoglycans.[46] The levels of enzymes that synthesize or degrade peptidoglycans in isolated envelopes of the minicells have been measured and compared with those in the envelopes obtained from rod-shaped cells of *E. coli* during the logarithmic phase of growth.[47]

C.d. and i.r. spectroscopy have indicated that the protein in the peptidoglycan–lipoprotein complex from the outer membrane of *E. coli* contains 64% of the α-helix and 15% of the β-structure.[48] The complex was hydrolysed by lysozyme,

[38] G. E. Bettinger, A. N. Chatterjee, and F. E. Young, *J. Biol. Chem.*, 1977, **252**, 4118.
[39] L. Glaser and B. Lindsay, *J. Bacteriol.*, 1977, **130**, 610.
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[47] E. W. Goodell and U. Schwarz, *European J. Biochem.*, 1977, **81**, 205.
[48] V. Braun, H. Rotering, J. P. Ohms, and H. Hagenmaier, *European J. Biochem.*, 1976, **70**, 601.

releasing a lipoprotein containing two or three mucopeptides at the CO_2H-terminus. The re-formation of the membranous structure of the outer membrane of sacculi, after dissociation with a detergent, has been investigated.[49]

Membrane preparations from *Gaffkya homari* catalysed the synthesis *in vitro* of a soluble, non-cross-linked peptidoglycan from spin-labelled UDP-*N*-acetylmuramylpentapeptide and UDP-2-acetamido-2-deoxy-D-glucose.[50] Signals in the e.s.r. spectrum of the spin-labelled peptidoglycan were extensively broadened by spin–spin exchange interactions, which were modified by changes in temperature, reduction of the free radicals by ascorbate, and degradation of the peptidoglycan with lysozyme. Complexation of the spin-labelled peptidoglycan with vancomycin resulted in appreciable reductions in the mobility of the probe and in spin–spin exchange.

The incorporation of peptidoglycan into the cell wall and the direction of transpeptidation have been studied using membrane preparations from *G. homari*, which catalysed the formation of peptidoglycan from UDP-2-acetamido-2-deoxy-D-glucose and either UDP-*N*-acetylmuramylpentapeptide (UDP-Mur*N*Ac-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala) or UDP-*N*-acetylmuramyltetrapeptide (UDP-Mur*N*Ac-L-Ala-D-Glu-L-Lys-D-Ala).[51] The peptidoglycan is incorporated into the cell wall by a transpeptidation reaction in which the D-alanyl-D-alanine sequences in the pre-existing cell wall function as donors, and the ε-amino-groups of the L-lysyl residues in the newly synthesized peptidoglycan function as acceptors, in the formation of N^{ε}-D-alanyl-lysine linkages. UDP-Mur*N*Ac-tetrapeptide is a slightly better substrate than UDP-Mur*N*Ac-pentapeptide in the formation of the cell-wall-bound peptidoglycan. The synthesis of both soluble and cell-wall-bound peptidoglycans from UDP-Mur*N*Ac-pentapeptide was competitively inhibited by UDP-Mur*N*Ac-tetrapeptide, and *vice versa*. It appears that the peptidoglycan formed from either UDP-Mur*N*Ac-pentapeptide or UDP-Mur*N*Ac-tetrapeptide is incorporated into the cell wall by the same catalytic sites of all the enzymes (*e.g.* transpeptidase) involved. DD-Carboxypeptidase appears to be essential for the synthesis of the cell walls of *G. homari*, since it controls the proportion of penta- and tetra-peptide units in the newly-synthesized peptidoglycan and, hence, the extent of peptide cross-linking in the completed cell-wall peptidoglycan. The incorporation of peptidoglycan into the cell wall of *G. homari* is inhibited by β-lactam antibiotics, which, by inhibiting the DD-carboxypeptidase, adversely affect the proportion of penta- and tetra-peptide units in the newly-synthesized peptidoglycan.[52] Since pentapeptide units are poor substrates for the transpeptidase, their incorporation into the cell wall is very much slower.

The peptidoglycan synthesized by a membrane cell-wall preparation from *Micrococcus luteus* either was cross-linked by transpeptidation to the pre-existing cell wall or remained associated, without cross-linking, with the membrane fraction.[53] The cross-linked peptidoglycan contained, on average, 66 disaccharide units, whereas the non-cross-linked peptidoglycan contained 26 disaccharide units.

[49] H. Yamada and S. Mizushima, *J. Biochem. (Japan)*, 1977, **81**, 1889.
[50] L. S. Johnston and F. C. Neuhaus, *Biochemistry*, 1977, **16**, 1251.
[51] W. P. Hammes and O. Kandler, *European J. Biochem.*, 1976, **70**, 97.
[52] W. P. Hammes, *European J. Biochem.*, 1976, **70**, 107.
[53] A. Weston, J. B. Ward, and H. R. Perkins, *J. Gen. Microbiol.*, 1977, **99**, 171.

Low levels (1 μg ml^{-1}) of benzylpenicillin inhibited cross-linking, but not the overall synthesis of glycan, whereas intermediate levels ($\leqslant 10$ μg ml^{-1}) slightly inhibited the overall synthesis of glycan. In the presence of high levels (100 μg ml^{-1}) of benzylpenicillin, the incorporation of disaccharide units into the pre-existing walls was significantly impaired—the residual incorporation taking place by extension of the peptidoglycan already cross-linked to the cell walls. Prolonged incubation with high levels of benzylpenicillin eventually resulted in a considerable loss of newly-synthesized peptidoglycan, owing to the expression of autolytic enzymes by the preparation following the action of the antibiotic.

The ability of human-milk D-galactosyltransferase to attach D-galactopyranosyl residues to O-4 of 2-acetamido-2-deoxy-D-glucopyranosyl residues at the ends of oligosaccharides has been used to label and to determine the chain-lengths of glycans in bacterial cell walls.[54] The average chain-length of the soluble peptidoglycan secreted by *M. luteus* on incubation with penicillin G and that of the peptidoglycan of *E. coli* sacculi were determined, based on the ratio of the number of bound D-galactopyranosyl residues to the total number of 2-acetamido-2-deoxy-D-glucopyranosyl residues. 2-Amino-2-deoxy-D-glucose 6-phosphate and muramic acid 6-phosphate have been isolated from lysozyme-resistant cell walls of *M. lysodeikticus*.[55] It was proposed that these sugar phosphates are involved in linking the antigenic polysaccharide to the peptidoglycan chains.

The closely related peptidoglycans synthesized by *Moraxella glucidolytica* and *M. lwoffi* growing on aliphatic (C_{12}—C_{18}) hydrocarbons are composed of muramic acid, 2-amino-2-deoxy-D-glucose, alanine, D-glutamic acid, and *meso*-diaminopimelic acid.[56] The peptidoglycans in both strains are resistant to the action of lysozyme, but they could be partially degraded with lysozyme after treatment with formamide, which solubilized a phosphate-containing polymer.

The peptidoglycan from *Pseudomonas aeruginosa* has been isolated following treatment of the cells with sodium dodecyl sulphate and proteolysis.[57] Analysis of the amino-acid sequence at the NH_2-terminus indicated that half of the peptide chains are cross-linked and that these linkages involve residues of alanine and diaminopimelic acid. Confirmation has been obtained that the peptidoglycan from *Streptomyces roseochromogenes* is composed of a backbone of 4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-acetylmuramyl repeating-units, to which peptide side-chains of L-alanyl-D-isoglutamyl-(glycyl)-LL-diaminopimelyl-D-alanine are attached.[58] A polysaccharide containing residues of D-mannose, D-galactose (molar proportions 5:1), 2-amino-2-deoxy-D-glucose, muramic acid, and phosphate was obtained from the polysaccharide–peptidoglycan complex of *S. roseochromogenes* following hydrolysis of the cells with lysozyme and a lytic enzyme from a *Flavobacterium* sp.[59] The polysaccharide is attached by a phosphoric diester linkage to O-6 of a muramic acid residue in the peptidoglycan.

[54] M. Schindler, D. Mirelman, and U. Schwarz, *European J. Biochem.*, 1976, **71**, 131.
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As many as four enzymes could participate in the cross-linking of peptidoglycans in *Streptomyces* species.[60] The enzyme systems of *Streptomyces* K15 and R61 appear to be similar, each containing an extracellular DD-carboxypeptidase, two cell-bound DD-carboxypeptidases, and a membrane-bound transpeptidase, whereas that of *S. rimosus* consists entirely of a membrane-bound transpeptidase. Immunological relationships exist among the various enzymes.[61] Kinetic parameters for the interactions of the membrane-bound transpeptidases of *Streptomyces* R61 and K15 and *S. rimosus* with peptides and β-lactam antibiotics have been reported.[62] However, it could not be decided whether the transpeptidase activity resides in a single enzyme or in a group of isoenzymes that exhibit identical kinetic properties. An immunologically homogeneous fraction obtained from a *Streptomyces* sp. acted as a D-alanyl-*meso*-diaminopimelic acid *endo*-peptidase on peptidoglycans.[63] Inhibition of the synthesis of peptidoglycan produced an immediate and massive secretion of lipids from several species of bacteria, including Streptococci, *Staph. epidermidis*, and *B. subtilis*, without lysis of the bacteria.[64] The secretion of lipids is specifically induced by inhibitors of peptidoglycan synthesis, so that the continued synthesis of peptidoglycan appears to be essential for integration of lipid material into, and retention of lipid material by, the plasma membrane. A relationship has been demonstrated between the composition of the cell walls of group H Streptococci and of *S. sanguis* and serological properties of the cells.[65] The cell walls of those strains containing the *a* antigen are homogeneous and contain little or no teichoic acid and a modest proportion of L-rhamnose. Inter-peptide linkages in these cells walls occur between two alanyl residues, whereas they occur between alanyl and lysyl residues in cell walls that do not contain the *a* antigen. Antibodies elicited against a synthetic immunogen (pentaglycylglycine covalently linked to albumin) cross-reacted with the interpeptide bridge of the peptidoglycan from *Staph. aureus*.[66]

The peptidoglycan sacculus has been obtained from *Vibrio parahaemolyticus* cells following treatment with detergent and then with proteolytic enzymes.[67] The proportions of amino-acids and amino-sugars in the peptidoglycan from this halophilic bacterium were shown to be similar to those in peptidoglycans from non-halophilic, Gram-negative bacteria. Roughly one-third of the diaminopimelic acid residues in the cell-wall peptidoglycan from *V. parahaemolyticus* are involved in cross-linkages.[68] A particulate enzyme fraction from *V. parahaemolyticus* catalysed a penicillin-sensitive transpeptidation reaction between UDP-*N*-acetylmuramylpentapeptide (labelled with D-[^{14}C]alanine) and UDP-2-acetamido-2-deoxy-D-glucose.

[60] M. Leyh-Bouille, J. Dusart, M. Nguyen-Distèche, J. M. Ghuysen, P. E. Reynolds, and H. R. Perkins, *European J. Biochem.*, 1977, **81**, 19.
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[65] B. Rosan, *Infection and Immunity*, 1976, **13**, 1144.
[66] P. H. Seidl and K. H. Schleifer, *European J. Biochem.*, 1977, **74**, 353.
[67] T. Tamura, K. Kato, S. Iwata, S. Kotani, and T. Kitaura, *Biken. J.*, 1976, **19**, 93.
[68] K. Kato, S. Iwata, H. Suginaka, K. Namba, S. Kotani, and T. Tamura, *Biken. J.*, 1976, **19**, 139.

The outer membrane proteins O-8 and O-9 of *E. coli* K-12 have been shown to bind to the peptidoglycan layer without the assistance of lipoprotein.[69] The binding is stimulated by the lipopolysaccharide.[70]

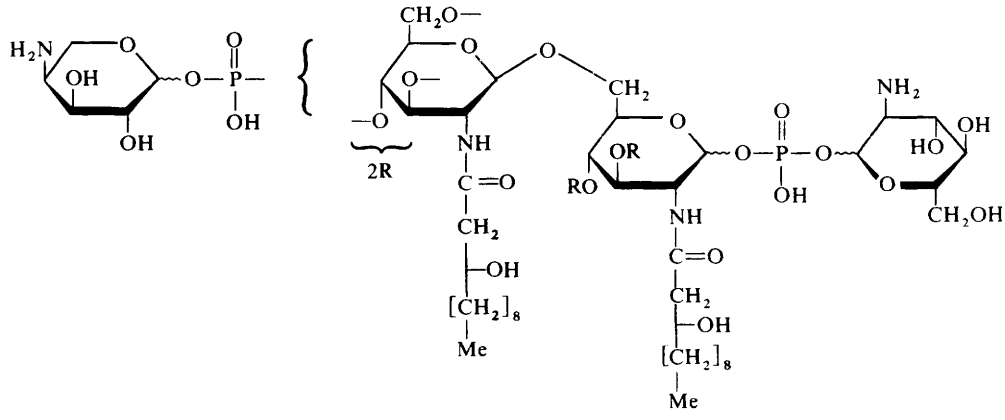
Lipopolysaccharides.—Recent reviews have dealt with the compositions and the structures of bacterial lipopolysaccharides,[71] the serological properties, chemistry, and genetics of the O antigens from *E. coli*,[72] the enterobacterial common antigen,[73] the involvement of lipids in the biosynthesis of lipopolysaccharides,[74] and the binding of bacteriophages to O antigens.[75]

Studies of the chemical composition and biology of the lipopolysaccharide from *Anacystis nidulans* have been reported.[76] The glycan component of this lipopolysaccharide is composed mainly of residues of D-mannose and small proportions of 3- and 4-*O*-methyl-D-mannose, D-galactose, D-glucose, L-fucose, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-mannose, and 3-deoxy-2-octulosonic acid. Methylation analysis and oxidation of the D-mannan chain with periodate revealed the presence of (1 → 3)- and (1 → 4)-linkages in the ratio 3:1. Lipopolysaccharides isolated from the membranes of *Acholeplasma* exhibit some of the physical characteristics of those from Gram-negative bacteria.[77] Acidic hydrolysis of the endotoxin from *Bordetella pertussis* released a disaccharide that was identified as 7-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-L-*glycero*-D-*manno*-heptose using chemical and enzymic techniques.[78] Characterization of the branched trisaccharide [*O*-β-D-glucopyranosyluronic acid-(1 → 2)]-*O*-2-(amino-2-deoxy-α-D-glucopyranosyl)-(1 → 7)-L-*glycero*-D-*manno*-heptose indicated that the endotoxin also contains residues of D-glucuronic acid.[79]

Lipopolysaccharides carrying identical O antigens have been extracted from *Bacteroides fragilis*, *B. melaninogenicus*, and *B. oralis* with aqueous phenol or aqueous H_4edta or trichloroacetic acid.[80] Differences in the proportions of neutral sugars in the extracted materials indicated that other carbohydrate-containing polymers might also be extracted with these solvents.

The structure (5) of the lipid-A component of the lipopolysaccharide from *Chromobacterium violaceum* has been elucidated by chemical and enzymic degradations, although the site of attachment of the 4-amino-4-deoxy-L-arabinopyranosyl phosphate residue and the distribution of the fatty-acid residues on the central disaccharide unit have still to be determined.[81] This lipid-A component differs from most others in that non-acylated amino-sugars are joined, by phosphoric diester linkages, to the central disaccharide unit.

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[70] F. Yu and S. Mizushima, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 1397.
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[73] P. H. Mäkelä and H. Meyer, *Bacteriol. Rev.*, 1976, **40**, 591.
[74] I. W. Sutherland, *Biochem. Soc. Trans.*, 1975, **3**, 840.
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[81] S. Hase and E. T. Rietschel, *European J. Biochem.*, 1977, **75**, 23.



(5) R = $COCHOH[CH_2]_9Me$, $CO[CH_2]_{10}Me$, $COCH_2CHOH[CH_2]_6Me$, or H

D-Glc*N*Ac*p*
D-Man*N*AcUA*p*
oligosaccharide
polysaccharide
}-(1 → 6)-D-Glc*p*-(1 → 2)-D-Glc*p*-(1 → 3)-D-Glc*p*-(1 → 3)-Hep*p*-(1 → 3)-Hep*p* → $(KDO)_3$ → lipid A

Glc*p* 6 → 1 D-Gal*p*

Hep*p* 7 → 1 Hep*p*

Hep*p* 7 → 1 Hep*p*

(8) KDO = 3-deoxy-D-*manno*-2-octulosonic acid; Hep = heptose;
D-Man*N*AcUA*p* = 2-acetamido-2-deoxy-D-mannopyranosyluronic acid

The lipopolysaccharides and phospholipids in unfractionated lysates of *E. coli* cells have been monitored by polyacrylamide gel electrophoresis.[82] This technique is suitable for measuring the amounts of various types of ^{32}P-containing macromolecules in cell lines and in cells growing in different media. Reductions in either the length of the polysaccharide chain of the lipopolysaccharide or the concentration of lipopolysaccharide increased the fluidity of the outer membrane of *E. coli*.[83]

Chemical and enzymic studies have shown that the O-specific side-chains of the lipopolysaccharide from *E. coli* O 69 are composed of the tetrasaccharide repeating-unit (6)[84] and that the hexose region of the lipopolysaccharide from *E. coli* C is composed of the pentasaccharide (7).[85]

$$\rightarrow 3)\text{-}\beta\text{-D-Glc}N\text{Ac}p\text{-}(1 \rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow 2)\text{-}\alpha\text{-D-Gal}p\text{-}(1 \rightarrow$$

(6)

$$\alpha\text{-D-Gal}p\text{-}(1 \rightarrow 2)\text{-}\alpha\text{-D-Gal}p\text{-}(1 \rightarrow 2)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow$$

$$\begin{array}{c} 3 \\ \uparrow \\ 1 \\ \alpha\text{-D-Glc}p \end{array}$$

(7)

The lipopolysaccharides have been isolated from two wild-type strains of *E. coli* K12, two core-deficient mutants, and an SR recombinant with *Salmonella typhimurium*.[86] Methylation analysis of the oligosaccharides released on dephosphorylation of the lipopolysaccharides revealed differences in the extents of completion of the core structures. The non-reducing end of the complete core structure of lipopolysaccharides from the *E. coli* K12 wild-type strains is substituted with either 2-acetamido-2-deoxy-D-glucose or, possibly, 2-acetamido-2-deoxy-D-mannopyranosyluronic acid, whereas the complete K12 core in the SR recombinant is substituted with an S-specific oligosaccharide of *S. typhimurium*. These substituents are attached to O-6 of the D-glucopyranosyl residue at the non-reducing end of the core oligosaccharide, as shown in (8).

Several heat-sensitive mutants of *E. coli* K12 have been isolated.[87] All the lipopolysaccharides isolated from mutants growing at different temperatures contained residues of D-glucopyranose, D-galactopyranose, D-galactofuranose, heptopyranose, and heptofuranose. However, the 6-linked D-glucopyranosyl residue at the non-reducing end of the core oligosaccharide was missing from one of the mutants. The lipopolysaccharides from *E. coli* K12 P678 and a mutant strain PM 61 have similar compositions.[88] Membrane-defective mutants of *E. coli* J5 have been isolated on the basis of their high sensitivity to novobiocin.[89] One of the mutant strains did not have a D-galactose-containing lipopolysaccharide in its outer membrane; chemical analyses of the mutant and wild-type polysaccharides

[82] S. C. Bailey and D. Apirion, *J. Bacteriol.*, 1977, **131**, 347.
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[87] M. Bruneteau and G. Michel, *European J. Biochem.*, 1977, **81**, 179.
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suggested that a UDP-D-glucosyltransferase, which transfers D-glucose to the growing lipopolysaccharide core, is defective in the mutant strain. Thus it appears that D-glucose must be added to the lipopolysaccharide core before D-galactose can be attached.

The lipid-A component of *Fusobacterium nucleatum*, like those of several unrelated bacteria (*e.g. Salmonella*, *Pseudomonas*, and *Rhodopseudomonas gelatinosa*), contains a 2-amino-6-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose disaccharide unit, to which two phosphate groups—one in glycosidic linkage and the other in ester linkage—are attached.[90] Thus it appears that the lipid-A components of certain groups of Gram-negative bacteria have similar structures, which have not been modified greatly during evolution of the bacteria.

The O-specific side-chains in *Klebsiella* O group 12 lipopolysaccharide are composed of the disaccharide repeating-unit (9) joined by (1 → 3)-linkages.[91] Two mutants that lack both the capsular and lipopolysaccharide O antigens

$$\rightarrow 3)\text{-}\beta\text{-D-Glc}N\text{Ac}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1 \rightarrow$$
(9)

have been isolated, by bacteriophage selection, from *K. aerogenes* type 2.[92] Comparison of polysaccharide fractions from the mutant lipopolysaccharides indicated that the D-galactan portion of the molecule is absent.

The proportions of D-glucose, D-galactose, and D-mannose in the lipopolysaccharides of five colonial types of *Neisseria gonorrhoeae* vary from strain to strain.[93] The virulent types 1 and 2 generally contain a higher proportion of hexose and higher ratios of D-mannose or D-galactose to 3-deoxy-D-*manno*-2-octulosonic acid than the avirulent types 3, 4, and 5.

Different serological determinants have been recognized on meningococcal lipopolysaccharides, although it was not possible to assign the determinants to the core region or to the O-specific side-chains of the lipopolysaccharides.[94]

Ribitol phosphate has been identified as a component of the lipopolysaccharide from *Proteus mirabilis* D52.[95] Acidic hydrolysis of the lipopolysaccharides from several strains of *P. mirabilis* from serogroups 016 and 033 liberated 1,4-anhydroribitol, which was identified by g.l.c.–m.s. of the peracetylated derivative.[96] Ribitol has a role in the serological specificities exhibited by these lipopolysaccharides.

The isolation and chemical and serological characterization of the O antigens from representatives of the genus *Phormidium* have been reported.[97] The lipopolysaccharides from *P. africanum*, *P. laminosum*, and *P. uncinatum* contain residues of D-galactose, D-glucose, D-mannose, D-xylose, L-arabinose, L-rhamnose, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and 3-deoxy-D-*manno*-2-octulosonic acid, but they do not contain residues of heptose. Lipopolysaccharides from seven serotype strains of *Pseudomonas aeruginosa* have been analysed for

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[91] C. Erbing, B. Lindberg, J. Lönngren, and W. Nimmich, *Carbohydrate Res.*, 1977, **56**, 377.
[92] I. R. Poxton and I. W. Sutherland, *J. Gen. Microbiol.*, 1976, **96**, 195.
[93] G. M. Wiseman and J. D. Caird, *Infection and Immunity*, 1977, **16**, 550.
[94] R. E. Mandrell and W. D. Zollinger, *Infection and Immunity*, 1977, **16**, 471.
[95] J. G. Meiner, *European J. Biochem.*, 1975, **58**, 621.
[96] J. G. Meiner, H. Mayer, I. Froume, K. Kotelko, and K. Zych, *European J. Biochem.*, 1977, **72**, 35.
[97] L. V. Mikheyskaya, R. G. Ovodova, and Y. S. Ovodov, *J. Bacteriol.*, 1977, **130**, 1.

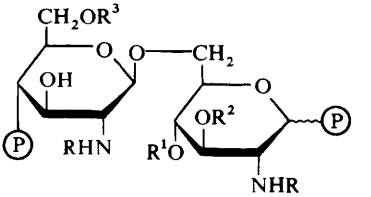
neutral sugars, amino-sugars, lipid, protein, 3-deoxy-D-*manno*-2-octulosonic acid, and phosphorus.[98] All the antigens contain 2-amino-2-deoxy-D-glucose and -D-galactose, together with 2-amino-2,6-dideoxygalactose, which was present as the DL form in the immunotype 2 antigen. As well as the foregoing amino-sugars, 2-amino-2,6-dideoxy-D-glucose is present in the immunotype 3, 4 (see below), and 5 antigens. Mild acidic hydrolysis of the antigens gave the lipid-A component containing all of the lipid and 2-amino-2-deoxy-D-glucose, together with lipid-A-free polysaccharides that contained principally carbohydrate.[99] The lipopolysaccharide antigen of *P. aeruginosa* immunotype 4 was freed from its lipid-A component, by mild acidic hydrolysis, to give a polysaccharide fraction that gave a mixture of neutral sugars and amino-sugars on further hydrolysis with acid. The amino-sugars were isolated and identified as 2-amino-2-deoxy-D-galactose, 2-amino-2,6-dideoxygalactose (as a 2:1 mixture of the D and L enantiomers), and 2-amino-2,6-dideoxy-D-glucose, which is also a constituent of the lipopolysaccharide antigens from immunotypes 3 and 5[99] and Habs serotypes.[100] Among the amino-sugars released on acidic hydrolysis of the lipopolysaccharide from *P. aeruginosa* NCTC 8505 were an aminodeoxy-L-galacturonic acid and 2,4-diamino-2,4,6-trideoxy-D-glucose, which may be a characteristic constituent of the O-specific chain of lipopolysaccharides in strains of *P. aeruginosa* belonging to Habs sero-group 3.[101] 3-*O*-Methyl-L-xylose has been isolated and identified as a component of the lipopolysaccharide fraction of *P. maltophilia*, which also contains residues of L-rhamnose and L-xylose.[102] Lipopolysaccharides have been detected in each of three outer layers of the cell envelope of a marine pseudomonad.[103] The O antigens of the taxonomically related bacteria *Rhodospirillum tenue* and *R. gelatinosa* have been compared; the *R. tenue* antigen has a characteristic chemical composition, which, despite many similarities, is readily distinguishable from that of the *R. gelatinosa* antigen.[104]

Conventional analytical procedures and ^{31}P n.m.r. spectroscopy have been used to determine the extents of phosphorylation of the lipid-A and oligosaccharide components of native and partially deacylated lipopolysaccharides from several strains of *Salmonella*.[105]

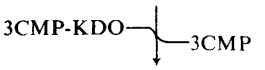
An incomplete lipid-A component has been purified from a mutant of *S. typhimurium*, whose growth and synthesis of 3-deoxy-D-*manno*-2-octulosonic acid 8-phosphate are temperature sensitive.[106] Pulse-chase experiments showed that the incomplete lipid-A component is an intermediate in the biosynthesis of the 3-deoxy-D-*manno*-2-octulosonic acid–lipid component of the lipopolysaccharide. Chemical studies identified the incomplete lipid-A component as a diphosphorylated 2-amino-6-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose carrying two *N*-substituted and two *O*-substituted residues of 3-hydroxymyristic acid. In contrast to the complete lipid-A component, the incomplete

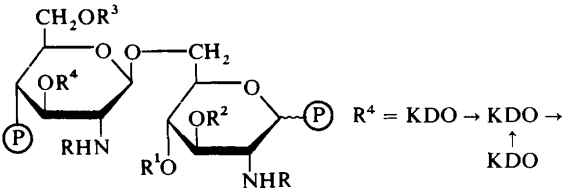
[98] D. Horton, G. Rodemeyer, and T. H. Haskell, *Carbohydrate Res.*, 1977, **55**, 35.
[99] D. Horton, G. Rodemeyer, and R. Rodemeyer, *Carbohydrate Res.*, 1977, **56**, 129.
[100] N. Suzuki, Y. Tsunematsu, H. Matsumoto, and T. Tazaki, *Infection and Immunity*, 1977, **15**, 292.
[101] S. G. Wilkinson, *Biochem. J.*, 1977, **161**, 103.
[102] F. Brown, D. J. Neal, and S. G. Wilkinson, *Biochem. J.*, 1977, **163**, 173.
[103] J. D. Nelson and R. A. MacLeod, *J. Bacteriol.*, 1977, **129**, 1059.
[104] J. Weckesser, G. Drews, R. Indira, and H. Mayer, *J. Bacteriol.*, 1977, **130**, 629.
[105] P. F. Mühlradt, V. Wray, and V. Lehmann, *European J. Biochem.*, 1977, **81**, 193.
[106] V. Lehmann, *European J. Biochem.*, 1977, **75**, 257.

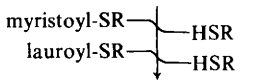
form contains neither 3-deoxy-D-*manno*-2-octulosonic acid nor non-hydroxylated fatty acids. The final steps of the biosynthesis of the 3-deoxy-D-*manno*-2-octulosonic acid–lipid A component of lipopolysaccharides presumably involve the reactions shown in Scheme 3. An identical scheme was invoked to account for the biosynthesis of this component of lipopolysaccharides following studies with another mutant strain of *S. typhimurium* that was unable to complete the synthesis of its lipid-A component.[107, 108] The presence or absence of either D-galactose or D-glucose as a constituent of the lipopolysaccharide core of *S. typhimurium* has been found to influence the resistance of the bacterium to several antibiotics.[109] Chromatography on concanavalin A cross-linked with glutaraldehyde has been used to separate two O antigens from *S. zuerich*.[110] Immunological differences between these antigens were related to the presence or absence of D-glucose as a constituent of the polysaccharides. The heterogeneity of the cell-wall O polysaccharide from *S. zuerich* does not appear to result from either mutation or heterogeneous bio-



R = $COCH_2CHOH[CH_2]_{10}Me$;
R^1 or R^2 or R^3 = R(2 mol.) or H:Ⓟ= OPO_3H_2







KDO–lipid A

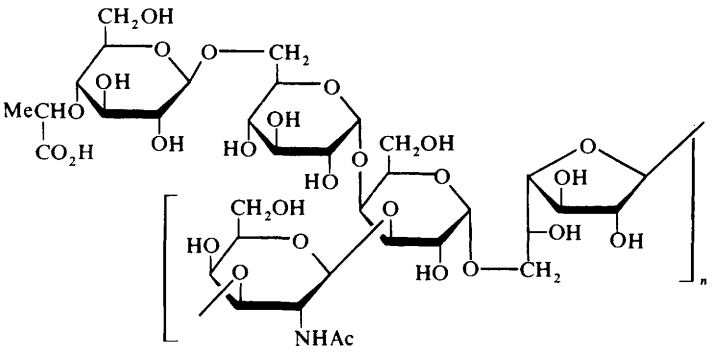
KDO = 3-deoxy-D-*manno*-2-octulosonic acid
HSR = hypothetical acyl-carrier protein

Scheme 3

[107] P. D. Rick and M. J. Osborn, *J. Biol. Chem.*, 1977, **252**, 4895.
[108] P. D. Rick, L. W. M. Fung, C. Ho, and M. J. Osborn, *J. Biol. Chem.*, 1977, **252**, 4904.
[109] R. J. Roantree, T. T. Kuo, and D. G. MacPhee, *J. Gen. Microbiol.*, 1977, **103**, 223.
[110] H. O. Nghiêm, *European J. Biochem.*, 1977, **75**, 613.

synthesis of the specific cell-wall O polysaccharides but rather from a form variation.[111]

A new acidic monosaccharide isolated from the O-specific lipopolysaccharide of *Shigella dysenteriae* type 3 has been identified as 4-*O*-[(*R*)-1-carboxyethyl]-D-glucose.[112] Previous investigations[113] and optical and ^{1}H n.m.r. measurements[114] have revealed the complete structure (10) of the pentasaccharide repeating-unit of the O-specific polysaccharide chain. The structure (11) of the repeating-unit of the O-specific antigen of the lipopolysaccharide from *Sh. dysenteriae* type 2 has been elucidated following de-*N*-acetylation and identification of the products of deamination.[115] Since the 2-acetamido-2-deoxy-α-D-glucopyranosyl residue attached to the main chain is not oxidized by periodate, it is assumed to be acetylated at either O-3 or O-4. Further studies of *Sh. flexneri* variant X (–; 7, 8), type 5a (V; 3,4), and type 5b (V; 7,8) have confirmed that the O-specific antigens are composed of a common tetrasaccharide repeating-unit that is variously substituted with one or two α-D-glucopyranosyl residues, as shown in (12), (13), and (14), respectively.[116] Most, but not always all, of the repeating-units carry α-D-glucopyranosyl residues at the positions indicated. α-D-Glucopyranosyl residues attached to O-3 of L-rhamnopyranosyl residues in the main chain are



(10)

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

(11)

[111] H. O. Nghiêm and A. M. Staub, *J. Bacteriol.*, 1977, **331**, 119.
[112] N. K. Kochetkov, B. A. Dmitriev, and V. L. Lvov, *Carbohydrate Res.*, 1977, **54**, 253.
[113] B. A. Dmitriev, L. V. Backinowsky, V. L. Lvov, N. K. Kochetkov, and I. L. Hofman, *European J. Biochem.*, 1975, **50**, 539.
[114] B. A. Dmitriev, V. L. Lvov, and N. K. Kochetkov, *Carbohydrate Res.*, 1977, **56**, 207.
[115] B. A. Dmitriev, Y. A. Knirel, N. K. Kochetkov, I. L. Hofman, and K. Čapek, *European J. Biochem.*, 1977, **76**, 433.
[116] L. Kenne, B. Lindberg, K. Petersson, E. Katzenellenbogen, and E. Romanowska, *European J. Biochem.*, 1977, **76**, 327.

→ 2)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 →
3
↑
1
α-D-Glc*p*

(12)

→ 2)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 →
3
↑
1
α-D-Glc*p*

(13)

→ 2)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 →
3 3
↑ ↑
1 1
α-D-Glc*p* α-D-Glc*p*

(14)

immunodominant in group 7,8 and type V antigens, while the position of the 2,3-disubstituted L-rhamnopyranosyl residue determines the serological specificity. For group 7,8 specificity, for example, the α-D-glucopyranosyl residue must be attached to the 2-linked α-L-rhamnopyranosyl residue next to the 2-acetamido-2-deoxy-β-D-glucopyranosyl residue, whereas it is located on the central 2-linked α-L-rhamnopyranosyl residue in the type V antigens. The O-specific antigen of the lipopolysaccharide from *Sh. dysenteriae* type 5 is composed of residues of D-mannose, 2-acetamido-2-deoxy-D-glucose, and 3-*O*-[(*R*)-1-carboxyethyl]-L-rhamnose and *O*-acetyl groups in the molar proportions 2:1:1:1.[117] Methylation analysis, Smith degradation, and oxidation with chromium trioxide indicated that the O-specific antigen is composed of the tetrasaccharide repeating-unit (15).

→3)-β-D-Glc*N*Ac*p*-(1 → 4)-[3-*O*-carboxyethyl-α-L-Rha*p*-(1 → 3)]-
α-D-Man*p*-(1 → 4)-2- or -3-*O*Ac-α-D-Man*p*-(1 →

(15)

The structure of the tetrasaccharide repeating-unit of the O-specific antigen of *Sh. flexneri* variant Y has been established as (16) on the basis of methylation analysis, ^{1}H n.m.r. spectroscopy, and analysis of the product of de-*N*-acetylation–deamination.[118]

→ 3)-β-D-Glc*N*Ac*p*-(1 → 2)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 →

(16)

2-Amino-2-deoxy-L-altruronic acid has been isolated from the lipopolysaccharide from *Sh. sonnei* following methanolysis and brief hydrolysis of the products with

[117] B. A. Dmitriev, L. V. Backinowsky, Y. A. Knirel, N. K. Kochetkov, and I. L. Hofman, *European J. Biochem.*, 1977, **78**, 381.
[118] L. Kenne, B. Lindberg, K. Petersson, and E. Romanowska, *Carbohydrate Res.*, 1977, **56**, 363.

acid.[119] Its identification followed from the isolation of 2-acetamido-2-deoxy-L-altrose from the methanolysate following *N*-acetylation, reduction of the carboxy-group, and hydrolysis with acid. No significant differences were observed in the chromatographic behaviours or the compositions of the polysaccharide components of lipopolysaccharides from two wild-type strains of *Sh. flexneri* and two *Sh. flexneri–E. coli* hybrids possessing the same specificities.[120] The compositions of the lipopolysaccharides from *Vibrio cholerae* types *cholerae* and *el tor* are identical, except that the *el tor* lipopolysaccharide contains an appreciably lower proportion of amino-sugars, which do not include 2-amino-2-deoxy-D-galactose.[121]

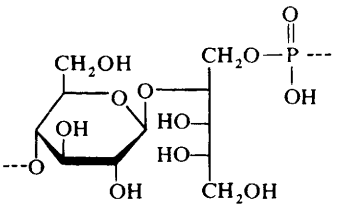
Capsular Polysaccharides

A book dealing with the surface carbohydrates of prokaryotic cells has discussed, among other topics, capsular polysaccharide antigens, immunological expression, the structures of bacterial polysaccharides,[122] and the biosynthesis of capsular polysaccharides.[123]

Immunological characterization of the capsular polysaccharides from *Clostridium perfringens* Hobbs 9 and related strains has indicated that they are similar, but not identical, and composed mainly of residues of D-glucose, D-galactose, and 2-amino-2-deoxy-D-galactose, some phosphate, and traces of peptide material.[124]

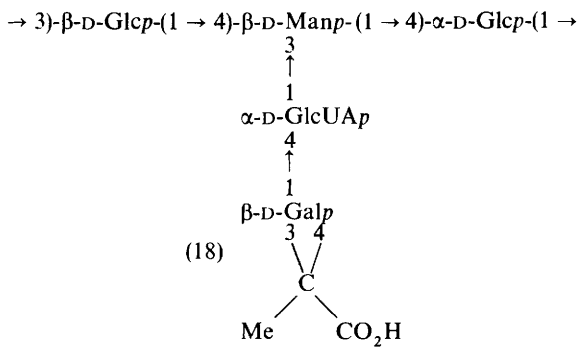
The morphology of the capsule of a strain of *E. coli* has been examined by freeze-etching techniques.[125] The effects of increasing concentrations of antibodies on the capsular structure, the location of the sites of synthesis of the capsular polysaccharide, and the 'export' of the capsular polysaccharide were reported for a temperature-sensitive mutant. In addition to a *his*-linked gene, a *trp*-linked gene is required for the synthesis of the complete K27(A) capsular polysaccharide of *E. coli* 08:K27.[126]

Chemical degradations and ^{13}C n.m.r. spectroscopy have shown that the capsular antigen (17) from *Haemophilus influenzae* type a is composed of residues of 4-*O*-β-D-glucopyranosyl-D-ribitol joined through phosphoric diester linkages

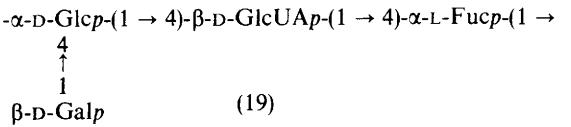


(17)

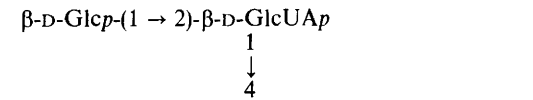
[119] T. Kontrohr, *Carbohydrate Res.*, 1977, **58**, 498.
[120] G. Seltmann and W. Beer, *Z. allgem. Chem.*, 1977, **17**, 381.
[121] M. Maiti, P. Sur, and S. N. Chatterjee, *Ann. Microbiol.*, 1977, **128A**, 35.
[122] K. Jann and B. Jann, in ref. 3, p. 247.
[123] A. Markovitz, in ref. 3, p. 415.
[124] R. Cherniak and H. M. Frederick, *Infection and Immunity*, 1977, **15**, 765.
[125] M. E. Bayer and H. Thurow, *J. Bacteriol.*, 1977, **130**, 911.
[126] G. Schmidt, B. Jann, K. Jann, I. Ørskov, and F. Ørskov, *J. Gen. Microbiol.*, 1977, **100**, 355.



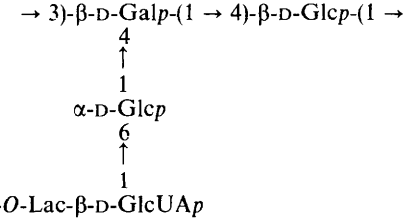
(18)



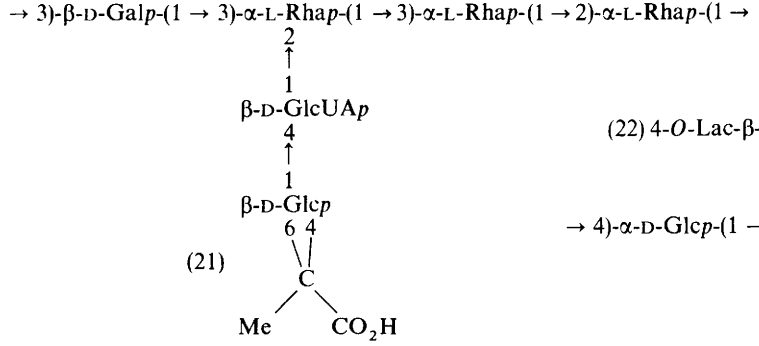
(19)



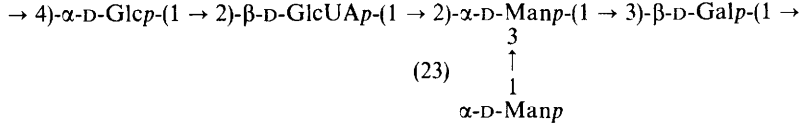
(20)



(22) 4-*O*-Lac-β-D-GlcUA = 4-*O*-[(*S*)-1-carboxyethyl]-β-D-glucuronic acid



(21)



(23)

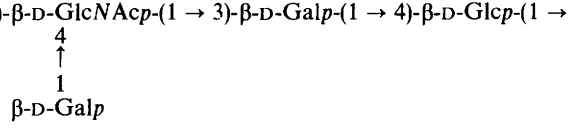
between O-4 of D-glucose and O-5 of D-ribitol.[127] This polysaccharide is closely related to the ribitol teichoic acid found in bacterial cell walls, except that the ribitol residues in the latter are linked through phosphoric diester linkages to O-1 and O-5. A capsular polysaccharide obtained from cultures of *H. influenzae* type b is composed principally of residues of D-ribose, ribitol, and phosphate.[128]

The primary structures of several *Klebsiella* capsular polysaccharides, including serotypes K13 (18),[129] K16 (19),[130] K25 (20),[131] K36 (21),[132, 133] K37 (22),[134] and K62 (23),[135] have been elucidated, mostly using a combination of methylation analysis, Smith degradation, partial hydrolysis with acid, and ^{1}H n.m.r. spectroscopy. In some cases (*e.g. Klebsiella* serotypes K13, K25, and K37), depolymerization of the polysaccharides was accomplished by glycanase activities associated with particles of *Klebsiella* bacteriophages. Serological cross-reactions also revealed similarities in the structures of certain capsular polysaccharides: for example, the polysaccharide from *Klebsiella* serotype K13 (18) cross-reacted with that from *Klebsiella* serotype K2, which has an identical structure except for the absence of the terminal 3,4-*O*-(1-carboxyethylidene)-β-D-galactopyranosyl residue.

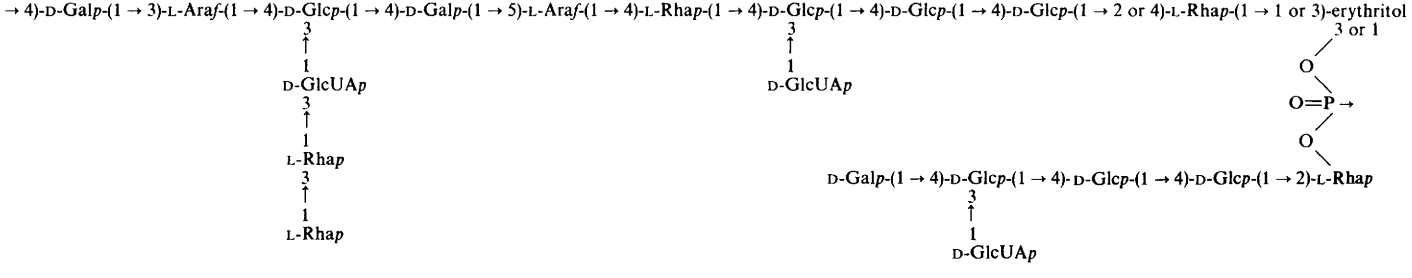
The disaccharide 9-*O*-α-*N*-acetylneuraminyl-*N*-acetylneuraminic acid has been isolated following partial acidic hydrolysis of the homopolysaccharide from *Neisseria meningitidis* serogroup C.[136] Serogroup C meningococcal polysaccharides aggregate in buffered solutions; the association–dissociation process is temperature-dependent and only a fraction of the aggregates dissociate in the presence of H_4edta.[137]

Aspects of the chemistry and the biology of pneumococcal capsular polysaccharides have been reviewed.[138] Methylation studies have established that the pyruvyl group is attached to O-2 and O-3 of the D-galactopyranosyl residues in the specific capsular polysaccharide from *Pneumococcus* type 4.[139] The structure of the capsular polysaccharide (S14) from *Pneumococcus* type 14 has been reinvestigated by methylation analysis, specific degradations, and ^{1}H n.m.r. spectroscopy.[140] The experimental data are compatible with a structure (24) for the tetrasaccharide repeating-unit, which includes several of the structural features previously proposed, although the absence of disaccharide side-chains and of a terminal α-D-galactopyranosyl residue in the revised structure is significant with regard to the reactions of S14 polysaccharide with antibodies and lectins. Thus, pneumococcal

[127] P. Branefors-Helander, C. Erbing, L. Kenne, and B. Lindberg, *Carbohydrate Res.*, 1977, **56**, 117.
[128] P. Anderson and D. H. Smith, *Infection and Immunity*, 1977, **15**, 472.
[129] H. Niemann, N. Frank, and S. Stirm, *Carbohydrate Res.*, 1977, **59**, 165.
[130] A. K. Chakraborty, H. Friebolin, H. Niemann, and S. Stirm, *Carbohydrate Res.*, 1977, **59**, 525.
[131] H. Niemann, B. Kwiatkowski, U. Westphal, and S. Stirm, *J. Bacteriol.*, 1977, **130**, 366.
[132] G. G. S. Dutton and K. L. Mackie, *Carbohydrate Res.*, 1977, **55**, 49.
[133] J. M. Berry, G. G. S. Dutton, L. D. Hall, and K. L. Mackie, *Carbohydrate Res.*, 1977, **53**, C8.
[134] B. Lindberg, B. Lindqvist, J. Lönngren, and W. Nimmich, *Carbohydrate Res.*, 1977, **58**, 443.
[135] G. G. S. Dutton and M. T. Yang, *Carbohydrate Res.*, 1977, **59**, 179.
[136] H. J. Jennings and A. K. Bhattacharjee, *Carbohydrate Res.*, 1977, **55**, 105.
[137] S. Fujime, F. C. Chen, and B. Chu, *Biopolymers*, 1977, **16**, 945.
[138] O. Larm and B. Lindberg, *Adv. Carbohydrate Chem. Biochem.*, 1976, **33**, 295.
[139] J. V. Lew and M. Heidelberger, *Carbohydrate Res.*, 1976, **52**, 255.
[140] B. Lindberg, J. Lönngren, and D. A. Powell, *Carbohydrate Res.*, 1977, **58**, 177.


→ 6)-β-D-GlcNAcp-(1 → 3)-β-D-Galp-(1 → 4)-β-D-Glcp-(1 →
4
1
β-D-Galp

(24)


→ 4)-D-Galp-(1 → 3)-L-Araf-(1 → 4)-D-Glcp-(1 → 4)-D-Galp-(1 → 5)-L-Araf-(1 → 4)-L-Rhap-(1 → 4)-D-Glcp-(1 → 4)-D-Glcp-(1 → 4)-D-Glcp-(1 → 2 or 4)-L-Rhap-(1 → 1 or 3)-erythritol
3 or 1
O
O=P →
O
3
1
D-GlcUAp
3
1
L-Rhap
3
1
L-Rhap
3
1
D-GlcUAp
D-Galp-(1 → 4)-D-Glcp-(1 → 4)- D-Glcp-(1 → 4)-D-Glcp-(1 → 2)-L-Rhap
3
1
D-GlcUAp

(25)

S14 polysaccharide readily precipitated wheat-germ agglutinin, which interacts with the O-4 and O-6 branched 2-acetamido-2-deoxy-β-D-glucopyranosyl residues, and the haemagglutinin from *Ricinus communis* seeds, which interacts with β- (or α-)D-galactopyranosyl residues, but not the lectin from *Bandeiraea simplicifolia*, which interacts only with terminal α-D-galactopyranosyl residues.[141] The interactions with *R. communis* lectin and wheat-germ agglutinin were abolished following exhaustive oxidation of pneumococcal S14 polysaccharide with periodate and reduction ($NaBH_4$) of the oxidized residues, owing to modification of the terminal β-D-galactopyranosyl residues as well as internal β-D-glucopyranosyl residues. Controlled oxidation of pneumococcal S14 polysaccharide with periodate and Smith degradation gave a linear polymer containing 6-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl residues, which precipitated only wheat-germ agglutinin.

The capsular polysaccharide of *Pneumococcus* type 22 is composed of residues of D-glucose, D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid, erythritol, and phosphate.[142] Structural studies of two fragments released on successive treatments of pneumococcal S22 polysaccharide with acid phosphatase and alkali suggested that it is possibly composed of the repeating-unit (25).

Extracellular and Intracellular Polysaccharides

The following topics have been reviewed: the structures and the biosynthesis of bacterial extracellular polysaccharides,[143] the binding of bacteriophages to extracellular polysaccharides,[144] and the functions of extracellular polysaccharides.[145] A symposium has been devoted to the production and the properties of extracellular microbial polysaccharides that are currently used, or are potentially useful, in industry.[146]

Both ^{13}C and ^{1}H n.m.r. measurements on a bacterial dextran and its acetylated derivative and on a synthetic tri-*O*-benzyldextran have revealed the main features of their linear α-(1 → 6)-linked structures.[147] Bacterial dextrans from *Streptococcus viridans* B-1351, *Streptobacterium dextranicum* B-1254, and four strains of *Leuconostoc mesenteroides* have been subjected to methylation analysis.[148] They differed with respect to the ease of methylation and subsequent hydrolysis of the methylated polysaccharides, owing to significant differences in the frequency and type of chain-branching. An enzymic approach has been used to obtain information on the frequency and distribution of chain-branching in dextrans.[149] Thus, B-512 native dextran was treated with an *endo*-dextranase (from *Pseudomonas* UQM 733) and the products were examined by quantitative t.l.c. The relative amount of each oligosaccharide (*i.e.* linear isomalto-oligosaccharides of DP 2–5

[141] S. Ebisu, J. Lönngren, and I. J. Goldstein, *Carbohydrate Res.*, 1977, **58**, 187.
[142] B. P. Chatterjee, S. Purkayastha, and C. V. N. Rao, *Indian J. Chem.*, 1976, **14**, 914.
[143] I. W. Sutherland, in ref. 3, p. 27.
[144] A. A. Lindberg, in ref. 3, p. 289.
[145] W. F. Dudman, in ref. 3, p. 357.
[146] Amer. Chem. Soc. Symposium Series, 1977, No. 45, ed. P. A. Sandford and A. I. Laskin.
[147] D. Gagnaire and M. Vignon, *Makromol. Chem.*, 1977, **178**, 2321.
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[149] M. T. Covacevich and G. N. Richards, *Carbohydrate Res.*, 1977, **54**, 311.

and branched oligosaccharides of DP 4–11 containing a single α-(1 → 3)-linkage) was then determined by reference to standard curves and the percentage of chain-branching from appropriate formulae. Rabbit anti-dextran serum has been separated into two fractions that interact specifically with (1 → 2)- or (1 → 6)-linked α-D-glucopyranosyl residues.[150] The former antibodies were used to fractionate dextran NRRL B1397, which contains α-(1 → 2)- and α-(1 → 6)-linkages, by stepwise precipitation. Most of the dextran fractions exhibited two specificities, which appear to be present in the same molecule. The nature of the molecular motions in a number of free and cross-linked dextrans have been studied by ^{13}C n.m.r. spectroscopy.[151]

A purified *endo*-dextranase from *Streptococcus mutans* acted on dextrans to give principally isomalto-triose, -tetraose, and -pentaose.[152] The hydrolysis of dextrans containing ≥ 96 % of (1 → 6)-α-D-glucopyranosyl residues—expressed as an apparent conversion into isomaltotriose—was virtually complete. D-Glucose was the principal product obtained on hydrolysis of insoluble (1 → 3)-linked α-D-glucans with a purified α-1,3-D-glucanase from *Cladosporium resinae*.[153] It was suggested that dextranase-CB, a bacterial *endo*-α-1,6-D-glucanase which does not release D-glucose from any substrate, could be used in combination with the α-1,3-D-glucanase from *C. resinae* to determine the relative proportions of (1 → 6)- and (1 → 3)-linked residues in the insoluble D-glucans synthesized by oral Streptococci.

Some properties of the free and cell-associated forms of the dextransucrase from *S. sanguis* have been reported; primer dextran, αα-trehalose, and maltose acted as acceptors of α-D-glucopyranosyl residues in the synthesis of soluble and insoluble dextrans from sucrose by both forms of the enzyme.[154] A sucrose-free medium containing D-fructose has been used to grow *S. mutans* OMZ 176.[155] Since a limit dextrin (prepared by treating dextran with an *exo*-dextranase) and a dextran with tri-isopropylbenzenesulphonylated non-reducing residues activated the dextransucrase of *S. mutans* OMZ 176, non-reducing chain-ends do not appear to be required for priming the biosynthesis of dextran, so that activation of dextransucrase by exogenous dextran presumably takes place by another mechanism. In the presence of increasing amounts of dextran, *S. mutans* dextransucrase synthesized a soluble, rather than an insoluble, dextran. A procedure for separating the extracellular activities of *S. mutans* that synthesize water-soluble or -insoluble D-glucans from sucrose has been reported.[156] The unique interactions of each activity with primer dextrans were described. It was proposed that the heterogeneity of native D-glucans produced from sucrose by *S. mutans* results from the concerted actions of dextransucrases and an *endo*-dextranase activity.

Tween 80 stimulated the D-glucosyltransferase activity and the synthesis of

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[151] A. J. Benesi and J. T. Gerig, *Carbohydrate Res.*, 1977, **53**, 278.
[152] A. Pulkownik and G. J. Walker, *Carbohydrate Res.*, 1977, **54**, 237.
[153] G. J. Walker and M. D. Hare, *Carbohydrate Res.*, 1977, **58**, 415.
[154] J. P. Klein, M. Schöller, and R. Frank, *Biochimie*, 1976, **58**, 1047.
[155] J. F. Robyt and A. J. Corrigan, *Arch. Biochem. Biophys.*, 1977, **183**, 726.
[156] G. R. Germaine, S. K. Harlander, W. S. Leung, and C. F. Schachtele, *Infection and Immunity*, 1977, **16**, 637.

dextrans by *S. mutans*.[157] A mutant of *S. mutans*, which synthesized a different extracellular polysaccharide from sucrose than that synthesized by the parent strain, has been used to study the role of extracellular polysaccharides in the development of dental caries.[158] The fibrillar extracellular D-glucan synthesized by the parent strain contains about 30% of α-(1 → 3)-linkages and seems to be necessary for adherence of the bacterium to hard surfaces and for the production of dental caries in test animals. Although an extracellular protein from a cariogenic strain of *S. mutans* did not exhibit either dextransucrase or dextranase activity, it was still able to bind dextran.[159] This dextran-binding protein, which is the first lectin-like molecule to be purified from a bacterial pathogen, seems to be involved in the adherence of the bacterium to dental surfaces. A mutant of *S. mutans*, which has a reduced level of D-glucosyltransferase activity, has been isolated.[160]

A purified D-glucosyltransferase activity from *S. sanguis* was inhibited by IgA fractions obtained from human saliva and dental plaque.[161] Three reproducible phases of D-glucosyltransferase activity have been detected during the growth of *S. sanguis*.[162] Dextran-like D-glucans containing different proportions of (1 → 3)- and (1 → 6)-α-D-glucopyranosyl residues were synthesized during these stages, supporting the view that a multi-enzyme system is involved in the synthesis of the D-glucans.

The uptake of $^{14}CO_2$ by the phototrophic prokaryote *Anacystis nidulans* produced uniformly labelled [^{14}C]glycogen, which yielded [^{14}C]malto-oligosaccharides, [^{14}C]maltose, or D-[^{14}C]glucose on hydrolysis with various amylases.[163] Granulose, an amylopectin-like polysaccharide, is the only D-glucan synthesized in significant amount by several wild-type strains of *Clostridium pasteurianum*.[164] Dextran was not detected (*cf.* ref. 165). The levels and the location of glycogen have been monitored during the life-cycle of *Dictyostelium discoideum*.[166]

The branching enzyme (α-1,4-glucan: α-1,4-glucan 6-glycosyltransferase) from *E. coli* B has been substantially purified by chromatography on 4-aminobutyl-Sepharose.[167] The interaction of the branching enzyme with phosphorylase and glycogen synthase in the formation of branched α-D-glucans was investigated. The subunit structure and the chemical composition of ADP-D-glucose pyrophosphorylase, an enzyme with a crucial role in the synthesis of glycogen by *E. coli* B, have been reported.[168] A purified ADP-D-glucose synthase (mol. wt. 2×10^5) from a mutant strain of *E. coli* B formed oligomeric species in the presence of its allosteric activator (D-fructose 1,6-diphosphate), whereas the corresponding enzyme from the parent strain did not.[169]

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[162] J. A. Beeley and P. M. Black, *Infection and Immunity*, 1977, **15**, 50.
[163] M. Lehmann and G. Wöber, *Carbohydrate Res.*, 1977, **56**, 357.
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The specificity of amylomaltase (the inducible 4-α-glucanotransferase of *E. coli* strain ML) has been redefined.[170] Hitherto the enzyme was regarded as a D-glucosyltransferase, catalysing the transfer of single D-glucopyranosyl residues, and maltose was considered to be its most important substrate. Amylomaltase is now known to exhibit both D-glucosyl- and 4-α-D-glucanosyl-transfer specificities: 4-α-D-glucanosyl chains containing as many as nine D-glucopyranosyl residues can be transferred. The transfer reaction maltose + maltose ⇌ maltotriose + D-glucose, by which the action of amylomaltase was originally expressed, does not take place and maltose has a restricted role as a substrate. The redefined action-pattern of amylomaltase is consistent with the utilization of exogenous and endogenous (1 → 4)-α-D-glucans by *E. coli*. A method has been developed for the isolation of mutant strains of *E. coli* whose ability to metabolize glycogen is impaired.[171]

Glycogen from the mycelia of *Neurospora crassa* has a molecular weight of 2×10^7, a chain-length of 10, a β-amylase limit of 66%, and an α-amylase limit of 42%.[172] It therefore has the highly-branched structure typical of animal and microbial glycogens. An enzyme from the mycelia of *N. crassa* catalysed the incorporation of D-[^{14}C]glucose from UDP-[^{14}C]glucose into two polymers of high molecular weight in the absence of added primer.[173] One of the polymers resembles glycogen, whereas the other is a glycoprotein containing chains of malto-oligosaccharides and a branched (1 → 4)-α-D-glucan. Prolonged incubation of the glycoprotein with the enzyme released the carbohydrate residues, which may then serve as primers for glycogen synthase. Structural-gene mutants of ADP-D-glucose pyrophosphorylase and glycogen synthase have been isolated from *Salmonella typhimurium* LT-2 and partially characterized.[174] An intracellular, granular polysaccharide has been isolated from *Ruminococcus albus*, an anaerobic bacterium that digests cellulose in the rumen of cattle.[175] This polysaccharide interacted strongly with concanavalin A and was hydrolysed by isoamylase and α-amylase.

A crude enzyme preparation from a soil *Bacillus* species contained a D-glucosyltransferase that catalysed the synthesis of cyclodextrins.[176] The properties and the purification of an inducible, extracellular cyclodextrin D-glucanotransferase [1,4-α-D-glucan:4-α-(1,4-α-glucano)transferase (cyclizing)] activity from *Klebsiella pneumoniae* M5 have been reported.[177] Potato starch and cyclohexa- or cyclohepta-amylose are the best substrates for inducing the synthesis of the enzyme, which acted on amylopectin to give cyclodextrins consisting of cycloheptaamylose (29%), cyclohexa-amylose (47%), and a branched cyclohexa-amylose (13%). D-Glucose 1-phosphate was liberated from cyclohexa-amylose by the actions of purified cyclodextrin D-glucanotransferase and maltodextrin phosphorylase from *K. pneumoniae* M5.[178] An amylomaltase-like enzyme, which

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is present in homogenates of the cells growing on cyclodextrins, is involved in disproportionation of the malto-oligosaccharides derived from the cyclodextrins. It appears that cyclodextrins are transported into the cells of *K. pneumoniae* and are degraded therein. Both α- and β-cyclodextrins in a mixture can be analysed by making use of differences in their abilities to complex with the fluorescent dye 2-(4-toluidinyl)naphthalene-6-sulphonate, whose fluorescence intensity increases on addition of cyclodextrins.[179] The uses of cyclodextrins in the pharmaceutical industry have been reviewed.[180]

A ^{13}C n.m.r. study of the resilient gel of a (1 → 3)-β-D-glucan (curdlan) from *Alcaligenes faecalis* var. *myxogenes* has been undertaken in an effort to understand the gel structure.[181] The very broad signals in the downfield region of the ^{13}C-spectrum were assigned to single-helical portions of the gel. It was concluded that the resilient gel is composed of many single-helical regions as well as multiple-helical junction zones wherein all of the ^{13}C-signals are lost because of conformational rigidity. Changes in the line-widths of the ^{13}C-signals upon the gradual addition of sodium hydroxide to an aqueous suspension of the D-glucan are characteristic of the transition from a helical to a random-coil conformation. The spontaneous mutation in the synthesis of polysaccharide by a strain of *A. faecalis*, which produces either a succinoglucan or curdlan, has been monitored by staining with Aniline Blue.[182]

Binding studies with myeloma proteins have shown that a levan from *Actinomyces viscosus* contains both (2 → 1)- and (2 → 6)-linked β-D-fructofuranosyl residues.[183] The levan and levansucrase (mol. wt. 2.2×10^5) of *A. viscosus* form a tightly-bound complex of molecular weight 1×10^8. A D-fructose–enzyme complex has been isolated following the reaction of *B. subtilis* levansucrase with sucrose.[184] The covalent linkage between D-fructose and the enzyme in the complex is cleaved with alkali, whereas proteolytic digestion of the complex released a D-fructosylaspartate—evidence was presented to show that D-fructose is in ester linkage with the β-carboxy-group of aspartic acid. Cytochemical and immunochemical techniques have been used to locate the sucrases elaborated by *S. mutans*.[185] Most of the invertase and D-fructosyltransferase activities in the strongly adherent and virulent parent strain are either extracellular or bound to the surface of the cell, whereas they are associated with extracellular polysaccharides in a mutant strain that is less adherent and less virulent.

A levan (mol. wt. 2×10^7) from *Corynebacterium levaniformans* containing 93% of β-(2 → 6)-linkages and 7% of β-(2 → 1)-linkages and plant levans containing higher proportions of β-(2 → 1)-linkages have been used in a study of the tolerogenicity of D-fructosans to murine B cells.[186] In order to retain tolerogenicity to the B cells, levans are required to have a molecular weight of $3–6 \times 10^3$, if the

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linkage is exclusively β-(2 → 6), or higher, depending on the proportion of β-(2 → 1)-linkages.

Studies with alginate lyases have revealed the presence of *O*-acetyl groups in bacterial alginates.[187] The *O*-acetyl groups may ensure that some of the D-mannopyranosyluronic acid residues do not epimerize during the biosynthesis of alginates.

A coryneform bacterium growing on either D-glucose or soluble starch synthesized a viscous polysaccharide (mol. wt. 2.5×10^5) composed of residues of D-mannose, D-galactose, D-glucose, D-glucuronic acid, and pyruvate (molar proportions 1:1:1:3:1).[188] The non-Newtonian flow and dynamic viscoelasticity of the polysaccharide resemble those of guar gum.[189] Analysis (g.l.c.–m.s.) of the products obtained on methanolysis, *N*-acetylation, and either *O*-trimethylsilylation or *O*-acetylation of partially methylated colominic acid (an extracellular polysaccharide from *E. coli*) has confirmed that adjacent *N*-acetylneuraminic acid residues are (2 → 8)-linked.[190]

Membrane fractions from a *lon* strain of *E. coli*, but not those from a wild-type strain, catalysed the incorporation of L-fucose from GDP-L-fucose and of D-glucose from UDP-D-glucose into an oligosaccharyl-lipid and a polysaccharide related to colanic acid, which is an extracellular polysaccharide composed of D-galactose, D-glucose, L-fucose, and D-glucuronic acid in the molar proportions 2:1:2:1.[191, 192] The oligosaccharyl-lipid was shown to be a (L-fucosyl)$_3$-(D-glucosyl)$_2$-D-glucosyl lipid pyrophosphate and is an intermediate in the synthesis of the polysaccharide. An extracellular polysaccharide containing residues of pyruvic acid has been isolated from *E. coli* 36M obtained from coligranuloma in chicken intestines.[193] Methylation analysis and acetal-exchange showed that the polysaccharide contains residues of 4,6-*O*-(1-carboxyethylidene)-D-glucopyranose, which are (1 → 4)-linked to residues of D-glucopyranosyluronic acid. The composition of an extracellular polysaccharide, containing residues of hexose, acetate, and pyruvate, elaborated by a *Pseudomonas* species in batch culture was not affected by the source of carbohydrate in the growth medium.[194]

A purified polysaccharide isolated from culture broths of a soil *Streptomyces* species (FERM-P1185) is composed of residues of D-glucose and D-mannose in the molar proportions 1.87:1.[195] Methylation analysis, oxidation with periodate, Smith degradation, and i.r. and ^{13}C n.m.r. spectroscopy indicated that this mannoglucan has a linear structure containing roughly equal proportions of (1 → 3)- and (1 → 4)-linked hexopyranosyl residues and an average chain-length of 46. Partial acetolysis of the extracellular polysaccharide (xanthan gum) from *Xanthomonas campestris* gave the constituent monosaccharides, cellobiose, and

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three neutral oligosaccharides thought to be derived from the branch-points.[196] Structural analysis of these oligosaccharides provided additional evidence for the presence of β-D-glucopyranosyl and α-D-mannopyranosyl residues in the polysaccharide. Electron microscopy has shown that native xanthan gum is composed of many strands of subunits arranged in a right-handed double- or triple-helix or as a coil.[197] The extracellular polysaccharides isolated from mutant strains of *X. fuscans* differ from that in the parent strain in as much as they contain higher proportions of D-mannose and also D-glucose and traces of L-rhamnose and D-ribose.[198] The rheological behaviour of xanthans has been ascribed to the ability of the polysaccharides to maintain a stiff, rod-like conformation in solution.[199] The ordered form can bind co-operatively to polysaccharides from the cell walls of certain plants, and this interaction may be important in determining the site of invasion of plant cells by the bacterial pathogen. Native xanthans and other rigid, ordered bacterial polysaccharides interacted quite differently with plant (1 → 4)-β-D-glycans.[200] Whereas xanthans associated strongly with these plant glycans, extracellular polysaccharides from *Arthrobacter* species did not show comparable interactions, providing further evidence for the view that the xanthan–glycan interaction has a role in pathogen–substrate recognition.

Miscellaneous Bacterial Polysaccharides

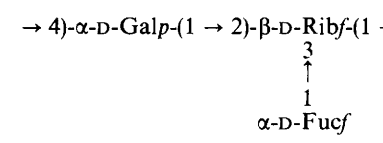
Morphological evidence from a study of *Acetobacter xylinum* and *A. acetigenus* cells has been integrated with previous biochemical and physiological evidence into a general scheme for the biosynthesis of cellulose by bacteria.[201] Cellulose synthesized within the cell is considered to be extruded into the external medium, where it associates with other cellulose chains to form nascent, highly-hydrated fibrils, which give crystalline microfibrils on desiccation. The structure of *A. xylinum* cells actively synthesizing cellulosic microfibrils has been studied.[202] Cellulose is synthesized *in vivo* by *A. xylinum* in the form of a ribbon projecting from the pole of the bacterial rod; the ribbon, which grows at a rate of 2 μm min^{-1}, consists of approximately 46 microfibrils with a cross-section of, on average, 1.6 × 5.8 nm.[203] Electron-microscopic techniques revealed the presence of roughly 50 sites that synthesize cellulose, which are organized in a row along the longitudinal axis of the bacterial rod and in close association with the outer envelope.

Oxidation with periodate and enzymic and lectin-binding studies have shown that the D-glucan from the microcyst walls of cyst-forming, Gram-negative bacteria is α-(1 → 3)-linked.[204] In addition to peptidoglycans, the cell walls of rods, cocci, and cystites of *Arthrobacter globiformis* contain about 60% of polysaccharide material composed of residues of D-glucose, D-galactose, and L-

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rhamnose.[205] A polysaccharide antigenically related to that from group C *Meningococcus* has been isolated from *E. coli* strain Bos-12.[206] The Bos-12 antigen, like groups B and C meningococcal polysaccharides, is composed entirely of sialic acid. ^{13}C N.m.r. studies on groups B and C meningococcal polysaccharides indicated that the residues of sialic acid are α-(2 → 8)-linked in the former and α-(2 → 9)-linked in the latter. Spectroscopic and chemical studies established that the Bos-12 polysaccharide contains alternate α-(2 → 8)- and α-(2 → 9)-linkages.

Digestion of *Eubacterium suburreum* cells with trypsin released an antigenic polysaccharide of high molecular weight.[207] This polysaccharide antigen, which contains equimolar proportions of D-galactopyranosyl, D-ribofuranosyl, and D-fucofuranosyl residues, is composed of the trisaccharide repeating-unit (26), although the β-configuration assigned to the D-ribofuranosyl residue is tentative.[208] Cell-wall hydrolysates of a *Halococcus* species (strain 24), an extremely halophilic



(26)

bacterium, contained amino-acids and amino-sugars, which include a novel 2-amino-2,6-dideoxyhexose-6-sulphonic acid.[209]

Four types of specific antigen have been isolated from the intact bacteria or from the cell walls of a large number of strains of *Lactobacillus acidophilus*.[210] Inhibition of antibody–antigen reactions with trehalose and maltose indicated that α-D-glucopyranosyl residues are immunodominant in these antigens.

A neutral D-manno-oligosaccharide (mol. wt. 3.2×10^3) with a structure similar to that of the polysaccharide component of yeast mannoprotein has been isolated from *Mycobacterium smegmatis*.[211] Methylation analysis, ^1H n.m.r. spectroscopy, and selective enzymic degradation indicated that it consists of an α-(1 → 6)-linked backbone, to which single (1 → 2)-linked α-D-mannopyranosyl residues are attached. The structure of the oligosaccharide is, therefore, similar to that of the outer chain of yeast D-mannan, although the molecule is smaller and the side-chains are restricted to single α-D-mannopyranosyl residues. A non-reducing polysaccharide containing residues of 3-*O*-methyl-D-mannose from *M. smegmatis* has been found to be a mixture of, at least, four components, which can be separated by gel filtration owing to differences in their sizes and extents of methylation.[212]

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[210] H. Shimohashi and M. Mutai, *J. Gen. Microbiol.*, 1977, **103**, 337.
[211] S. K. Maitra and C. E. Ballou, *Biochem. Biophys. Res. Comm.*, 1976, **73**, 1101.
[212] S. K. Maitra and C. E. Ballou, *J. Biol. Chem.*, 1977, **252**, 2459

Although 3-*O*-methyl-D-mannose is the principal constituent of the polysaccharides, all of them contain a small proportion of D-mannose. Controlled acidic hydrolysis of the [^{3}H-methyl]polysaccharides released 6% of the methyl groups as [^{3}H]methanol at a rate characteristic of that of the hydrolysis of methyl α-D-mannopyranoside. ^{1}H N.m.r. spectroscopy of the polysaccharides and the Smith-degraded polysaccharides confirmed that the reducing end of the chains are substituted with a methyl group in glycosidic linkage. This and other chemical studies suggested that the methylated polysaccharides are composed of linear chains of 11–14 (1 → 4)-linked 3-*O*-methyl-α-D-mannopyranosyl residues, each terminated by a single D-mannopyranosyl residue at the non-reducing end and by a methyl aglycone at the reducing end. A polysaccharide composed of residues of 3-*O*-methyl-D-mannose and D-mannose in the approximate molar ratio of 10:1, and which also contains *O*-acetyl groups, has been isolated from *Streptomyces griseus*.[213] This acetylated polysaccharide resembles the polysaccharide from *M. smegmatis* in size and composition, in the absence of acidic or basic groups, and in the lack of a reducing end. ^{1}H N.m.r. spectroscopy, methylation analysis, and Smith degradation established that the *S. griseus* polysaccharide has a terminal α-D-mannopyranosyl residue (1 → 4)-linked to a linear chain of ten consecutive (1 → 4)-linked residues of 3-*O*-methyl-α-D-mannopyranose: the 3-*O*-methyl-D-mannopyranosyl residue at the reducing end of the chain exists, at least in part, as its methyl α-glycoside. The positions at which the *O*-acetyl groups are attached were not determined.

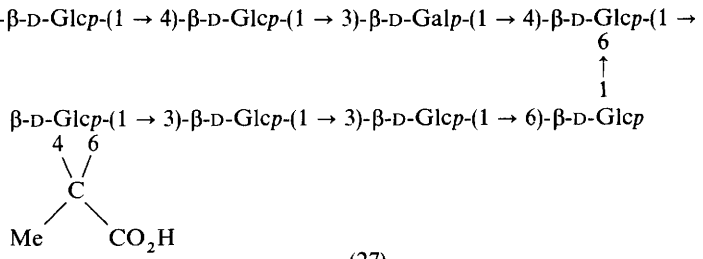
Mycobacterial polysaccharides are able to accelerate the diffusion of long-chain acylated derivatives of coenzyme A away from fatty-acid synthase.[214] A general mechanism has been proposed to account for variations in the rates and products of reactions catalysed by the fatty-acid synthase from *M. smegmatis* over a wide range of experimental conditions.[215] Mycobacterial polysaccharides are considered to form ternary complexes with enzyme-bound coenzyme A, causing the rapid release of fatty-acid derivatives.

Two strains of *Methanosarcina barkeri* that lack peptidoglycans possess thick, rigid cell walls with a high content of carbohydrate, in which the principal component is an acidic heteropolysaccharide composed of residues of 2-amino-2-deoxy-D-galactose, neutral sugars, and hexuronic acid.[216] The formation of myxospores from bacillary forms of *Myxococcus xanthus* is accompanied by loss of the polysaccharide component from the lipopolysaccharide in the cell wall.[217] Preliminary investigations suggested that the lipopolysaccharide in the cell wall is replaced by another polysaccharide in this and other fruiting species of Myxobacteria. A polysaccharide of high molecular weight has been isolated following acidic hydrolysis of a lipopolysaccharide fraction from the cell walls of *Rhizobium leguminosarum*.[218] The polysaccharide is composed of residues of D-glucose (90%) and small proportions of D-mannose, D-galactose, and hexuronic acid; it may be

[213] L. S. Harris and G. R. Gray, *J. Biol. Chem.*, 1977, **252**, 2470.
[214] R. J. Banis, D. O. Peterson, and K. Bloch, *J. Biol. Chem.*, 1977, **252**, 5740.
[215] W. I. Wood, D. O. Peterson, and K. Bloch, *J. Biol. Chem.*, 1977, **252**, 5745.
[216] O. Kandler and H. Hippe, *Arch. Mikrobiol.*, 1977, **113**, 57.
[217] I. W. Sutherland, *Nature*, 1976, **259**, 46.
[218] K. Planqué and J. W. Kijne, *F.E.B.S. Letters*, 1977, **73**, 64.

related to antigenic polysaccharides in the rhizobial cell wall, but it does not appear to be derived from lipopolysaccharide material since it does not contain residues of heptose. Hydrolysis of a slow-growing *Rhizobium* polysaccharide has led to the isolation and identification of 3-*O*-methyl-D-ribose.[219] This appears to be the first time that this sugar has been found in a natural product.

Sequential degradation of the extracellular polysaccharide from *R. meliloti* has shown that it is composed of the octasaccharide repeating-unit (27).[220] Thus, after removal of pyruvic acid residues from the methylated polysaccharide, the four β-D-glucopyranosyl residues in the side-chain were removed by oxidation, β-elimination, and, where necessary, hydrolysis with acid (see Vol. 10, p. 18). The sequence of hexopyranosyl residues in the main chain of the polysaccharide was determined by a modified Smith degradation in which the polyalcohol was methylated prior to hydrolysis with acid.



(27)

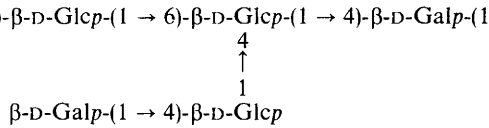
The melting points and heats of junction formation of curdlan gels depend on the concentration and DP of the polysaccharide [a (1 → 3)-β-D-glucan from *Alcaligenes faecalis*].[221] The junction zones of the gels appear to form in regions of aggregation of intertwined helices. *X*-Ray diffraction studies have revealed that curdlan assumes a triple-helical structure.[222]

It has been proposed that the biosynthesis of the group A-specific polysaccharide of *Streptococcus pyogenes* takes place on the cell membrane by the random addition of L-rhamnopyranosyl and 2-acetamido-2-deoxy-D-glucopyranosyl residues to a neutral lipid acceptor.[223] A polysaccharide serotype *c* antigen composed of residues of D-glucose and L-rhamnose has been obtained from the cell walls of *S. mutans*.[224] α-D-Glucopyranosyl residues appear to be immunodominant. The disaccharide 2-acetamido-3-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-2-deoxy-D-galactopyranose has been isolated from a group C streptococcal polysaccharide.[225] This group C polysaccharide is probably composed of a linear backbone of (1 → 2)- and (1 → 3)-linked L-rhamnopyranosyl residues, to which

[219] L. D. Kennedy, *Carbohydrate Res.*, 1976, **52**, 259.
[220] P. E. Jansson, L. Kenne, B. Lindberg, H. Ljunggren, J. Lönngren, U. Ruden, and S. Svensson, *J. Amer. Chem. Soc.*, 1977, **99**, 3812.
[221] T. Kuge, N. Suetsugu, and K. Nishiyama, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 1315.
[222] H. Marchessault, Y. Deslandes, K. Ogawa, and P. R. Sundarajan, *Canad. J. Chem.*, 1977, **55**, 300.
[223] V. M. Reusch and C. Panos, *J. Bacteriol.*, 1977, **129**, 1407.
[224] J. R. Wetherell and A. S. Bleiweis, *Infection and Immunity*, 1975, **12**, 1341.
[225] J. E. Coligan, B. A. Fraser, and T. J. Kindt, *J. Immunol.*, 1977, **118**, 6.

side-chains of 2-acetamido-3-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-2-deoxy-D-galactopyranose are attached at O-3 of (1 → 2)-linked residues. Sequential extraction and fractionation of group B streptococcal polysaccharides have yielded the group- and type-specific polysaccharides and an acidic polysaccharide composed of residues of D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and sialic acid.[226] Immunochemical evidence indicated that the acidic polysaccharide has a structure similar to that of the type-specific antigen, except for the presence of the immunodominant residues of sialic acid, and may represent the native capsular material.

A *S. faecalis* heteroglycan composed of residues of D-glucose and D-galactose has been shown to contain D-galactopyranosyl residues at non-reducing, terminal positions using a micro-method employing galactostix test-strips.[227] This evidence supports the structure (28) originally proposed for the repeating-unit of this heteroglycan.[228] A typical molecule of the heteroglycan is composed of 18 repeating-units joined by (1 → 4)-linkages, although the lactosyl side-chains are replaced by cellobiosyl side-chains in a few of the repeating-units. Surprisingly, less than 5% of the D-glucopyranosyl residues are oxidized with sodium periodate. Methylation analyses on the native, the periodate-oxidized, and the periodate-oxidized and

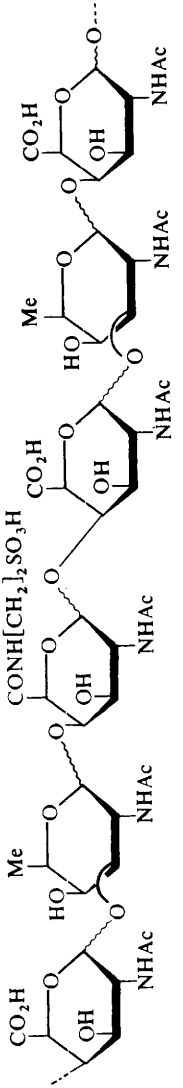


(28)

reduced heteroglycan led to an understanding of this unusual behaviour.[229] The presence of 2,6-di- and 2-*O*-methyl-D-glucose in the hydrolysate of the periodate-oxidized and methylated heteroglycan showed that O-3 of the D-glucopyranosyl residues is substituted in the periodate-oxidized heteroglycan. Since these methylated products were not obtained when the aldehydo-groups were reduced with sodium borohydride prior to methylation, it is clear that HO-3 of the D-glucopyranosyl residues forms a hemiacetal linkage with the aldehydo-groups generated by oxidation of the D-galactopyranosyl residues.

A hexasaccharide repeating-unit (29), which contains 2-acetamido-2-deoxy-D-galactopyranosyluronic acid, 2-acetamido-2,6-dideoxy-D-galactose (*N*-acetyl-D-fucosamine), and taurine in the molar proportions 4:2:1, has been proposed for a surface polysaccharide from *Staph. aureus* M.[230] The disaccharide 2-amino-3-*O*-(2-amino-2-deoxy-D-galactopyranosyluronic acid)-2,6-dideoxy-D-galactopyranose was isolated and characterized following hydrolysis of the polysaccharide with acid. The configurations of the linkages in (29) are not known at present.

[226] J. A. Kane and W. W. Karakawa, *J. Immunol.*, 1977, **118**, 2155.
[227] J. H. Pazur, H. R. Knull, and G. E. Chevalier, *J. Carbohydrates, Nucleosides, and Nucleotides*, 1977, **4**, 129.
[228] J. H. Pazur, A. Cepure, J. A. Kane, and C. G. Hellerqvist, *J. Biol. Chem.*, 1973, **248**, 279.
[229] J. H. Pazur and L. S. Forsberg, *Carbohydrate Res.*, 1977, **58**, 222.
[230] D.-F. Liau and J. H. Hash, *J. Bacteriol.*, 1977, **131**, 194.



(29)

Fungal Polysaccharides

Cell Walls.—Aspects of the biosynthesis of fungal cell walls, including the roles of chitin and chitin synthase, have been reviewed.[231]

The degradation of the cell walls of yeast and of germinated fungal spores with microbial depolymerases has been examined.[232] Three β-1,3-D-glucanases exhibited various degrees of activity towards yeast cells—an enzyme preparation from *Oerskovia xanthineolytica* being most effective in releasing spheroplasts from yeast. Some commercially available enzymes used in the preparation of fungal cell walls have been shown to be contaminated with glycohydrolases.[233]

Electron-dense markers of a size suitable for use with transmission- and scanning-electron microscopy have been prepared by depositing a monolayer of specific macromolecules on gold granules.[234] The labelled granules were used to locate polysaccharides and glycoproteins in the cell walls of *Candida utilis* and *Saccharomyces cerevisiae.*

Glucans.—The structure and conformation of lentinan (an anti-tumour, branched (1 → 3)-β-D-glucan from *Lentinus edodes*), and of acid-degraded fractions obtained from it, have been investigated by ^{13}C n.m.r. spectroscopy.[235] The ^{13}C n.m.r. spectra are considerably changed, depending on the molecular weight; for example, a water-soluble fraction (mol. wt. 3.6×10^3) gave well-resolved ^{13}C n.m.r. spectra, from which it is possible to assign ^{13}C-signals to the (1 → 3)-β-D-glucan and the branch-points at O-6, whereas the ^{13}C-signals of lentinan (mol. wt. 1×10^6) and a fraction of high molecular weight (1.6×10^4), which readily formed gels in water, are considerably broadened (or suppressed), due to immobilization of the main chain and the side-chains in an ordered conformation. The ^{13}C-signals observed for the gels were assigned to the side-chains of the disordered conformation. The ordered conformation of both the β-D-(1 → 3)-linked main chain and the side-chains was identified as the single-helix conformation, which tends to form multiple helices as junction zones for the gel structure.

The D-glucan synthases of *Saprolegnia monoica* have been located in cell-wall and microsomal fractions.[236] Cellobiose and Mg^{2+} ions both stimulated the enzymic activities, and high concentrations of UDP-D-glucose increased the production of insoluble D-glucans, which could be degraded by cellulase to cellobiose. The action of aculeacin A, a new anti-fungal agent, on *S. cerevisiae* cells has been examined.[237] Most of the cells were degraded in the presence of aculeacin A, with the release of intracellular components from the tips of the buds. The antibiotic inhibited the incorporation of D-glucose into the cell-wall D-glucan. Differences in the susceptibilities of intact cells and isolated cell walls of *S. cerevisiae* to lysis by an inducible enzyme system from *Arthrobacter* GJM1 have confirmed that there are marked differences in the compositions of outer and inner surfaces

[231] G. W. Gooday, *J. Gen. Microbiol.*, 1977, **99**, 1.
[232] T. W. Jeffries, D. E. Eveleigh, J. D. Macmillan, F. W. Parrish, and E. T. Reese, *Biochim. Biophys. Acta*, 1977, **499**, 10.
[233] T. E. Davis and J. E. Domer, *Analyt. Biochem.*, 1977, **80**, 593.
[234] M. Horisberger and J. Rosset, *J. Histochem. Cytochem.*, 1977, **25**, 295.
[235] H. Saitô, T. Ohki, N. Takasuka, and T. Sasaki, *Carbohydrate Res.*, 1977, **58**, 293.
[236] M. Fèvre and C. Dumas, *J. Gen. Microbiol.*, 1977, **103**, 297.
[237] J. Mizoguchi, T. Saito, K. Mizuno, and K. Hayano, *J. Antibiotics*, 1977, **30**, 308.

of the cell walls.[238] Lysis of the intact cells was shown to involve an *endo*-β-1,3-D-glucanase and an alkaline protease.[239] The protease may attack glycoproteins in the cell wall and expose the inner layer of β-D-glucan to hydrolysis by the lytic β-D-glucanase, which releases laminaripentaose as the principal product of hydrolysis. Proteolysis was not required for lysis of isolated cell walls by the *endo*-β-1,3-D-glucanase. Most of the D-mannan of *S. cerevisiae* is attached to the cell wall *via* a D-glucan and is released following lysis of isolated cell walls by either β-1,3- or β-1,6-D-glucanases.[240]

The amounts of polysaccharides in the cell walls of synchronously growing *S. cerevisiae* have been investigated.[241] The amounts of D-mannan and total D-glucan increased linearly throughout the cell-cycle, although they remained fairly constant during division of the cells. The increase in D-glucans is characteristic insofar as the amounts of alkali-soluble and -insoluble D-glucans increase mainly during the first or the second half of the cell-cycle, respectively.

A mixture of polysaccharides, including a galactomannan, (1 → 3)-α-D-glucan, and a branched (1 → 3)-β-D-glucan, has been extracted with dilute alkali from the cell walls of *Schizosaccharomyces pombe*.[242] The alkali-insoluble residue contained a lightly branched (1 → 3)-β-D-glucan and small amounts of an extremely highly branched (1 → 6)-β-D-glucan. The synthesis of an alkali-insoluble β-D-glucan containing (1 → 3)- and (1 → 6)-linkages and of an alkali-soluble (1 → 3)-α-D-glucan continues during development of the fruit bodies of the Basidiomycete *Schizophyllum commune*, whereas that of a water-soluble β-D-glucan containing (1 → 3)- and (1 → 6)-linkages terminates at an early stage.[243] The extent of lysis of the cell walls of *Sclerotinia fructigena* varied with the age of the culture.[244] β-1,3-D-Glucanase activity was detected in the culture medium after autolysis had begun and it was also found in mycelial extracts and in cell walls during exponential and stationary phases of growth, confirming that there is a balance between the synthesis and lysis of cell-wall D-glucans during growth. The balance can be displaced towards lysis of the cell-wall D-glucans when synthesis stops owing to lack of nutrients.

A mutation in *Aspergillus nidulans* resulted in loss of both melanin and (1 → 3)-α-D-glucan (a principal cell-wall polysaccharide) and prevented the formation of cleistothecia.[245] The cell walls of the mutant strain contained more (1 → 3)-β-D-glucan and D-galactans than those of the parent strain. Examination of the cell walls of *A. niger* and *A. awamori* by *X*-ray methods, electron microscopy, and enzymic techniques showed that chains of nigeran occupy, at least, three domains or configurations in the hyphal walls and that they form a highly crystalline arrangement *in situ*.[246]

[238] M. Vršanská, Z. Krátky, and P. Biely, *Z. allgem. Mikrobiol.*, 1977, **17**, 391.
[239] M. Vršanaká, P. Biely, and Z. Krátky, *Z. allgem. Mikrobiol.*, 1977, **17**, 465.
[240] G. H. Fleet and D. J. Manners, *J. Gen. Microbiol.*, 1977, **98**, 315.
[241] M. Hayashibe, N. Abe, and M. Matsui, *Arch. Mikrobiol.*, 1977, **114**, 91.
[242] D. J. Manners and M. T. Myer, *Carbohydrate Res.*, 1977, **57**, 189.
[243] M. N. Schwalb, *Arch. Mikrobiol.*, 1977, **114**, 9.
[244] F. Reyes and R. Lahoz, *J. Gen. Microbiol.*, 1977, **98**, 607.
[245] I. Polacheck and R. F. Rosenberger, *J. Bacteriol.*, 1977, **132**, 650.
[246] T. F. Bobbitt, J. H. Nordin, M. Roux, J. F. Revol, and R. H. Marchessault, *J. Bacteriol.*, 1977, **132**, 691.

The D-glucosyltransferase from *A. niger* is able to catalyse the synthesis of 1,6-anhydro-β-D-glucopyranose from maltose.[247] The formation of the intramolecular ether linkage presumably involves displacement of the enzyme from a D-glucose–enzyme complex by HO-6 of the hexose, which must undergo a change of conformation. Methylation analysis and other studies have revealed that an alkali-soluble polysaccharide from purified mycelial walls of *Coprinus lagopus* is a β-D-glucan containing principally (1 → 3)-linkages and about 14% of (1 → 6)-linkages.[248] Partial acidic hydrolysis of the Smith-degradation product gave laminaribiose and gentiobiose, suggesting that the branches are generally longer than a single D-glucopyranosyl residue.

A strain of the yeast-like form of *Paracoccidiodes brasiliensis* on subculturing *in vitro* gradually lost its ability to synthesize a (1 → 3)-α-D-glucan in the cell wall, although this ability was partly recovered on growing the strain *in vivo*.[249] Since a mutant strain of *P. brasiliensis*, which contained a (1 → 3)-linked D-mannan instead of (1 → 3)-α-D-glucan in the cell wall, is much less virulent than the parent strain, the presence of (1 → 3)-α-D-glucan in the cell wall of the yeast-like form may contribute to the pathogenicity of the fungus (*e.g.* by acting as a protective layer against enzymes present in the leucocytes of the host). Synthesis of the (1 → 3)-α-D-glucan in the cell wall of *P. brasiliensis* could be induced *in vitro* by adding foetal calf serum to the growth medium.[250]

Mannans.—The chemistry, structures, and biosynthesis of D-mannans in the cell-wall envelopes of yeasts have been reviewed.[251]

Specific lactoperoxidase-catalysed iodination of the plasma membrane of intact protoplasts from *Candida albicans* has permitted relatively pure preparations of the plasma membrane to be isolated.[252] The specific activity of the labelled membrane was very low, indicating that only a few proteins are exposed on the outer surface of the membrane. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate, in conjunction with autoradiography, indicated that only two glycosylated proteins are exposed on the outer surface of the membrane, whereas all membrane proteins can be labelled in preparations of isolated plasma membranes. Purified plasma membranes incorporated D-mannose from GDP-D-mannose into an endogenous protein acceptor, and the addition of exogenous cell-wall D-mannoprotein increased the extent of incorporation. Approximately 12% of the incorporated D-mannosyl residues were removed by β-elimination with alkali and are presumably attached to the protein by *O*-glycosidic linkages. Phosphoric diester linkages in the cell-wall D-mannan from *S. cerevisiae* have been shown not to participate in the formation of Ca^{2+} ion-bridges during flocculation of this yeast.[253] Lysates of protoplasts from *S. cerevisiae* transferred D-mannose from either GDP-D-mannose or dolichyl D-mannopyranosyl phosphate to a polymer of high molecular weight.[254] Radiolabelling of polysome and mem-

[247] J. H. Pazur, Y. Tominaga, and L. S. Forsberg, *Arch. Biochem. Biophys.*, 1977, **182**, 774.
[248] H. P. Schaefer, *Arch. Mikrobiol.*, 1977, **113**, 79.
[249] G. San-Blas, F. San-Blas, and L. E. Serrano, *Infection and Immunity*, 1977, **15**, 343.
[250] G. San-Blas and D. Vernet, *Infection and Immunity*, 1977, **15**, 897.
[251] C. E. Ballou, *Adv. Microbial Physiol.*, 1976, **14**, 93.
[252] M. S. Marriott, *J. Gen. Microbiol.*, 1977, **103**, 51.
[253] P. M. Jayatissa and A. H. Rose, *J. Gen. Microbiol.*, 1976, **96**, 165.
[254] G. Larriba, M. V. Elorza, J. R. Villanueva, and R. Sentandreu, *F.E.B.S. Letters*, 1977, **71**, 316.

brane preparations and the release of incorporated D-mannosyl residues with alkali suggested that dolichyl D-mannopyranosyl phosphate participates in the D-mannosylation of hydroxylated amino-acids in the polysomes. This view is supported by other work, which examined the ability of enzymes in the light membranes (endoplasmic reticulum), the Golgi-like vesicles, and the plasma membrane to transfer D-mannosyl residues from GDP-D-mannose to D-mannoproteins and to dolichyl phosphate.[255] The first D-mannosyl residue of the *O*-glycosidically linked D-manno-oligosaccharides is incorporated with highest specific activity by the endoplasmic reticulum, while incorporation of the second, third, and fourth D-mannosyl residues is also catalysed, with increasing specific activity, by the Golgi-like vesicles and the plasma membrane. These results strongly suggest that D-mannosyl residues are attached to the protein component during its synthesis or shortly thereafter.

The binding of concanavalin A (tetrameric) and succinylated concanavalin A (dimeric) to a yeast (*Schizosaccharomyces pombe*) mannan gel has been studied.[256] D-Mannans on thin sections of *S. cerevisiae* and *C. utilis* cells can be located with either the homologous antibodies or concanavalin A labelled with colloidal gold.[257] Fully synthesized D-mannan was detected in the cell walls, on the plasmalemma, and in the cytoplasm, sometimes associated with vesicles and vacuoles.

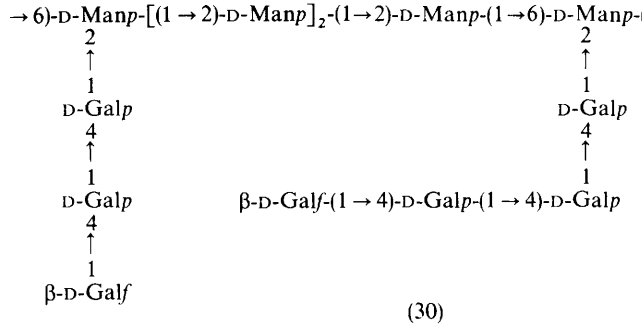
Chitin.—The presence of chitin in cell walls of the rumen organisms *Neocallimastix frontalis*, *Piromonas communis*, and *Sphaeromonas communis* was revealed by the release of 2-acetamido-2-deoxy-D-glucose with chitinase.[258] Wheat-germ agglutinin has been used to locate chitin or chito-oligosaccharides in bud scars and cell walls of *S. cerevisiae* and *C. utilis*.[257] Chitin and D-mannans could be located simultaneously in the cell walls using lectins labelled with colloidal gold. The cell walls of the edible mushroom *Agaricus bisporus* contain chitin (43%), an alkali-soluble D-glucan (14%), and an alkali-insoluble β-D-glucan (27%).[259] The growth of this fungus was inhibited by the antibiotic polyoxin D, which specifically inhibited the chitin synthase activity.[260] The biosynthesis of chitin by *A. fumigatus* appears to take place primarily at a membrane-bound site in the tips of hyphae and is inhibited by polyoxin D.[261] A high proportion of the chitin synthase activity in growing cultures of *A. nidulans* exists as an inactive zymogen, which is activated by proteolysis.[262] The fine structure of chitin synthase particles (chitosomes) from *Mucor rouxii* and that of the elaborated chitin microfribils have been examined by electron microscopy.[263] Chitin synthase has been isolated from the supernatant fraction of disrupted yeast cells of *M. rouxii* and purified; it is present as an inactive zymogen that is activated by proteolysis.[264] Most of the properties of this chitosomal chitin synthase are similar to those of the crude enzyme, except that the zymogen is less readily activated and inhibited. The extent

[255] L. Lehle, F. Bauer, and W. Tanner, *Arch. Mikrobiol.*, 1977, **114**, 77.
[256] M. Horisberger, *Carbohydrate Res.*, 1977, **53**, 231.
[257] M. Horisberger and M. Vonlanthen, *Arch. Mikrobiol.*, 1977, **115**, 1.
[258] C. G. Orpin, *J. Gen. Microbiol.*, 1977, **99**, 215.
[259] G. O. Michalenko, H. R. Hohl, and D. R. Rast, *J. Gen. Microbiol.*, 1976, **92**, 251.
[260] D. A. Wood and J. B. W. Hammond, *J. Gen. Microbiol.*, 1977, **98**, 625.
[261] D. B. Archer, *Biochem. J.*, 1977, **164**, 653.
[262] N. S. Ryder and J. F. Peberdy, *J. Gen. Microbiol.*, 1977, **99**, 69.
[263] C. E. Bracker, J. Ruiz-Herrera, and S. Bartnicki-Garcia, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 4570.
[264] J. Ruiz-Herrera, J. E. Lopez-Romero, and S. Bartnicki-Garcia, *J. Biol. Chem.*, 1977, **252**, 3338.

of invasion of stored grain by fungi can be assessed by measuring the amount of chitin, as 2-amino-2-deoxy-D-glucose, in the grain.[265]

Miscellaneous Cell-wall Polysaccharides.—The cell walls of *Agaricus bisporus* contain chitin and D-glucans.[266] Mild hydrolysis of the cell walls with acid released D-glucose, gentiobiose, and a branched tetrasaccharide composed of D-glucopyranosyl residues in (1 → 3)- and (1 → 6)-linkage. The carbohydrate compositions of isolated cell walls of meiospores of *Allomyces arbuscula* have been determined at various stages of growth.[267] Chitin was identified as the principal constituent of the cell walls of cysts, rhizoids, and hyphae, which also contain polysaccharides composed of D-galactose, D-glucose, D-mannose, L-arabinose, D-xylose, L-fucose, and L-mannose. The ratio of D-galactose to D-glucose in these polysaccharides decreases sharply during the differentiation of ungerminated cysts into germlings with rhizoids and hyphae, and is accompanied by a decrease in the proportions of D-xylose or L-fucose or both and D-galactose.

A galactomannan (30) extracted with alkali from the cell walls of *Aspergillus niger* is composed of chains of (1 → 2)-linked α-D-mannopyranosyl residues, which are joined through O-6 and to which oligosaccharide side-chains containing three or four D-galactosyl residues are attached.[268] Most of the side-chains are terminated by a β-D-galactofuranosyl residue.



(30)

A polysaccharide–fatty acid complex isolated from the surface of cells of *Candida tropicalis* (a yeast that grows on aliphatic hydrocarbons) is considered to form part of the hydrocarbon-binding system.[269] By comparison with other yeasts of the same genus, the cell walls of a *Candida* strain growing on aliphatic hydrocarbons contain less neutral sugars but more proteins and 2-amino-2-deoxy-D-glucose; part of the amino-sugar occurs as chitin, which represents 22% of the constituents of the cell wall.[270]

Studies with partially purified glycosidases have confirmed the presence of chitin and (1 → 3)-β-D-glucan in the cell walls of *Histoplasma capsulatum* chemo-

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types I and II.[271] Degradation of the cell walls of *H. capsulatum* chemotype I with a β-1,3-D-glucanase and pronase released a polysaccharide (mol. wt. 1.2×10^5) composed of residues of D-glucose, D-mannose, 2-amino-2-deoxy-D-glucose, and D-galactose, whereas 2-amino-2-deoxy-D-glucose was not found in a polysaccharide released by similar treatment of the cell walls of *H. capsulatum* chemotype II.[272]

Electron microscopy has revealed that there are two distinct layers in the cell walls of conidia and chlamydospores from *Fusarium sulphureum*.[273] The amounts of D-glucose and 2-acetamido-2-deoxy-D-glucose incorporated into polymers in the innermost layer increases appreciably during the differentiation of conidia to chlamydospores. D-Glucuronic acid is also found in polysaccharides from both layers.

Immunochemical studies on galactomannans isolated from the mycelia and culture media of *Hormodendrum pedrosoi*, *H. compactum*, and *H. dermatitidis* have indicated that acid-labile D-galactofuranosyl residues are responsible for precipitating antibodies.[274] The galactomannans and their respective core components precipitated anti-(*S. cerevisiae*) serum, which interacts only with (1 → 6)- or (1 → 2)-linked α-D-mannopyranosyl residues. At least three types of polymer have been extracted from the cell walls of *Mucor rouxii* with H_4edta, aqueous alkali, or acetic acid.[275] They consist of acidic polysaccharides of high molecular weight composed of residues of D-glucuronic acid, D-galactose, D-mannose, and L-fucose and small amounts of protein or peptide; glycopeptides of low molecular weight containing principally residues of D-mannose; and glycoproteins of high molecular weight containing high proportions of D-mannose and glycine. The synthesis of cell-wall D-mannan and D-glucan by a mutant strain of *S. cerevisiae* has been measured following inhibition of the synthesis of RNA and protein.[276] The results indicated that the mRNAs of cell-wall D-mannan–peptides and of D-glucan and D-mannan synthases are very stable (long-lived), thus accounting for the continuous synthesis of cell-wall polysaccharides during the entire cell-cycle.

The compositions of polysaccharides in the cell walls of spores, mycelia, and sporangiophores of *Phycomyces blakesleeanus* have been analysed.[277] Polysaccharides in spore cell walls contain more than 50% of D-glucose and roughly 20% of hexuronic acid, 10% of D-mannose, and 10% of amino-sugars. The polysaccharides in the hyphae contain amino-sugars (46%) and significant proportions of hexuronic acid, L-fucose, D-glucose, and D-galactose, whereas those in sporangiophores contain mainly (90%) 2-amino-2-deoxy-D-glucose (as chitin or chitosan) and 6% of hexuronic acid. Traces of L-rhamnose were detected in all of the cell-wall preparations.

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[277] A. J. van Laere, A. R. Carlier, and J. A. van Assche, *Arch. Mikrobiol.*, 1977, **112**, 303.

Differences have been observed in the morphology of the hyphae of *Picnoporus cinnabarinus* growing on acetate and on D-glucose.[278] Chemical and enzymic analyses of the compositions of the cell-wall polysaccharides revealed that those of the acetate-grown organism contain a higher proportion of amino-sugars and a higher ratio of 2-amino-2-deoxy-D-glucose to 2-amino-2-deoxy-D-galactose. The source of carbon in the growth medium also influenced the types of cell-wall polysaccharide synthesized by *Torulopsis* yeasts.[279] Chitin and D-mannan are the principal constituents of the cell walls when *Torulopsis* yeasts grow in the presence of methanol, whereas D-glucan and glucomannan preponderate when they grow in the presence of ethanol.

Yeast-forms and conjugation tube-forms of *Tremella mesenterica* contain similar amounts of α-(1 → 3)- and β-(1 → 3 and 6)-linked D-glucans and chitin.[280] Two highly branched polysaccharides from the fruit bodies of *T. fuciformis* each contains residues of D-xylose, D-mannose, D-glucuronic acid, and acetate.[281] Although there are differences in their structures, each polysaccharide is composed of a main chain of (1 → 3)-linked α-D-mannopyranosyl residues, which has D-glucopyranosyluronic acid and D-xylopyranosyl end-groups and to which side-chains containing (1 → 2)- and (1 → 3)-linked β-D-xylopyranosyl residues are attached at O-2. *O*-Acetyl groups are probably located on hexuronic acid residues.

The principal constituents of the cell walls of *Trichophyton mentagrophytes* are a neutral polysaccharide, probably (1 → 3)-β-D-glucan, and chitin.[282]

Tritiated 2-acetamido-2-deoxy-D-glucose was incorporated into the tips, hyphal walls, and septa of the lichens *Xanthoria parietina*, *Tornabenia intricata*, and a *Sarcogyne* sp. and into the free-living fungus *Trichoderma viride*, indicating that chitin is the principal component of the cell walls.[283] This result was confirmed by the binding of a fluorescein-conjugated agglutinin from wheat germ to the cell walls.

Extracellular Fungal Polysaccharides.—Neither of the two polysaccharides associated with the acid protease from *Aspergillus oryzae* is bound to the protein by a covalent linkage, although they may stabilize the protease during secretion.[284]

Nuclease P_1 from *Penicillium citrinum* is produced in a form of complex with malonogalactan (a D-galactan composed of (1 → 5)-linked β-D-galactofuranosyl residues that are esterified with malonic acid at O-3) in wheat-bran cultures.[285] The complex was not dissociated by purification procedures, which included fractionations with acetone and ammonium sulphate, gel filtration, and chromatography on DEAE-cellulose. The malonogalactan–nuclease P_1 complex was demalonylated by a carboxyesterase found in cultures of *P. citrinum* growing on wheat bran; the resulting D-galactan–nuclease P_1 complex was partially dissociated by chromatography on DEAE-cellulose. The properties of the malonogalac-

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tan–nuclease P_1 complex towards RNA and heat-denatured DNA were compared with those of the polysaccharide-free enzyme.[286]

Although the presence of tartrate in the growth medium did not affect the synthesis of an extracellular polymer containing 2-acetamido-2-deoxy-D-galactopyranosyl residues by *A. parasiticus*, the acetyl content of the polymer decreased, due, possibly, to the production of an acetylase.[287] Treatment of *Aureobasidium pullulans* with ethidium bromide produced several mutant strains, which, on culturing, formed a high proportion of yeast-like cells.[288] Comparison of several mutant strains with the wild-type strain suggested that the yeast-like cells are responsible for the synthesis of pullulan.

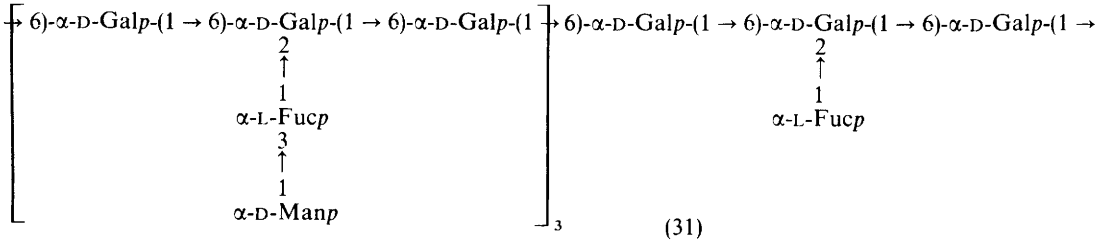
Partial acidic hydrolysis of a homogeneous D-glucan (pullulan) from the fruit bodies of *Cyttaria harioti* Fischer yielded principally isomaltose, whereas acetolysis, *etc.*, gave maltose and maltotriose.[289] Hydrolysis with amyloglucosidase and pullulanase, oxidation with periodate, and methylation analysis showed that this polysaccharide has a structure based on maltotriose residues joined by α-(1 → 6)-linkages. The structure (31) of the repeating-unit of a mannofucogalactan from the fruit bodies of *Flammulina velutipes* has been elucidated by methylation analysis, Smith degradation, and acetolysis.[290] This structure is similar to those of the mannofucogalactans obtained from other fungi of the *Basidiomycetes* group.

An extracellular polysaccharide (D-glucan) fraction from culture filtrates of *Fomes fomentarius* exhibited anti-tumour activity towards Ehrlich ascites carcinoma.[291]

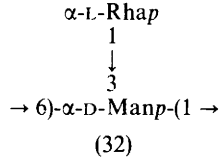
A viscous, extracellular, branched D-glucan from *Monilinia fructigena*, which also contains small proportions of D-mannose and D-galactose, is polydisperse over the molecular-weight range 5×10^3—8×10^4.[292] Oxidation of the polysaccharide with periodate showed that the D-glucopyranosyl residues are predominantly (1 → 4)-linked.

Enzymic hydrolysis of an α-D-glucan from *Polyporus circinatus* with α- and β-amylases indicated that it is a highly branched type of glycogen.[293] The effect of different extraction procedures on the yields of water-soluble and -insoluble glycogen fractions from a number of *Saccharomyces* strains has been investigated.[294] The similarities of the yields obtained using the different procedures showed that neither form of glycogen is an artifact. Variations in the relative amounts of glycogen in the two fractions during cell growth and in various strains of yeast suggested that two distinct pools of glycogen exist in the *Saccharomyces*. The water-insoluble glycogen is linked to a component, possibly a β-D-glucan, of the cell wall. Glycogen from the mycelia of a wild-type strain of *Neurospora crassa* has a molecular weight $>2 \times 10^7$, an average chain-length of 10, and

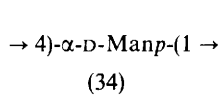
[286] M. Fujimoto, A. Kuninaka, and H. Yoshino, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 1121.
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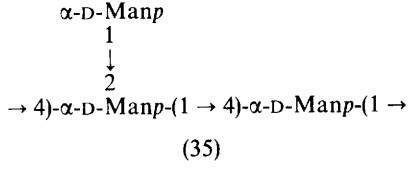
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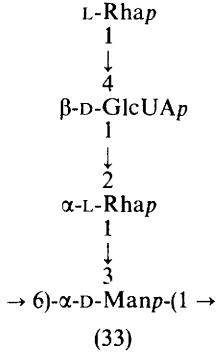
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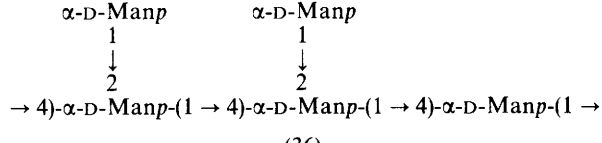
(34)



(35)



(33)



(36)

α- and β-amylolysis limits similar to those of typical animal and microbial glycogens.[295]

Both ^{13}C and ^{1}H n.m.r. studies of D-mannose-containing polysaccharides from *Sporothrix schenckii* and *Ceratocystis stenoceras*, two closely related fungi, have been extended.[296] Extracellular filtrates of *C. stenoceras* were shown to contain mainly a D-galactan and smaller amounts of a rhamnomannan (32) having single-unit α-L-rhamnopyranosyl side-chains, an acidic rhamnomannan (33) containing D-glucuronic acid residues, and amylose. Spectroscopic and chemical evidence was obtained for the presence of the structures (34), (35), and (36) in the extracellular polysaccharide of *S. schenckii*.

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5

Glycoproteins, Glycopeptides, and Animal Polysaccharides

BY B. J. CATLEY

Microbial Glycoproteins

X-Ray diffraction studies have shown that the haemagglutinin from the membrane of Hong Kong influenza virus has the unit-cell dimensions $a = 164$ and $c = 178$ Å.[1] The molecular weight of a glycoprotein from vesicular stomatitis virus and those of related molecules have been determined: that of the non-glycosylated precursor is 6.3×10^4, that of the desialylated glycoprotein is 6.7×10^4, and that of the complete glycoprotein is 6.9×10^4.[2] The fusion glycoprotein in the envelope of Sendai virus is responsible for the formation of the target antigen on cell walls coated with an inactivated virus.[3] A structure has been proposed for Semliki forest virus.[4] One of the glycoproteins (B_2) synthesized by a mutant strain of herpes simplex virus has been found to promote the fusion of membranes of human cells infected with the virus, whereas another viral membrane glycoprotein (C_2) suppresses the union.[5] The matrix protein from a nuclear polyhedrosis virus of the cabbage looper (*Trichoplusia ni*) is associated with small amounts of carbohydrate consisting of residues of D-mannose, D-glucose, D-galactose, and 2-amino-2-deoxy-D-galactose, although evidence for a covalent linkage between the carbohydrate and protein components was not presented.[6] The principal glycoprotein (mol. wt. $5.2. \times 10^4$) from murine mammary tumour virus has been obtained in a highly purified form; it associates readily with an accompanying viral glycoprotein of molecular weight 3.6×10^4.[7] Two glycoproteins, gp 70 and gp 45, from the envelope of Rauscher murine leukaemia virus are composed of essentially identical protein chains, but gp 70 (mol. wt. 6.75×10^4) contains 32% of carbohydrate whereas gp 45 (mol. wt. 4.75×10^4) contains 6–9%.[8]

Nitrate reductase, a membrane-bound respiratory enzyme of *E. coli* K12, is a glycoprotein containing neutral sugars and amino-sugars.[9] A luciferase from the luminescent bacterium *Photobacterium leiognathi* is also a glycoprotein, whereas an accompanying luciferase, which accounts for 20% of the enzymic activity, is not glycosylated.[10]

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The nature of the interactions with concanavalin A showed that the extracellular *endo*-β-1,3-D-glucanase from *Saccharomyces cerevisiae* is a D-mannoprotein similar to the extracellular invertase and acid phosphatase, whereas the *exo*-β-D-glucanase is a glycoprotein that does not contain a highly-branched D-mannan component.[11] Most of the carbohydrate component was removed from the extracellular invertase of *S. cerevisiae* by an *endo*-β-D-2-acetamido-2-deoxyglucosidase from *Streptomyces plicatus*.[12] The carbohydrate-depleted protein is composed of two subunits (mol. wt. 6×10^4), which are probably identical. An examination of two invertases produced by a synchronously grown mutant strain of *Saccharomyces* showed that, whereas the synthesis of the smaller enzyme is periodic, the synthesis of the larger enzyme (a glycoprotein) is continuous.[13] Tunicamycin inhibited the synthesis of the larger enzyme only. Both the intra- and extra-cellular forms of the α-D-galactosidase from *Saccharomyces carlsbergensis* appear to be identical glycoproteins.[14] A glycoprotein from *Epidermophyton floccosum*, which contains 60% of carbohydrate consisting almost entirely of residues of D-mannose, has a strong affinity for human C-reactive protein.[15] A purified α-D-galactosidase obtained from culture filtrates of *Aspergillus niger* contains residues of D-mannose and 2-acetamido-2-deoxy-D-glucose in the molar proportions 3 or 4:1; the carbohydrate component is probably linked to the polypeptide backbone through an asparagyl residue.[16] An intracellular β-D-mannosidase (a glycoprotein) from *A. niger* contains residues of D-mannose and 2-acetamido-2-deoxy-D-glucose in the ratio of 2.5 or 3:1.[17] A purified β-1,4-D-glucan cellobiohydrolase (mol. wt. 4.8×10^4) from *Trichoderma viride* contains D-mannose (26.4), D-glucose (4.8), D-galactose (2.4), and 2-amino-2-deoxy-D-glucose (3.4 residues).[18] Analysis of the saccharides released with alkaline borohydride showed that the cellobiohydrolase contains, on average, 8.8 mono-, 1.8 di-, 4.6 tri-, 1.2 tetra-, and 0.4 pentasaccharide chains, which are attached to seryl or threonyl residues in the polypeptide backbone. An examination of four D-glucan hydrolases from *T. viride* showed that, despite differences in their electrophoretic mobilities, they have similar u.v. absorptions, amino-acid and amino-sugar compositions, thermal stabilities, molecular weights, and specific activities; they differ only in the contents and compositions of the neutral hexose residues.[19] ^{1}H N.m.r. measurements on anti-tumour glycoproteins extracted from the mycelia of *Coriolus versicolor* showed that the carbohydrate components contain α- and β-(1 → 3)-, α- and β-(1 → 4)-, and α- and β-(1 → 6)-linkages.[20]

An investigation of the changing patterns of proteins and glycoproteins on the plasma membrane of *Dictyostelium discoideum* suggested that glycoproteins are involved in aggregation of the cells.[21] The level of 2-acetamido-2-deoxy-D-

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[20] Y. Inoue and R. Chûjô, *Carbohydrate Res.*, 1977, **56**, 351.
[21] S. Hoffman and D. McMahon, *Biochim. Biophys. Acta*, 1977, **465**, 242.

glucosyltransferase activity in isolated cell nuclei from the slime mould *D. discoideum* has been determined, and the roles of glycoproteins and glycosaminoglycans in the structure and in transcriptional and replicative processes of *D. discoideum* have been discussed.[22] Novel linkages between the protein and carbohydrate components of cutinase, an extracellular enzyme from *Fusarium solani f. pisi*, have been proposed; they involve the hydroxy-groups of β-hydroxyphenylalanyl and β-hydroxytyrosyl residues, as well as those of seryl and threonyl residues.[23] Further studies have indicated that the glycyl residue at the NH_2-terminus forms an amide linkage with D-glucuronic acid.[24] An extracellular anionic polysaccharide isolated from cultures of the unicellular red alga *Porphyridium cruentum* contains a small proportion of protein, which is linked through a seryl or threonyl residue to a D-xylosyl residue in the carbohydrate chain.[25] The carbohydrate compositions of glycoproteins isolated from several variants of *Trypanosoma equiperdum* at various stages of infection of rats appeared to change very little.[26]

Plant Glycoproteins

Glycoproteins in aqueous extracts of the leaves of *Cannabis sativa* of South African origin have been separated by chromatography on DEAE-cellulose into two fractions that contain residues of L-arabinose, D-galactose, D-glucose, D-mannose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-galactose, and, in one, D-galacturonic acid.[22,28] Methylation analysis and Smith degradation of the carbohydrate components suggested that there is a high degree of branching at the D-glucosyl and D-galactosyl residues, and that L-arabinofuranosyl residues occur at non-reducing termini and, possibly, as (1 → 5)-linked units in the main chain. The interior of the carbohydrate chains is composed mainly of (1 → 3)-linked D-galactopyranosyl residues. The carbohydrate chains are probably linked to the protein through the hydroxy-group of hydroxyprolyl and seryl residues. Glycoproteins from the leaves of *C. sativa* of Thailand origin have also been investigated.[29] Although there are minor differences in the quantitative compositions of the various sugars in the glycoproteins from plants of South African and Thailand origins, the most noticeable difference is the absence of hydroxyproline from glycoproteins in the latter source. Sequencing of the amino-acids of horseradish peroxidase revealed that neutral carbohydrate chains are attached to asparagine-13, -57, -158, -186, -198, -214, -255, and -268, which are located in the sequon Asn-*X*-Thr(Ser).[30] An analogous sequence involving the amino-acids 286—288 is apparently inaccessible to glycosylation. An hydroxyproline-rich glycoprotein from the spent media of cultured tobacco XD-6 cells is composed of 94% of carbohydrate and 6% of protein; the carbohydrate component consists of residues of D-galactose (44%), L-arabinose (30%), L-rhamnose (5%), and hex-

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[29] A. Hillestad, J. K. Wold, and T. Engen, *Phytochemistry*, 1977, **16**, 1953.
[30] K. G. Welinder, *F.E.B.S. Letters*, 1976, **72**, 19.

uronic acids (21 %), part of which is present as the methyl ester.[31] If the molecule is assumed to have a rod-like shape, its molecular weight was estimated to be 6×10^5. A rutin-like glycoprotein (mol. wt. 1.8×10^4) isolated from tobacco leaves and from condensates of cigarette smoke activated factor XII in human plasma *in vitro*, generating clotting, fibrinolytic, and kinin activities.[32] β-Conglycinin (a reserve protein in soybeans that consists of six principal isomers) has been dissociated and separated by ion-exchange chromatography into three principal subunits and a minor subunit.[33] Two of the principal subunits each contains two carbohydrate components, whereas the other contains only one.

Lectins

Reviews have appeared on the involvement of plant lectins in the recognition of, and defence against, bacteria[34, 35] and on the structures of plant lectins and the uses of plant lectins in studies of cell-surface components.[36, 37]

Extensive homologies have been noted among the amino-acid sequences at the NH_2-terminus of the β-chains of lectins from leguminous plants.[38] A comparative study of 2-acetamido-2-deoxy-D-galactose-binding lectins from *Helix pomatia*, soybeans, lima beans, and *Dolichos biflorus* has been reported.[39] It showed that the size of the carbohydrate-binding site of each lectin is relatively restricted and also yielded information on the specificities of the lectins and the groups on 2-acetamido-2-deoxy-D-galactopyranose that are in contact with the carbohydrate-binding site of the lectins.

Affinity electrophoresis has been used to determine the dissociation constants of lectin–sugar complexes, which were calculated from the relation between the electrophoretic mobility of the lectin and the concentrations of immobilized and free sugar in the polyacrylamide gel.[40] Affinity electrophoresis also showed that an acetylated (non-haemagglutinating) derivative of the lectin from lentil (*Lens esculenta*) seeds and the photo-oxidized and sulphenylated derivatives of the lectin from peas (*Pisum sativum*) retain their sugar-binding properties.[41] Affinity chromatography using reductively aminated lactose, melibiose, maltose, or di-*N*-acetylchitobiose has been used to purify lectins from peas, castor and jack beans, lentils, wheat germ, and *Bandeiraea simplicifolia*.[42] The use of butane-1,4-diol diglycidyl ether to couple saccharides to Sepharose should prove useful in the preparation of affinity matrices for the purification of lectins.[43] 6-Aminohexyl β-D-mannopyranoside bound to Sepharose did not interact with concanavalin A.[44]

The results of kinetic studies on the binding of 4-methylumbelliferyl α-D-manno-

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[44] M. A. E. Shaban, D. K. Podolsky, and R. W. Jeanloz, *Carbohydrate Res.*, 1976, **52**, 129.

pyranoside to the tetrameric form of concanavalin A are consistent with a single-step bimolecular association that is not diffusion controlled.[45,46] No kinetic evidence was obtained for any interaction between the saccharide-binding sites or for a conformational change in the protein on binding the glycoside. Related investigations on the binding of this glycoside to the dimeric form of concanavalin A again indicated that a single-step bimolecular association takes place.[47] Glycopeptides from transferrin that contain two peripheral Neu*N*Ac*p*-D-Gal*p*-D-Glc*N*Ac*p* units linked to the core protein were bound by Sepharose–concanavalin A, whereas a glycopeptide from fetuin that contains three of the trisaccharide units was not; this difference in behaviour appears to reside in the substitution pattern of D-mannopyranosyl residues in the core pentasaccharide.[48] Measurements of the kinetic parameters for the binding of 4-nitrophenyl α-D-mannopyranoside to concanavalin A and for dissociation of the complex showed that the entropies of activation are much smaller than those usually associated with changes in the conformation of the protein chain.[49] Unequivocal assignments to the H-2 signal of specific histidyl residues in the amino-acid sequence have been made in the 220 MHz ^{1}H n.m.r. spectra of complexes of concanavalin A with Co^{2+} and Zn^{2+} ions.[50] Glycolipids (*e.g.* D-glucosyl- and lactosyl-ceramide) incorporated into planar bilayer membranes interacted with concanavalin A, depending on the positions of D-glucopyranosyl residues in the carbohydrate moiety.[51]

A lectin with a specificity directed towards 2-acetamido-2-deoxy-α-D-glucopyranosyl residues has been extracted from *Bandeiraea simplicifolia* seeds.[52] Five isolectins that interact with α-D-galactopyranosyl residues have also been extracted from the seeds.[53] All of them are tetramers composed of two subunits; one of the subunits interacts with both 2-acetamido-2-deoxy-α-D-galactopyranosyl and α-D-galactopyranosyl residues, whereas the other interacts with α-D-galactopyranosyl residues only. A second haemagglutinin isolated from *B. simplicifolia* seeds interacted with type III polyagglutinable receptors present on 'acquired-B' red cells.[54]

Lectin is preferentially released during the first 8 h of the uptake of water by soybean seeds.[55] Azide ion, a potent inhibitor of oxidative phosphorylation and cellular metabolism, did not inhibit the release of lectin, which may indicate that soybean lectin is not a cellular component, as has been suggested. A highly-specific and sensitive assay for $\geqslant 0.1$ μg of soybean agglutinin, which could, in principle, be applied to the assay of other lectins, has been reported.[56] The high specificity of the assay derives from competition for the carbohydrate-binding sites on the agglutinin between 2-acetamido-2-deoxy-D-galactopyranose (as a conjugate with Sepharose) and ^{125}I-labelled antibodies raised against the lectin.

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Dimeric wheat-germ lectin (as determined by *X*-ray analysis at 2.2 Å resolution) consists of two closely associated protomers (each containing 164 amino-acid residues) centred about the crystallographic two-fold axis.[57] Two carbohydrate-binding sites of the lectin were identified.

The association of lectins with albumins and globulins in cotyledons from mature seeds of *Phaseolus vulgaris* has been examined, and the interactions of the lectins with macromolecules present in extracts of the seeds have been discussed.[58] The phytohaemagglutinin from *P. vulgaris* consists of a group of five tetrameric proteins composed of all possible combinations of two subunits, which appear to be synthesized independently *in vivo* before they combine randomly to form the phytohaemagglutinin.[59] The dissociation and the recombination of the subunits have been studied.[60] These lectins are strongly inhibited by the trisaccharide *O*-β-D-galactopyranosyl-(1 → 4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-D-mannopyranose.[61] A lectin (mol. wt. 1×10^5) from a Brazilian cultivar of *P. vulgaris* contains neutral sugars (9.1 %) and amino-sugars (1.4 %).[62]

A galactomannan (from guar gum) embedded in a polyacrylamide gel has been used as the affinity matrix in the isolation of peanut agglutinin.[63] Immunological studies showed that peanut agglutinin interacts most strongly with the disaccharide 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactopyranose.[64] The pattern of precipitation of peanut agglutinin with blood-group substances resembles that of anti-I cold autoagglutinins. Besides being useful in diagnosing T-polyagglutination, peanut agglutinin should prove to be an important reagent in structural studies of glycoproteins and cell-surface receptors.

The structures of a lectin and the toxin ricin D from castor beans (*Ricinus communis* L.) have been compared.[65] The protomers (mol. wts. 3.4×10^4 and 3.1×10^4) of the tetrameric lectin are homologous with, but not identical to, those of the dimeric toxin. Microcalorimetric measurements of the interaction between ricin D and D-galactose or lactose afforded values for ΔG, ΔH, and ΔS.[66] The binding constants indicated that lactose binds more strongly than D-galactose. Lectins have been shown to be plant-membrane components, and it was proposed that they participate in the initial stages of recognition and contact between different intracellular membranes in the fusion processes that form the basis of endo-membrane flow within the cell.[67] Abrin A, a toxic lectin from *Semen jequiriti* (the seeds of *Abrus precatorius* L.), inhibited not only the elongation of peptide chains but also the initiation of peptide synthesis in cell-free systems from wheat germ.[68]

Two haemagglutinins (mol. wts. 1.3×10^5 and 1.05×10^5) from *Bordetella*

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pertussis are glycoproteins containing 6.8% and 25% of carbohydrate, respectively.[69] The symbiotic relationship between the clams *Tridacna maxima* or *T. gigas* and algae containing D-galactans is thought to be mediated by anti-D-galactan precipitins in the haemolymph of the clams.[70] A specificity for sialic acid residues has been found in a haemagglutinin that occurs in tissue fluids of the Pacific oyster *Crassostrea gigas*.[71] A β-D-galactoside-specific lectin from the thigh muscles of chicken embryos is now available in a highly purified form; since it contains traces of carbohydrate, it may be a glycoprotein.[72] Two lectins—one membrane-bound and the other a soluble form—specific for β-D-galactopyranosyl residues have been found in calf's heart; the soluble form is a dimer composed of subunits of molecular weight 9×10^3.[73] A lectin displaying similar specificity has been found in calf's lung.

Antibodies have been raised in rabbits against di-*N*-acetylchitobiose and purified in order to obtain cell-surface probes that are monovalent.[74] The biological and structural properties of these specific antibodies allow new approaches to the study of cell-surface carbohydrates.

Blood-group Substances

A glycoprotein (mol. wt. 1×10^5) that exhibits blood-group A activity has been isolated from oyster viscera; it contains residues of D-galactose (12.8%), L-fucose (9.9%), 2-amino-2-deoxy-D-glucose (4.6%), 2-amino-2-deoxy-D-galactose (16.6%), and D-glucose (3.3%), but no residues of sialic acid.[75]

The α-D-galactosyltransferase (the group-B gene-specified transferase) from human sera has been purified (10^5-fold) by affinity chromatography on blood-group O red-cell membranes and elution with 2′-*O*-α-L-fucopyranosyl-lactose.[76] Blood-group B specificity can be converted into blood-group H specificity using a purified α-D-galactosidase from soybean meal.[77] A trisaccharide{*O*-α-D-galactopyranosyl-(1 → 3)-[*O*-α-L-fucopyranosyl-(1 → 2)]-D-galactopyranose} found in the urine of individuals from blood-group B was also excreted by a Rhesus monkey (serogroup B) that had been fed D-galactose.[78] The use of D-[^{14}C]galactose (administered by mouth or through an intestinal vein) showed that this trisaccharide is synthesized in the intestines.

Contrary to previous evidence, the H-determinant disaccharide 2-*O*-α-L-fucopyranosyl-D-galactopyranose is now thought to occur only on the type-1 chain, rather than on both type-1 and -2 chains, of the branched core structure.[79] The case histories of a family with fucosidosis have demonstrated the importance of α-L-fucosidases in the catabolism of Le antigens on the red cells.[80] The high

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levels of Le^b antigens—in contrast to the normal levels of Le^a antigens—suggested that there is a deficiency of α-1,2-L-fucosidase in these particular cases.

Collagens

The view that the adhesion of platelets to collagen is mediated by the formation of a complex between a D-glucosyltransferase on the platelet membrane and incomplete carbohydrate chains in collagen is no longer tenable, since it has been reported that native collagen is not a substrate for the collagen D-glucosyltransferase from platelets, which requires the conformation of the collagen substrate to be non-helical.[81] A purified D-glucosyltransferase from chicken embryos probably consists of a single polypeptide chain of molecular weight $7.2—7.8 \times 10^5$.[82] Purification of the collagen D-glucosyltransferase from either chicken embryos or human foetal tissues has been achieved by affinity chromatography on agarose–concanavalin A; hydrophobic interactions appear to contribute to the affinity of the enzyme for agarose–concanavalin A.[83] D-Glucosyltransferase activity, but not D-galactosyltransferase activity, is present in human sera and may originate from extravascular tissues.[84] Chicken-embryo tissues producing type II collagen contain higher levels of collagen glycosyltransferase activities than those producing type I or III collagen, and it is clear that the glycosyltransferases help to regulate the carbohydrate content of the collagen synthesized by growing tissues.[85] The high levels of glycosyltransferase activities found in the leg tendon of chicken embryos are probably related to the high rate of synthesis of collagen in these tissues. That the glycosyltransferases and amino-acid hydroxylases involved in the synthesis of collagen are not controlled in an identical manner was inferred from the 4—6-fold increase in the transferase activities in human foetal skin compared with that in adult skin, which is less than those occurring in the hydroxylase activities.[86] Cortisol diacetate inhibited both hydroxylase and glycosyltransferase activities in embryonic chicken tendon, thus decreasing the amount of collagen synthesized.[87] It has been shown that once collagen forms a triple helix further hydroxylation of lysyl residues and the glycosylation of hydroxylysyl residues already present are prevented.[88]

An improved method for determining hydroxylysyl, D-galactosylhydroxylysyl, and D-glucosyl-D-galactosylhydroxylysyl residues has shown that the collagen composition of long-standing scar tissues gradually reverts to normal.[89] The number of glycosylated amino-acid residues in chicken-skin and -bone collagens decreases with age from about nine to four and from seven to four, respectively.[90] The hydroxylysyl residue in the non-helical region at the NH_2-terminus of the α(I)-chain of embryonic collagens is not extensively glycosylated. Compatible with the reluctance of this hydroxylysyl residue to undergo glycosylation, the glycosylated

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residue occurs entirely (in bone) or mostly (in skin) as D-galactosylhydroxylysine.

Tunicamycin significantly inhibited the incorporation of D-mannosyl residues into procollagen by cultures of chicken bone and fibroblasts, whereas the synthesis of collagen decreased by only 10—20%.[91] Procollagen synthesized in the presence of tunicamycin was secreted normally and its immunological characteristics were unchanged. However, tunicamycin inhibited the conversion of procollagen into collagen, resulting in the accumulation of biosynthetic intermediates containing disulphide-bridge extensions at the CO_2H-terminus. These findings support the view that more than one enzyme is involved in the limited proteolysis that converts procollagen into collagen.

The difficulties encountered in characterizing glycoproteins in glomerular basement membranes by immunological methods have been discussed.[92]

Glycogen

The structure of glycogen from the livers of rats that had been fasted and then intubated with D-glucose differed little, if at all, from that from control animals.[93] The glycogen (mol. wt. 5×10^8) associated with rat-liver lysosomes is larger than, and has a different molecular-weight distribution from, that in the cytosols.[94] The hydration and hydrodynamic properties of rabbit-liver glycogen have confirmed that it has a high molecular weight.[95] There is evidence that rabbit-liver glycogen is attached to a protein containing disulphide linkages.[96] Protein-bound glycogen of high molecular weight and an excess of glycogen of low molecular weight were found in the livers of rabbits on refeeding after starvation.[97] This result suggests that glycogen is synthesized by two pathways: one affording a polysaccharide of high molecular weight which is attached to a protein backbone capable of forming disulphide linkages, and another affording a polysaccharide of low molecular weight which either contains no protein or is attached to a protein backbone that is unable to form disulphide linkages. The number of B chains in shellfish glycogen exceeds that of the A chains, and there is evidence indicating that the B chains occur at the periphery of the molecule.[98]

A possible role for carcase glycogen as a source of D-glucose during short-term starvation has been investigated.[99] When deprived of food for 19 h, rats mobilized 64% of their liver glycogen and 36% of their carcase glycogen.

Glycerol-gradient centrifugation has been used in the purification of rabbit-muscle glycogen particles, which were obtained completely free from sarcoplasmic reticulum membranes.[100] The identification and assay of glycogen by routine procedures are of dubious value unless attention is paid to the variable appearance of glycogen particles and unless verification is sought using specific cytochemical

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procedures.[101] A preparation of rat hepatocytes that maintains *in vitro* the characteristic responsiveness to insulin has been reported.[102]

The actions of adrenalin, [8-arginine]vasopressin, and angiotensin have confirmed that the degradation of liver glycogen is stimulated by a wide variety of hormones.[103] The effects of hormones on the turnover of glycogen in perfused rat liver has been examined, particularly the control of glycogenolysis by hormones that may or may not be cAMP-dependent.[104] Chlorothiazide inhibited the accumulation and utilization of glycogen by slices of canine aorta *in vitro*.[105]

The ability of liver cells, isolated from rats on a controlled feeding schedule, to synthesize glycogen has been examined.[106] The results indicated that different fractions of the cell population may be involved in the synthesis of glycogen at any particular time of day. *In vitro* studies with rat skeletal muscle have shown that insulin stimulates the phosphorylation of exogenous uridine, thereby increasing the cellular concentration of UTP and the rate of synthesis of glycogen.[107] This provides a mechanism by which insulin can stimulate the synthesis of glycogen that is additional to the well-established activation of glycogen synthase. Little difference was found between the metabolism of muscle glycogen by diabetic rats and by normal rats during exercise.[108]

Large doses of 2-amino-2-deoxy-D-galactose induced acute hepatic injury in mice, largely due to the synthesis and toxic effects of 'aminoglycogens'.[109] An increased availability of fatty acids reduced the rate of depletion of glycogen in the slow and fast red muscles of rats during exercise, although it had no effect on the glycogen in fast white muscles.[110] The amount of glycogen in the livers of animals with increased fatty-acid levels decreased by 23% during exercise, compared with that of 80% in controls.

An improved assay for glycogen synthase in rat-liver homogenates, which avoids any degradation of glycogen, used an anion-exchange resin to separate glycogen from phosphorylated compounds and other electrolytes.[111] A rapid procedure for assaying glycogen phosphorylases *a* and *b* was also described. The cycle of fasting and feeding that controls the amount of glycogen in rat liver has been studied in relation to the levels of phosphorylase and synthase activities.[112] The participation of these enzymes in the metabolism of glycogen has also been examined in the livers of developing rats.[113] The results suggest that the ratio of the active forms of phosphorylase and synthase may be important in regulating the storage of glycogen in liver and that, under basal conditions, there could be a continuous turnover of glycogen.

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Despite proteolysis, the trichloroacetic acid-insoluble glycogen (desmo-glycogen) from rat liver could not be freed from peptide material, which remained in close association with the polysaccharide throughout a number of purification procedures.[114] The occurrence of protein-bound glycogen lends substance to the view that a protein may prime the synthesis of glycogen. Short chains of protein-bound malto-oligosaccharides could then be extended by glycogen synthase. The kinetic constants for the reactions of a series of D-glucosyl acceptors (*e.g.* D-glucose, maltose, malto-oligosaccharides, and amylopectin) with the I form of rabbit-muscle glycogen synthase has led to the proposal that the enzyme possesses two sites—a polysaccharide-binding site and a separate catalytic site where the reaction occurs.[115] Insulin stimulated the synthase D phosphatase in rat heart by inducing a stable modification of either the enzyme itself or its substrate glycogen synthase D, making it a better substrate for the reaction.[116] Insulin also appears to stimulate the glycogen synthase activity of fat cells by at least two mechanisms, but only one of them requires D-glucose.[117] Human polymorphonuclear leucocytes might control the conversion of glycogen synthase D into the I form by two mechanisms: one relies on inhibition by a combination of glycogen and magnesium-ATP, which is counteracted by D-glucose 6-phosphate, whereas the other depends on inhibition of the synthase D phosphatase by phosphorylase *a*.[118] Glycogen synthase D from human placenta can be activated by manganese sulphate in the absence of D-glucose 6-phosphate, although Mn^{2+} and sulphate ions had no effect separately.[119] At physiological concentrations of UDP-D-glucose and D-glucose 6-phosphate, concentrations of <1 mmol l^{-1} of either Mn^{2+} ions or manganese sulphate increased the glycogen synthase D activity by as much as did 8 mmol l^{-1} of D-glucose 6-phosphate. The high concentrations and wide distribution in rat tissues of cAMP-independent glycogen synthase kinases, which account for most of the glycogen synthase kinase activity in many tissues, suggest that these enzymes are of physiological importance.[120] A cAMP-independent protein kinase from rabbit skeletal muscle is able to phosphorylate glycogen synthase I, giving the D form.[121] The properties of the soluble cAMP-dependent protein kinases from bovine cardiac muscle have been reviewed.[122] Glycogen synthase phosphatase and phosphorylase phosphatase from rat liver have been separated by chromatography on DEAE-cellulose; they differ in their stabilities to heat, proteases, and 2-mercaptoethanol.[123]

Three protein phosphatase activities have been isolated from rabbit skeletal muscle: protein phosphatase I (mol. wt. 3×10^5, an active histone phosphatase) exhibited only little glycogen synthase phosphatase-1 and -2, phosphorylase

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kinase phosphatase, and phosphorylase phosphatase activities; protein phosphatase II (mol. wt. 1.7×10^5, another active histone phosphatase) was active towards the α-subunit of phosphorylase kinase and accounted for 10–15% of the glycogen synthase phosphatase-1 and -2 activities, < 5% of the activity towards the β-subunit of phosphorylase kinase, and 1—2% of the phosphorylase phosphatase activity; protein phosphatase III exhibited 95% of the phosphorylase phosphatase and β-phosphorylase kinase phosphatase activities and 75% of the glycogen synthase phosphatase-1 and -2 activities.[124] Its properties suggested that protein phosphatase III catalyses each of the dephosphorylation reactions that either inhibit glycogenolysis or stimulate the synthesis of glycogen. Protein phosphatase III was separated into two components (mol. wts. 7.5×10^4 and 4.6×10^4) of identical specificity by chromatography on Sephadex G-200. Both forms could be specifically inhibited by a purified, heat-stable protein from rabbit skeletal muscle.[125] The specificity of this inhibitor can be used to distinguish protein phosphatase III from protein phosphatases I and II. Two heat-stable inhibitors of phosphorylase phosphatase—one of which can be phosphorylated—are present in rabbit skeletal muscle; they are distinguishable from the inhibitor of cAMP-dependent protein kinase.[126] The phosphorylase phosphatase in dog liver exists in two forms: either as a complex with glycogen or in the cytoplasm.[127] The enzymes exhibit different properties and do not interconvert.

A review has discussed the ligand-binding sites and the subunit interactions of muscle phosphorylase *a* that have been deduced from the crystal structure.[128] The catalytic activities of tetragonal crystals of cross-linked muscle phosphorylases *a* and *b* have been examined, since the conformation of either enzyme in the crystals might be totally or partly unrelated to the 'active' conformation of the enzyme in solution.[129] Kinetic data suggested that the binding of substrates is similar for the crystalline and soluble enzymes, although the crystalline enzymes are less active, owing either to differences in the active sites of the enzymes caused by crystallization or to the crystal restricting a change of conformation that is essential for catalysis. ^{31}P N.m.r. spectroscopy has been used to study the glycogen phosphorylase *b* from rabbit muscle.[130] The use of adenosine 5′-*O*-thiophosphate and arsenate (instead of inorganic phosphate) enabled three distinct forms of enzyme-bound pyridoxal 5′-phosphate to be observed. These forms can be interconverted by the allosteric transition induced by AMP or adenosine 5′-*O*-thiophosphate or, more efficiently, by phosphorylation of the enzyme to give phosphorylase *a*. Phosphorylase *b* inactivated with 5-diazo-1*H*-tetrazole has been labelled by reaction with either 4-(iodoacetamido)salicylic acid (a fluorescent probe) or *N*-(2,2,6,6-tetramethyl-4-piperidinyl-1-oxide)iodoacetamide (a spin-labelled probe.[131] Use of these probes indicated that inactivation of phosphorylase

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b does not interfere with either the binding of AMP and D-glucose 6-phosphate or the resulting conformational changes of the protein chain, although it does affect the binding of D-glucose 1-phosphate and glycogen. Further studies confirmed that inactivation of phosphorylase *b* occurs by modification of a carboxylic acid group that is involved in catalysis. A ^1H n.m.r. investigation of the conformations adopted by nucleoside 5′-phosphates bound to the active site of glycogen phosphorylase *b* showed that the purine base of such strong activators as AMP has an *anti* orientation, whereas it assumes a *syn* orientation with such weak activators as inosine 5′-phosphate.[132]

Glycogen phosphorylases *a* and *b* from human liver have been purified.[133] Phosphorylase *b* has a low affinity for D-glucose 1-phosphate, which is not affected by AMP, and a low catalytic activity, which becomes equal to that of phosphorylase *a* in the presence of AMP. Lyotropic anions (*e.g.* fluoride and sulphate) stimulated phosphorylase *b* and inhibited phosphorylase *a* by modifying their affinities for D-glucose 1-phosphate. Both forms of the enzyme are easily saturated with glycogen. At least five molecular forms of phosphorylase from various organs of rats and pigs have been separated by electrophoresis.[134] The ability of AMP and other nucleotides to activate the isoenzymes of glycogen phosphorylase *b* from rabbit muscle, brain, and liver has been studied.[135] Muscle, brain, and hybrid muscle-brain isoenzymes were strongly and specifically activated by AMP and its analogues, whereas the liver isoenzyme was little affected. Antibodies raised in rabbits against a purified glycogen phosphorylase *b* from porcine skeletal muscle were able to inactivate not only homologous and heterologous antigens, but they also cross-reacted with the phosphorylase *b* of the immunized animal.[136] Kinetic experiments showed that the antibodies and autoantibodies act as non-competitive inhibitors of these phosphorylases with respect to AMP and D-glucose 1-phosphate, and that they exhibit a mixed type of inhibition with respect to glycogen. Kinetic studies of the inhibition of rabbit-muscle phosphorylase *b* and of potato phosphorylase by D-glucopyranosyl fluoride, D-glucose, D-glucopyranosylamine, 1- and 5-thio-D-glucopyranose, and 5-amino-5-deoxy-D-glucopyranose (nojirimycin) indicated that D-glucose 1-phosphate binds in the $^4C_1$ conformation to the enzymes, forming a hydrogen bond between O-1 and a hydrogen donor of the enzyme.[137]

Synthetic peptide variants of the sequence Ser-Asp-Gln-Glu-Lys-Arg-Lys-Gln-Ile-Ser-Val-Arg-Gly-Leu, which is found in phosphorylase *b*, have been used to reveal the structural features that are important for the interaction between rabbit-muscle phosphorylase *b* and phosphorylase *b* kinase.[138] The results indicated that the important determinants in phosphorylase *b* recognized by phosphorylase *b* kinase reside in the linear sequence of amino-acids, particularly in the sequence Lys-Gln-Ile-Ser-Val-Arg. Although the precise role of these residues in the interaction with phosphorylase *b* kinase is not known, it appears that the

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kinase prefers a spatially organized structure in its substrates—this is suggested by differences between the reactivities of the synthetic peptides and phosphorylase *b* as substrates. Since the synthetic peptides were also phosphorylated by cAMP-dependent protein kinase, the failure of this kinase to phosphorylate phosphorylase *b* might be due to a feature of the secondary structure of phosphorylase *b* which exposes a residue, probably arginine-16, that inhibits recognition by the protein kinase. A relationship between the enzymic activity and the extent of phosphorylation of rabbit-muscle phosphorylase, which has already been established *in vitro* (P. J. Roach, Y. Takeda, and J. Larner, *J. Biol. Chem.*, 1976, **251**, 1913; P. J. Roach and J. Larner, *J. Biol. Chem.*, 1976, **251**, 1920), has been confirmed *in vivo* for rabbits administered insulin.[139] An investigation of the enzyme cascade that stimulates glycogenolysis has shown that activation of the phosphorylase in rat liver cannot always be related to a change in the activity of phosphorylase kinase.[140] The stimulation of glycogenolysis by angiotensin, vasopressin and phenylephrine, which act independently of cAMP, has been compared with that by glucagon, which depends on cAMP.[141] Calcium ions participate in the processes that stimulate these hormones[141] and in the α-adrenergic stimulation of rat-liver phosphorylase.[142] Administration of insulin to anaesthetized rabbits resulted in rapid inactivation of both phosphorylase and phosphorylase kinase, but did not change either the concentration of cAMP or the activity of the cAMP-dependent protein kinase.[143] The stimulation of glycogenolysis in perfused rat heart by sodium salicylate and aspirin is associated with an increase in the concentration of phosphorylase *a*.[144]

The activity of branching enzymes from muscle may be controlled by RNA present in the extract.[145]

An improved fluorometric assay for the 'acid' α-1,4-D-glucosidase in leucocytes has facilitated the detection of Pompe's disease (glycogen-storage disease type II).[146] Possible interference by 'neutral' α-D-glucosidase in the diagnosis of Pompe's disease in cultured cells has been investigated.[147] The 'neutral' α-D-glucosidase activity could be removed completely by preliminary precipitation at pH 5, without affecting the 'acid' α-D-glucosidase activity. The 'neutral' α-D-glucosidases from various human tissues have been separated by electrophoresis into two components, the mobilities of which differed from those of the enzymes from tissues of adults with Pompe's disease.[148] A method that distinguishes between the 'neutral' and 'acid' α-1,4-D-glucosidases in human tissues is based on the effects of potassium chloride on their activities at pH 4; the lysosomal ('acid') form is stimulated, whereas the 'neutral' form is inhibited, by potassium chloride at this pH.[149] A micro-assay for the 'acid' α-1,4-D-glucosidase in cultured skin fibroblasts,

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etc., uses [^{14}C]maltose as the substrate in a total assay volume of 8 μl; D-[^{14}C]-glucose released is separated (by t.l.c. on cellulose) and counted.[150] Low levels of phosphorylase kinase, which is virtually absent from haemolysates of patients with glycogen-storage disease type VI, have been detected in the patients' leucocytes, while the level of the enzyme in their muscles appeared to be normal.[151] Some patients have low levels of total phosphorylases *a* and *b*, while others have high levels of phosphorylase kinase activity. The levels of phosphorylase phosphatase were also monitored. Glycogen synthase, which was deficient in the liver of a male patient with hypoglycaemia, was found in his erythrocytes and leucocytes, indicating that the analysis of blood cells could give misleading results in the diagnosis of such disorders.[152] Platelets from patients with multiple myeloma were shown to have increased levels of glycogen and glycogen synthase *a* and a low level of phosphorylase *a*.[153] Prior incubation of the patients' platelets in a control plasma increased the rate of activation of glycogen synthase and abolished the activation of phosphorylase. Fluctuations in the levels of glycogen synthase activity and glycogen during inflammation of human gingival epithelia have been studied.[154] A model polysaccharide-storage disease can be produced by intraperitoneal administration of dextran to rats, one result of which is to increase the level of dextran in their livers.[155] Intravenous administration of dextranase trapped in liposomes rapidly decreased the amount of accumulated dextran.

Glycosaminoglycuronans and Glycosaminoglycans

The chemistry of heparin, the crystal structures of sodium and calcium heparinates, the clinical uses, *etc.*, of heparin,[156] and the biosynthesis and structures of dermatan sulphate, heparin, and heparan sulphate[157] have been reviewed. The structures and the conformations of glycosaminoglycans are discussed in a book dealing with the shapes of polysaccharides.[158]

Analytical Methods.—Individual glycosaminoglycans in 250 mg of aortic tissue have been determined following electrophoresis on cellulose acetate, enzymic digestion, and assay of the released 2-acetamido-2-deoxy-D-glycoses.[159] Radiolabelled glycosaminoglycans in cell-culture media have been measured following digestion with papain and precipitation with cetyl pyridinium chloride onto filtration membranes of a cellulose ester, which can be washed to remove other radiolabelled materials.[160] Hyaluronic acid can be eluted from the filtration membrane with dilute hydrochloric acid, whereas other glycosaminoglycans are retained. Glycosaminoglycans have been separated by one- or two-dimensional

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electrophoresis on agarose gels in the presence of diamine buffers; 1–10 μg suffice for separation and detection.[161] Similar amounts of chondroitin 4- and 6-sulphates can be determined by enzymic hydrolysis and oxidation of the released disaccharides with periodate.[162] Chondroitin 4- and 6-sulphates can also be determined using the thiobarbituric acid procedure, whereafter chondroitin 6-sulphate can be determined specifically using alkaline 4-dimethylaminobenzaldehyde. The unsaturated disaccharides released on enzymic hydrolysis of the chondroitin sulphates can be separated efficiently by t.l.c. on cellulose.[163] The ratio of *N*- to *O*-sulphate has been used to characterize heparan ^{35}S-sulphates.[164] An almost completely desulphated, *N*-acetylated heparin was obtained by brief treatment of an *N*-desulphated and *N*-acetylated heparin with DMSO containing 10% of methanol at 100 °C. Other glycosaminoglycuronan sulphates were also readily desulphated on treatment of their pyridinium salts with DMSO containing 10% of water or methanol at 80–100 °C.[165] The flavobacterial glycuronidases that act on unsaturated disaccharides derived from the glycosaminoglycuronans seem to be specific for the position of the linkage rather than, as previously supposed, for the anomeric configuration of the linkage.[166]

Occurrence, Isolation, and Structure.—Galactosaminoglycans predominate among the glycosaminoglycans of low molecular weight in human plasma, while the principal constituents of high molecular weight are hyaluronic acid and under-sulphated chondroitin sulphates.[167] The high proportion of under-sulphated chondroitin 4-sulphates in human plasma has been confirmed using coagulation factor IX.[168] Dissociated proteoglycans from the articular cartilage of baboons (*Papio papio*) were separated by gel electrophoresis into three main types that have different gel-electrophoretic mobilities, buoyant densities, and abilities to aggregate.[169] The chondroitin sulphate in bovine femoral articular cartilage is polydisperse.[170] The amount of hyaluronic acid in bovine ear cartilage exceeds that required for optimal aggregation of the proteoglycans.[171] Most of the hyaluronic acid is associated with elastin and appears to participate in the formation of elastic cartilage. Chondroitin sulphates and heparan sulphate have been identified as constituents of ovine brain tissues.[172, 173] The amounts of heparan sulphate, dermatan sulphate, and chondroitin sulphates in the aortas and coronary arteries of hypophysectomized dogs were reduced, while the amount of hyaluronic acid was normal in their coronary arteries and was reduced in their aortas.[174] Replacement therapy with somatotrophin had no significant effect on the amounts of sulphated glycosaminoglycans in the coronary arteries of hypophysectomized

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dogs, but it increased the amounts of sulphated glycosaminoglycans (chiefly chondroitin sulphate) in diverse aortic segments. Collagen is associated with dermatan sulphate in tensional regions of rabbit tendon, whereas the proteoglycan–collagen complex in sesamoid regions contains mainly chondroitin sulphate.[175]

Purified nuclei from rat liver contain glycosaminoglycans equivalent to 0.2–0.3 μg of hexuronic acid for each mg of DNA.[176] Hyaluronic acid (the principal constituent of nuclear glycosaminoglycans) and chondroitin 4- and 6-sulphates are associated with the chromatin fraction. Heparan sulphate on the surface of rat-liver cells has a half-life of <5 h.[177]

Sulphated glycosaminoglycans, with the exception of heparin, in the livers, kidneys, lungs, ilea, aortas, skin, and pancreas of guinea pigs, rabbits, pigs, and humans have characteristic distributions and the same types of tissue from different mammals have identical types and proportions of sulphated glycosaminoglycans, although the molecular weights of the sulphated glycosaminoglycans can vary.[178] The results suggest that the composition of sulphated glycosaminoglycans in mammalian tissues is tissue specific rather than species specific. The distributions of sulphated glycosaminoglycans in diverse mammalian tissues during growth and in cancerous tissues have also been reported.[179] Characteristic changes in the glycosaminoglycan composition of several tissues were noted during the growth of mammals and there is an appreciable increase in the amounts of chrondroitin 4- and 6-sulphates in tumoural tissues. Heparan sulphate and chondroitin 4-sulphate are minor constituents of bovine vitreous bodies, but chondroitin 6-sulphate was not detected.[180]

Hyaluronic acid of molecular weight $2{\cdot}5 \times 10^4$ represents approximately 20% (w/w) of the hexuronic acids contained in the organic matter of chicken-egg shells.[181]

The amounts of various glycosaminoglycans in blowflies (*Calliphora erythrocephala*),[182] lobsters (*Homarus vulgaris*),[183] hagfish, lampreys, and *Chimaera*[184] have been determined. The distributions of glycosaminoglycans in the skin and notochord of amphioxus are more akin to those of vertebrates than to those of cephalopods.[185] Comparisons of the distributions of sulphated glycosaminoglycans in 22 species of invertebrates belonging to the phyla *Arthropoda*, *Mollusca*, *Annelida*, *Tunicata*, *Echinodermata*, *Coelenterata*, and *Porifera* showed that each species has a characteristic distribution.[186]

The isolation and the fractionation of urinary glycosaminoglycans have been achieved by partitioning complexes of the glycosaminoglycans and quaternary ammonium salts between a two-phase system of butan-1-ol and aqueous salt

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solutions.[187] Various sulphated glycosaminoglycans have been isolated by using a partially purified lectin from *Phaseolus vulgaris* to precipitate them from solution.[188] The interaction between heparin and concanavalin A was inhibited by 2-acetamido-2-deoxy-D-glucose, suggesting that the monosaccharide and heparin interact with the same site on the lectin. Proteoglycans should be extracted from bovine nasal cartilage at low temperature in the presence of protease inhibitors if unnecessary degradation is to be avoided.[189] Despite such precautions, disaggregation of the proteoglycans occurred during 6-months' storage at $-20\,^{\circ}C$. Selective precipitation with cetyl pyridinium chloride has been used to obtain hyaluronic acid from bovine nasal cartilage.[190] The chondroitin sulphates and dermatan sulphate are associated with collagen, and heparan sulphate with elastin, in bovine aorta.[191] The hyaluronate (mol. wt. 3×10^5) in fresh rat skin has been isolated quantitatively by homogenization (in an Edebo press), extraction with buffered saline, selective elution from DEAE-cellulose, and gel chromatography on Sephadex G-200.[192] Heparin (and protamine) can be removed rapidly from human plasma by adsorption onto ECTEOLA-cellulose, thereby facilitating accurate screening tests for coagulation.[193] Affinity chromatography of heparin on antithrombin–Sepharose gave two distinct fractions; the more strongly adsorbed fraction possessed potent anticoagulant activity, whereas the other was virtually inactive.[194] No structural difference could be found between the two fractions. There is some doubt whether electrofocusing can be used to separate heparin into distinct molecular species.[195]

The amino-acid composition of the link protein (mol. wt. 4×10^4) from swarm rat chondrosarcoma differs from that of the core protein.[196]

Polarized i.r. spectra have been recorded for oriented, crystalline specimens of hyaluronates, chondroitin 4- and 6-sulphates, dermatan sulphate, and a cartilage proteoglycan.[197] The dichroism data for the vibrational modes of the amide and carboxylic acid groups were interpreted with respect to the molecular structures. In bovine nasal and tracheal cartilages, chains of keratan sulphate are located close to the site where hyaluronic acid binds to the proteoglycan, whereas chains of chondroitin sulphate are located some distance from this site.[198] Variations in the length of the chains of chondroitin sulphate account for differences in the size of cartilage proteoglycans, the size of the other components remaining fairly constant.[199] The ability of uncharged, open-coil dextrans to penetrate tissues without changing their molecular conformation can be used to investigate the structures and distribution of proteoglycans *in vivo*.[200] Proteoglycans remaining

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after digestion of bovine heart with collagenase were not in a highly aggregated form.[201] The structures of the D-galactosaminoglycans synthesized by human foetal lung differ from those synthesized by skin fibroblasts when both tissues grow under identical conditions in monolayer cultures.[202] The ^{13}C n.m.r. spectra of intact bovine nasal cartilage, a proteoglycan aggregate, and chondroitin 4-sulphate indicated that the protein chain, unlike chains of the glycosaminoglycan, has restricted mobility.[203]

Small-angle *X*-ray scattering and viscosimetric studies have shown that sodium hyaluronate is approximately 4 nm long in sodium chloride solution.[204] Sodium, lithium, calcium, and magnesium ions induced local, rather than gross, changes in the conformation of hyaluronic acid.[205] It has been shown that ε-amino-groups of lysyl residues are involved in binding hyaluronic acid to proteoglycans.[206] Acetylation of these groups prevented reaggregation or dissociated existing aggregates of high molecular weight. Traces of L-arabinose have been found in the hyaluronic acid from bovine brain.[207]

Analysis of the products obtained on depolymerization with chondroitinase showed that the chondroitin sulphate (mol. wt. 1.5×10^4) from whale cartilage is composed of three types of oligosaccharide, namely dodeca-, hexa-, and tetrasaccharides containing five, two, and one 4-sulphated disaccharide unit(s), respectively, for every 6-sulphated disaccharide unit.[208] The polymer contains, on average, 3 mol of each type of oligosaccharide per mol of chondroitin sulphate. Each molecule of the chondroitin sulphate (mol. wt. 5.5×10^4) from shark cartilage contains, on average, five 4-sulphated disaccharide units. A tetrasaccharide composed of 4-sulphated and 6-sulphated disaccharide units was isolated following treatment with a chondroitinase, showing that the 4-sulphated disaccharide units are not linked together but are distributed throughout the molecule. Chondroitin sulphates containing residues of D-glucose have been isolated from squid cartilage; the hexose is attached to O-6 of some of the 2-acetamido-2-deoxy-D-galactopyranosyl residues.[209] These chondroitin sulphates have higher molecular weights than usual. ^{13}C N.m.r. measurements have supported earlier evidence as to the main structural features of the chondroitin sulphates and dermatan sulphate, but they also revealed that many chondroitin sulphate preparations are substantially heterogeneous in composition.[210]

The c.d. properties of extensively aggregated complexes of Acridine Orange and chondroitin sulphate or dermatan sulphate are markedly different from those of soluble or partially soluble complexes.[211] Aggregation is favoured at high concentrations of the dye and the glycosaminoglycan and in solutions where the anionic sites on the glycosaminoglycan are effectively complexed with the dye.

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It is likely that long-range order is present in the aggregates. The structures of disaccharides released from human umbilical cord indicated the presence of a copolymer of dermatan sulphate and chondroitin sulphate.[212]

Feasible models for the sodium heparan sulphate molecule have been computer-generated, based on previous *X*-ray data and a repeating sequence of (1 → 4)-linked 2-amino-2-deoxy-α-D-glucopyranosyl and β-D-glucopyranosyluronic acid residues.[213] An optimum two-fold helical conformation, which incorporates stabilizing intra-chain hydrogen bonds across the glycosidic linkages, was proposed. The highly extended nature of the chain resembles those of other glycosaminoglycans. Most of the L-idopyranosyluronic acid residues in heparan sulphates from porcine mucosa and bovine lung are sulphated, whereas the 2-amino-2-deoxy-D-glucopyranosyl residues are not so extensively sulphated.[214] The sulphated L-idopyranosyluronic acid residues occur almost entirely in a single block. Heparan sulphate that accumulates in human fibroblasts deficient in specific sulphatases contains only D-glucopyranosyluronic acid at its reducing end, providing evidence for the presence of an *endo*-D-glucuronidase that acts on heparan sulphate.[215]

The disaccharides 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-L-idopyranosyluronic acid and 4-*O*-(2-amino-2-deoxy-α-D-glucopyranosyl)-D-glucopyranosyluronic acid have been isolated following hydrolysis of purified porcine heparin with acid.[216] The interaction of heparin with cationic polypeptides [*e.g.* poly(lysyltyrosine) or poly(lysylphenylalanine)] caused them to assume either a β-structure or an α-helical structure, depending on the nature of the amino-acid residues adjacent to the cationic residues and on the proclivity of these residues to form part of α-helical or β-structures in polypeptides.[217] Related interactions between a number of glycosaminoglycans and other cationic polypeptides have been studied.[218] A new species of proteochondroitin sulphate, which differs from those previously isolated in its molecular weight and amino-acid composition, has been found in chicken embryos before chondrogenesis takes place.[219]

Biosynthesis.—The patterns of incorporation of [^{35}S]sulphate and 2-amino-2-deoxy-D-[^{3}H]glucose into bovine rib cartilage suggested that the synthesis of chondroitin sulphate and hyaluronic acid takes place either in different compartments of the cell or in different types of cartilage cell.[220] Studies using guinea-pig rib cartilage have revealed two metabolic pools of proteoglycans having half-lives of 3 and 70 days.[221] Aggregated forms of proteoglycans increase between stages 24 and 35 of the differentiation of limb-bud cartilage, whereas non-aggregated or monomeric forms decrease.[222] Only one link protein is found in limb buds at stage 24, whereas two are found in those at stage 35, suggesting that the synthesis

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of link proteins may be a factor regulating limb chondrogenesis. The synthesis of glycosaminoglycans was increased by 80—100% following acute hepatic injury in rats; the relative rates of synthesis of heparan sulphate, heparin, the chondroitin sulphates, and hyaluronic acid were markedly different and changed disproportionately during hepatic injury.[223] The accumulation of sulphated proteoglycans in cultured human chondrocytes in the presence of L-ascorbate has been attributed to inhibition of the aryl sulphatase A and B activities.[224] L-Ascorbate also stimulated the deposition of newly-synthesized sulphated proteoglycans in the cell layer and reduced their secretion into the culture medium. Low concentrations (10^{-9} molar) of retinoic acid inhibited the synthesis of glycosaminoglycans by chondrocytes.[225] Mouse Balb/c 3T3 cells transformed with simian virus 40 showed a marked decrease in their capacity to synthesize extracellular proteoglycans, which contained more heparin or heparan sulphate or both (65—75%) and less chondroitin sulphate (15%) than those synthesized by untransformed cells.[226]

Studies of the biosynthesis of glycosaminoglycans in developing chicken corneas have shown that the synthesis of keratan sulphate commences eight days before the onset of corneal transparency, while the rate of synthesis of heparan sulphate increases or remains the same and the ratio of chondroitin 4-sulphate to chondroitin 6-sulphate increases during the same period.[227] Keratan sulphate becomes more highly sulphated at later stages of development. Suspension cultures of chondrocytes from the larynges of adult pigs synthesized proteoglycans that interacted with hyaluronic acid to form aggregates large enough to be excluded from Sepharose 2B.[228]

A polyprenyl derivative of 2-acetamido-2-deoxy-4-*O*-(D-glucopyranosyluronic acid)-D-glucopyranose isolated from human fibroblasts transformed by simian virus 40 may be involved in the synthesis of the disaccharide repeating-unit of such proteoglycans as heparin.[229] Heparan sulphate has been identified as the principal sulphated glycosaminoglycan synthesized *in vitro* by acinar cells from rat pancreas in the presence of sulphate ions, although the levels of heparan sulphate synthesized were very low.[230]

Hyaluronic acid can be re-incorporated into some cells that synthesize it.[231] This adsorptive pinocytosis occurs with mesenchymal cells, but not with either skin fibroblasts or arterial smooth-muscle cells. cAMP appeared to stimulate the synthesis of hyaluronic acid by human fibroblasts[232] and confluent rat fibroblasts.[233]

4-Methylumbelliferyl β-D-xylopyranoside, which inhibits the synthesis of chondroitin sulphate, may act either by inhibiting one or more of the enzymes involved in elongation of the chain or in sulphation of the glycosaminoglycan

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or by initiating the synthesis of new chains, causing a decrease in the average chain-length.[234] These proposals are supported by the fact that embryonic chicken sternum synthesized two types of chondroitin sulphate, which differed in their chain-length and extent of sulphation, on incubation with a low concentration of 4-methylumbelliferyl β-D-xylopyranoside.[234, 235] Serum factors were shown to stimulate the synthesis of proteochondroitin sulphates without affecting the chain-length or degree of sulphation. On the other hand, the effect of 4-methylumbelliferyl β-D-xylopyranoside has been ascribed to inhibition of a D-galactosyltransferase activity that participates in the synthesis of the linkage region of the proteoglycan.[236] Hormones can influence the synthesis of proteochondroitin sulphates by modifying the D-xylosyl- and D-galactosyl-transferase activities.[237] Thus, the reduced amounts of proteoglycan in the costal cartilage of diabetic or thyroidectomized or hypophysectomized rats were attributed to inhibition of the D-xylosyltransferase that is required for synthesis of the linkage region. Hydrocortisone also inhibited the synthesis of proteochondroitin sulphates by reducing the activity of a D-galactosyltransferase.

Degradation.—Heparan [^{35}S]sulphate, like heparin, was distributed among a number of sites after intravenous injection into rats.[238] The metabolism of heparan sulphate at these sites led to the formation of inorganic sulphate; these processes were impaired when heparin was injected simultaneously, indicating that some of the sites are common to both sulphated glycosaminoglycuronans.

Properties.—The viscosity of heparin in water under conditions of low shear stress (0.019—0.222 dyne cm^{-2}) varied with the nature and the concentration of cations (Na^+, K^+, Cs^+, Mg^{2+}, Ba^{2+}, and Cu^{2+}) present; it decreased with increasing shear stress, but this dependence is reduced as the ionic strength of the solution is increased.[239] Differences in the viscosities of heparin solutions in the presence of various cations appear to be related to the binding affinity of heparin for the cation. C.d. measurements indicated that heparin binds Cu^{2+} ions in a different way from the other cations. Heparin from the peritoneal mast cells of rats has a low coagulating activity, a molecular weight that varies between 2×10^5 and 7×10^5, and is not degraded by proteolytic enzymes.[240] Heparin released from the peritoneal mast cells of rats by immunologic challenge is the same size as the residual cellular heparin.[241] Although free carboxylic acid groups in heparin are not essential for its binding to antithrombin III, they are important in increasing the rate of the interaction between antithrombin III and thrombin.[242] *N*-Sulphamido-groups, on the other hand, are essential for the interaction between heparin and antithrombin III, which has been shown (c.d. and other measurements) to result in a conformational change that decreases the proportion of β-structure, with a compensatory increase in the proportion of random-coil structure, of

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[242] I. Danishefsky, M. Ahrens, and S. Klein, *Biochim. Biophys. Acta*, 1977, **498**, 215.

antithrombin III.[243] The processes that bind porcine mucosal heparin and rat-liver heparan sulphate to rat-liver cells have been shown to be time-dependent and reversible and to exhibit saturation kinetics, indicating that there are a limited number of cell-associated binding sites.[244]

New experimental evidence has shown that glass capillaries filled with potassium hyaluronate solutions do not contain electrochemical activity gradients between the ends of the capillaries.[245] Depending on the method of extraction, hyaluronic acid from rabbit skin has a molecular weight between 1.7×10^4 and 1.3×10^6.[246]

Functions.—Heparin fractionated by gel filtration appeared to bind to two sites on antithrombin III (association constants 0.6×10^{-6} and 0.2×10^{-6} mol l^{-1}), whereas heparin prepared by affinity chromatography on matrix-bound antithrombin III appeared to bind to only one site (association constant 2.3×10^{-6} mol l^{-1}).[194,247] These results suggest that one of the binding sites on antithrombin III does not bind the most active heparin components, but accommodates heparin-like molecules which, although similar in size to the active heparin components, have little or no anticoagulant activity. Heparins with high or low affinities for antithrombin III exhibited no differences in their abilities to bind lipoprotein lipase.[248] Studies of the interaction between the lipoprotein lipase from cow's milk and Sepharose-immobilized heparin have shown that heparin is polydisperse.[249] Whereas heparin facilitated complex formation between α-thrombin and antithrombin III, it had little effect on the interaction between β-thrombin and antithrombin III.[250]

Fractionation of porcine mucosal heparin by gel chromatography yielded a preparation that enhanced the rate of inhibition of thrombin by heparin co-factor without affecting the relative extent of thrombin inhibition.[251] A highly purified proteoglycan from the arterial wall of bovine aorta was able to prolong thrombin-induced clotting and to inhibit thrombin-induced aggregation, suggesting that it participates in regulating the response of circulating blood to vascular injury.[252] The long-held view that heparin binds to and activates antithrombin III in the coagulation of blood may need some revision.[253] Stopped-flow techniques have shown that heparin interacts rapidly with thrombin, probably inducing a conformational change in the enzyme that would facilitate complex formation with antithrombin III.

Heparin seems to exert two antagonistic effects on the association between platelets and foreign substances; it acts directly on platelets to increase their adhesiveness, while acting on foreign substances to reduce their retention by the platelets.[254] The addition of heparin to rat-liver chromatin induced the forma-

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tion of a fraction that sedimented more slowly; the amount of this fraction depended on the concentration of heparin.[255] The sedimentation behaviour of heparin-treated chromatin is associated with significant redistribution of chromosomal proteins among the fractions obtained by differential centrifugation.

Heparin exerts its effect in stimulating transcription in isolated rat-liver nuclei by increasing the rate of polynucleotide elongation of RNA polymerase B, possibly by removing protein bound specifically or non-specifically to chromatin DNA.[256] The synthesis of poly(phenylalanine) by rat-liver ribosomes was strongly inhibited by heparin, whereas other naturally occurring glycosaminoglycans had no significant effect.[257] Heparin is also a potent inhibitor of an elongation factor (EF-1) isolated from the embryos of brine shrimps (*Artemia salina*).[258]

The reduced activities of 30 different lysosomal enzymes *in vitro* in the presence of a chondroitin sulphate support the view that the lysosomal activity of leucocytes is regulated by interactions between the enzymes and chondroitin sulphates.[259] The proteoglycan in porcine laryngeal cartilage, although not inhibiting collagenase directly, is thought to provide a physical barrier that prevents the enzyme from reaching its substrate.[260] The amounts, types, and molecular sizes of sulphated glycosaminoglycans in neonate, adult, and tumoural tissues have been reported.[261] The results suggest that sulphated glycosaminoglycans may be involved in cell recognition and cell–cell adhesion.

Pathology.—The enzyme deficiencies in mucopolysaccharidoses and the participation of enzymes in excretion and recapture processes in the lysosomes of cultured fibroblasts have been discussed.[262] A comprehensive account of the chemistry, biochemistry, and physiology of proteoglycans in health and disease has appeared.[263]

A combination of agarose gel electrophoresis, enzymic degradation, and determination of the molecular weights of urinary glycosaminoglycans suffices to distinguish among Hunter's, Hurler's, and Sanfilippo's A and B diseases.[264] Two-dimensional electrophoresis has revealed that there are abnormally high amounts of dermatan and heparan sulphates in amniotic fluids from pregnancies affected by Hurler's disease and of heparan sulphate in amniotic fluids from pregnancies affected by Sanfilippo's disease.[265] Excellent discrimination between normal and affected pregnancies is provided by the ratios of dermatan sulphate:chondroitin sulphate (Hurler's disease) and heparan sulphate:chondroitin sulphate (Sanfilippo's disease) in amniotic fluids. Two-dimensional electrophoresis on cellulose acetate has been recommended for the separation of glycosaminoglycans.[266] The accumulation of glycosaminoglycans inside human glial cells

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[259] J. L. Avila and J. Convit, *Biochem. J.*, 1976, **160**, 129.
[260] D. E. Woolley and J. M. Evanson, *Biochim. Biophys. Acta*, 1977, **497**, 144.
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growing in media supplemented with ammonium chloride simulated that in cultures of the cells from patients with mucopolysaccharidoses.[267] Analysis of the glycosaminoglycans in urine appears to be a reliable method for detecting Sanfilippo's disease.[268] An excess of heparan sulphate is synthesized and stored both in the fibroblasts and glial cells of patients with this disease.[269] Despite some limitations, assay of the sulphamidase activity in leucocytes can be used to detect heterozygous carriers of Sanfilippo's disease.[270] Sulphated glycosaminoglycans in cultures of fibroblasts from patients with Sanfilippo's type B or Hurler's disease are associated with the cell nuclei.[271]

The diagnosis of mucopolysaccharidosis type I has been facilitated by the use of a novel radiolabelled substrate.[272] Thus the action of α-L-iduronidase on 2,5-anhydro-4-*O*-(α-L-idopyranosyluronic acid)-D-[1-^{3}H]mannitol afforded, as one of the products, 2,5-anhydro-D-[1-^{3}H]mannitol, which can be separated and assayed. A study of the β-D-glucuronidase activity in hybrids of mouse cells and fibroblasts from a patient with mucopolysaccharidosis type VII showed that the subunit of the mutant enzyme in the fibroblasts associates with the normal murine subunit to produce a viable β-D-glucuronidase.[273] Enzyme immunoassays have revealed the presence of material that cross-reacts with β-D-glucuronidase in the fibroblasts of four patients with a deficiency of this enzyme.[274] There are no differences in the contents and the distributions of cellular glycosaminoglycans extracted from the lymphocytes of controls and of patients with cystic fibrosis.[275] The high concentration of hyaluronic acid in pleural fluids associated with mesothelioma producing hyaluronic acid is diagnostic of this disease.[276]

Chondroitin 4-sulphate is the principal proteoglycan found in the media of cultured B16 mouse melanoma cells.[277] The epiphysial cartilage of four-week-old chickens that had been fed a Mn^{2+}-deficient diet contained low levels of glycosaminoglycans, owing to a decrease in the D-xylosylation of core proteins and an increase in the degradation of glycosaminoglycans.[278]

Mammalian Cell and Tissue Glycoproteins

References to macromolecules present on cell surfaces have been collected together in a separate section (see p. 337).

A review of the glycoproteins of connective tissues has discussed methods for isolating them from skin, cornea, cartilage, synovial fluid, tendon, cardiovascular tissue, *etc.*[279] Some properties of the principal L-fucosylated glycoprotein released

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into the medium by human-skin fibroblasts have been reported.[280] It was proposed that collagen and this L-fucosylated glycoprotein may be associated *in vivo* in a pericellular matrix which constitutes the immediate environment of connective-tissue cells and which may play an important part in the development of tissue morphology. A glycoprotein isolated from thromboplastin (factor III) in human-brain tissues contains L-fucose (2.4), D-mannose (6.0), D-galactose (7.4), 2-acetamido-2-deoxy-D-glucose (2.6), and *N*-acetylneuraminic acid (0.7 residue(s) for each molecule of protein).[281] An intact glycoprotein (mol. wt. 9.6×10^3) isolated from human gastric juice contains 69% of carbohydrate which is *N*- and *O*-glycosidically linked and composed of residues of 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, D-galactose, D-mannose, L-fucose, and sialic acid in the molar proportions 2:10:7:4:12:1.[282] The 4-L-aspartyl-β-D-glucosylamine amidohydrolase from human liver has been purified (17 500-fold).[283] The levels of this enzyme in the livers, brains, and kidneys of patients with aspartylglucosaminuria are very much lower than normal and the products of enzymic hydrolysis appear to be modified further.[284] Human pancreatic lipase (mol. wt. 4.8×10^4) contains 2-acetamido-2-deoxy-D-glucose (4.7), D-mannose (2.8), L-fucose (2.9), D-galactose (3.0), and D-glucose (1.1 residues for each molecule of protein).[285] Porcine lipase has been separated into two glycoprotein fractions by gel electrophoresis.

The 'acid' phosphatase I (mol. wt. 8.9×10^4) from human prostrate glands is a glycoprotein containing L-fucose (3), D-galactose (4), D-mannose (11), 2-acetamido-2-deoxy-D-glucose (15), and sialic acid (7—8 residues for each molecule of protein).[286] β-D-2-Acetamido-2-deoxyhexosidases A and B from human placenta have been separated and purified; each has a molecular weight of 1.1×10^5.[287, 287a] β-D-2-Acetamido-2-deoxyhexosidase A is a tetramer ($\alpha_2\beta_2$) containing 2-acetamido-2-deoxy-D-glucose (4) and neutral sugars (8 residues) in each β-subunit and 2-acetamido-2-deoxy-D-glucose (2 residues) in each α-subunit. The data obtained are also consistent with the subunit structures β_4 and α_4 for β-D-2-acetamido-2-deoxyhexosidases B and S, respectively.

A glycoprotein from the lung lavage of patients with alveolar proteinosis contains sialic acid (3), D-mannose (4), D-galactose (4.2), L-fucose (1), and 2-acetamido-2-deoxy-D-glucose (5.8 residues for each molecule of protein).[288] The carbohydrate compositions and structures of the oligosaccharide chains of the principal glycoprotein (mol. wt. 3.6×10^4) from the alveoli of patients with alveolar proteinosis have been established.[289] Pronase digestion of the glycoprotein and gel filtration and ion-exchange chromatography gave two glycopeptides containing sialic acid (1, 2), D-galactose (2, 2), D-mannose (2, 2), 2-acetamido-2-deoxy-D-glucose (3, 3), and L-fucose [0, 1 residue(s), respectively]. The oligosaccharide chains of each

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glycopeptide are attached to asparagyl residues in the polypeptide chain *via* 2-acetamido-2-deoxy-D-glucosyl residues. The structure of the oligosaccharide chains of one of the glycopeptides is similar to that of the receptor site for phytohaemagglutinin on human erythrocytes. The tyrosinase extracted with detergents from human malignant melanomas has been separated into a number of fractions by affinity chromatography on immobilized concanavalin A.[290] The enzymic properties of intestinal sucrases from normal individuals and from patients with intestinal metaplasia are similar.[291] Antithrombin III, α_1-acid glycoprotein, and α_2-macroglobulin have been found in malignant tissues.[292] The presence of either 2-deoxy-D-*arabino*-hexose or 2-amino-2-deoxy-D-glucose in the culture medium inhibited the synthesis of interferon by human fibroblasts.[293] Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate indicated that altered and unaltered interferons are synthesized in the presence of partially inhibitory concentrations of either of these monosaccharides.

Bovine tendon glycoprotein has been shown to exert two effects on fibrillogenesis: it retards the assembly of collagen molecules and, if present early enough in the aggregation process, decreases the diameter of the collagen fibrils.[294] Two of the principal glycoproteins in myelin from bovine peripheral nerve tissues are also found in similar tissues from pigs, rabbits, and guinea-pigs.[295] A glycopeptide isolated from bovine rhodopsin contains two carbohydrate components, which are attached to asparagyl residues in the polypeptide chain.[296]

A structural glycoprotein that participates in fibrogenesis and differentiation of the cells has been isolated from the media of pig aorta.[297] The carboxamidomethylated protein interacted with the soluble, undegraded component of calf-skin collagen. Renin from the kidneys of pigs contains 3.2 residues of 2-amino-2-deoxy-D-glucose for each molecule of protein.[298]

The pancreas of coypus contains one ribonuclease component, whereas the pancreas of chinchillas contains two ribonuclease components: the carbohydrate components of coypu and chinchilla ribonucleases are attached to the polypeptide backbone at asparagine-34.[299]

Cultures of baby-hamster kidney cells adapted to grow in suspension produced and released a glycoprotein that is similar to, if not identical with, the Tamm–Horsfall glycoprotein in hamster urine.[300] The amount of this glycoprotein increases during the G2 phase of the cell-cycle and then decreases before physical division of the cells.

The glycoproteins in transformed mouse cells have been identified using ^{125}I-labelled lectins or antibodies in sodium dodecyl sulphate gels and auto-

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radiography.[301] Marked differences in the cell-surface glycoproteins were revealed when normal and virus-transformed cells were compared. Cells transformed with simian virus 40 and a polyoma transformant contained as much or more of a large, external, transformation-sensitive (LETS) protein as do normal cells, suggesting that the accumulation of LETS protein within the cells is related to their incomplete expression on the cell surface. The results of fractionation and methylation analysis of glycopeptides derived from papain digestion of delipidized brain, kidney, and liver tissues from rats indicated that the glycoproteins in these tissues contain four structurally distinct types of oligosaccharide chains which resemble one another in the substitution pattern of the interior D-mannopyranosyl residues (probably derived from the core region), although there are considerable differences in the peripheral regions of oligosaccharides from the different tissues.[302] α-D-Mannosidase (mol. wt. 3×10^5)[303] and α-L-fucosidase[304] have been isolated from the Golgi membrane and the lysosomes, respectively, of rat liver.

A rapid procedure for the selective isolation of the principal glycoprotein in rat-brain myelin involved initial solubilization of the glycoprotein with lithium 3,5-di-iodosalicylate.[305] The residual glycoproteins were separated into two main and several minor components by polyacrylamide gel electrophoresis.[306] The identity and amounts of sugars in glycoproteins of myelins from rat brain and rat sciatic nerve have been determined with the aid of *in vivo* labelling of the glycoproteins with L-[^{3}H]fucose and 2-amino-2-deoxy-D-[^{14}C]glucose.[307] Both central and peripheral myelins contain many of the sugars that are characteristic of glycoproteins. Glycoproteins are present in larger amounts in peripheral myelin than in central myelin, and most of the D-galactosyl and sialic acid residues present in glycoproteins from central myelin are found in a fraction of high molecular weight. Glycoproteins from the synaptic plasma membranes and junctions and postsynaptic densities of rat brain have been characterized.[308] The structures of sulphated glycoproteins in a purified fraction from synaptic plasma and myelin membranes of rat brain differ from those of the sulphated glycosaminoglycans in rat brain.[309] They have high molecular weights and contain residues of D-glucose, D-mannose, L-fucose, D-galactose, 2-amino-2-deoxy-D-glucose, and *N*-acetylneuraminic acid. Assays of the glycosaminoglycans and glycoproteins in rat-brain nuclei have indicated the presence of a sulphated glycoprotein.[310] The disaccharide unit 2-acetamido-2-deoxy-3-*O*-α-D-galactopyranosyl-D-galactose appears to occur only as a component of the glycoproteins in animal brain tissues, since it is not found in those from livers, kidneys, gastric and small intestinal mucosa, or erythrocytes.[311] Affinity chromatography on immobilized concanavalin A has been used

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to fractionate the glycoproteins extracted from the brains of rats.[312] Some of these glycoproteins contain α-*N*-acetylneuraminyl-(2 → 8)-*N*-acetylneuraminyl residues as part of the carbohydrate component.[313] The structures (1) and (2) have been identified in rat-brain glycopeptides on the basis of treatment with sodium hydroxide–sodium borohydride and methylation analysis.[314] A hormone-like substance, which is required for the proliferation of granulocyte-macrophage progenitor cells *in vitro*, has been isolated from the culture media of a subline of mouse cells.[315] It was identified as a sialoglycoprotein, although the sialic acid residues are not essential for its activity.

α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 3)-[α-Neu*N*Ac*p*-(2 → 6)]-D-Gal*N*Ac*p*

(1)

β-D-Gal*p*-(1 → 3)-[α-Neu*N*Ac*p*-(2 → 6)]-D-Gal*N*Ac*p*

(2)

Cell-surface Glycoproteins

The binding of large and small extracellular substances to receptors on cell surfaces, particularly the interactions with paramyxovirus and the functions of sialoglycoproteins on erythrocyte membranes, has been reviewed.[316] The roles of glycoproteins in the membranes of mitochondria, milk-fat globules, and lysosomes and the interactions of lectins associated with intracellular membranes have been discussed.[317]

Various detergents have been tested for the solubilization of HLA antigens from a cultured line of human lymphoblastoid cells.[318] Glycoproteins containing disulphide cross-linkages from human-platelet membranes have been separated by two-dimensional gel electrophoresis.[319] Removal of residues of sialic acid from these glycoproteins did not affect their ability to bind thrombin.[320] Only one glycoprotein (mol. wt. 8.9×10^4) was removed from human platelets by the action of thrombin, which released a soluble glycoprotein (mol. wt. 7×10^4).[321] Three glycopeptides isolated from human peripheral lymphocytes that had been stimulated with a mitogen from *Wistaria floribunda* contained two types of carbohydrate components: one of them contained residues of sialic acid, D-galactose, 2-acetamido-2-deoxy-D-glucose, D-mannose, and L-fucose in the molar proportions 2:2:4:2:1, whereas the other two contained tri- or tetra-saccharide chains composed of residues of sialic acid, D-galactose, and 2-acetamido-2-deoxy-D-galactose in the molar proportions 1 or 2:1:1, respectively.[322] Glycoproteins of high molecular

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weight and with electrophoretic mobilities similar to that of a glycoprotein from rat myelin have been found in human and bovine myelins at all stages of development.[323] The adherence of *E. coli* to human mucosal tissues is mediated by a D-mannose-like receptor on the surface of epithelial cells.[324]

Glycoproteins on the external surface of chromaffin granules in bovine adrenal medulla may be involved in exocytosis.[325] Gangliosides and glycoproteins are both components of the receptor for thyrotrophin on bovine thyroid membranes.[326] A substantial purification of the membrane-bound 2-acetamido-2-deoxy-D-galactosyltransferase from porcine submaxillary gland has been achieved; it is a glycoprotein (mol. wt. 1×10^5) composed of two subunits (mol. wts. 4.6×10^4 and 5.7×10^4).[327] The properties of this transferase, including its ability to transform human type O erythrocytes into type A erythrocytes, have been examined.[328] One of the principal glycoproteins from the zymogen granules of canine pancreas contains 15% of carbohydrate.[329]

A major glycoprotein (mol. wt. $7.4—7.9 \times 10^4$) has been identified in the plasma membranes of fat cells from the perirenal adipose tissues of rabbits, rats, and calves, although substantial differences were apparent among polypeptide components of the species.[330] The plasma membrane from rat adipose tissues also contains a prominent glycoprotein of molecular weight 8.8×10^4. Gel electrophoresis revealed that, for each species, all membrane fractions from the microsomal pellet contain the same principal glycoprotein band(s), a finding that may have a significant bearing on the biogenesis of the membrane. Most of the carbohydrate chains of glycoproteins in the plasma membrane from rabbit fat cells are linked through 2-acetamido-2-deoxy-D-glucopyranosyl residues to asparagyl residues in the polypeptide backbone.[331]

Removal of the sialic acid residues from a specific hepatic binding glycoprotein from rabbits virtually abolished its ability to bind certain asialoglycoproteins.[332] It appears that D-galactosyl residues exposed by desialylation compete for the binding sites on the desialylated glycoprotein. The hepatic binding glycoprotein is also a mitogen for human peripheral lymphocytes.[333] The interactions of concanavalin A with purified plasma membranes from rabbit thymocytes have been analysed.[334] The data suggested that concanavalin A induces the formation of dimers, or possibly oligomers, of a glycoprotein (mol. wt. 5.5×10^4) through a co-operative process that leads to increased affinity for the lectin, which, at saturation, binds mostly through low-affinity associations that do not involve specific membrane components.

Several clones of ovary cells from Chinese hamsters have been selected for their

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resistance to the toxic effects of wheat-germ agglutinin.[335] The variant cells failed to carry out specific glycosyl transfers *in vivo*, although they possess the appropriate nucleotide-linked sugars, glycoprotein and glycolipid acceptors, and glycosyltransferases. A related investigation with four sub-clones of the cells also suggested that the loss of lectin-induced agglutinability results from a reduction in the amount of glycoprotein at the surface of the cells.[336] Culturing of ovary cells from Chinese hamsters with 2-phenylethanol modified the cell-surface glycoproteins, rendering the cells less effective hosts for vesicular stomatitis virus.[337] The uptake of colchicine by hamster ovary cells may be mediated by a phosphorylation–dephosphorylation cycle involving, at least, two cell-surface glycoproteins.[338] The transformation of hamster lymphocytes by simian virus 40 caused changes in the glycoprotein composition of the nuclear membrane.[339] The sensitivity of baby-hamster kidney (BHK) fibroblasts to certain lectins was reduced following growth in the presence of 2-deoxy-D-*arabino*-hexose, owing to disruption of the glycosylation of cell-surface glycoproteins.[340] Prolonged treatment of the cells with ethanol (100 mmol l^{-1}) also caused changes in these membrane components.[341] However, some disruption of the organization and the composition of cell-surface glycoproteins was also apparent in BHK fibroblasts that were resistant to 2-deoxy-D-*arabino*-hexose, indicating that such alterations cannot in themselves play an essential role in the cytotoxicity of 2-deoxy-D-*arabino*-hexose.[342]

The carbohydrate components of several glycoproteins in cellular membranes from rat brain and liver contain α-*N*-acetylneuraminyl-(2 → 8)-*N*-acetylneuraminyl units.[313, 314, 343] L-Fucose-containing glycoproteins in the plasma membrane of regenerating rat liver do not differ appreciably from those in the normal liver.[344] The metabolism and the turnover of membrane glycoproteins have been investigated with membranes of neuroblastoma cells growing in culture.[345] Glycoproteins therein were found to turn over at non-uniform rates and many cell-surface glycoproteins were metabolized either more rapidly or more slowly than most other cell-surface components. The L-fucose-containing glycopeptides on the cell surface of murine and human neuroblastomas have been compared.[346] Similarities were found among the membrane glycopeptides at the surface of these murine and human cells, and it was proposed that these similarities are related to the ability of the cells to perform the differentiated functions of neuronal cells or to exist as tumours. A protein that specifically binds desialylated serum glycoproteins has been isolated from the Golgi complex, the lysosomes, and the smooth microsomes, as well as from the external plasma membrane, of rat liver.[347] The

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receptor for Shiga toxin on rat-liver membranes is a glycoprotein containing 2-acetamido-2-deoxy-D-glucose.[348] The receptor for concanavalin A present on the glomerular basement membranes of rats has been identified as nephritogenoside—a water-soluble glycoprotein containing α-D-glucopyranosyl residues at the non-reducing end of the oligosaccharide chains.[349] Differences have been found between the carbohydrate composition of a glycoprotein from the epithelial basement membranes of normal murine kidney cells and that of a glycoprotein from murine teratocarcinoma cells.[350] The glycoprotein from normal cells contains more L-fucose, D-mannose, 2-acetamido-2-deoxy-D-glucose, and sialic acid; in addition, both glycoproteins contain D-galactose, D-glucose, and 2-acetamido-2-deoxy-D-galactose. Sialoglycopeptides obtained by papain digestion of normal and of neoplastic rat-liver tissues differed both in molecular size and lectin-binding ability.[351] A D-galactosyltransferase on the surface of rat-spleen lymphocytes transferred D-galactosyl residues from UDP-D-galactose to endogenous cell-surface glycoproteins.[352] A membrane-bound glycoprotein (mol. wt. 1.5–2.0 × 10^5) appears to contain the receptor for horse anti-(lymphocyte globulin) on murine thymus lymphocytes.[353] The receptor (a cell-surface glycoprotein) for polkweed mitogens has been isolated from the bone-marrow and thymus lymphocytes of mice (strain C3H/HE).[354] Mouse thymocytes stimulated with concanavalin A incorporated L-fucose and D-galactose into membrane glycoproteins without any transformation of the sugars, apart from some (10%) isomerization of D-galactose to D-glucose.[355] Labelled D-mannose was partly converted into L-fucose, and labelled 2-amino-2-deoxy-D-glucose was distributed among sialic acid (25%), 2-amino-2-deoxy-D-glucose (40%), 2-amino-2-deoxy-D-galactose (30%), and neutral sugars (5%) on incorporation into the stimulated thymocytes. An epidermal growth-factor controls the expression of a principal glycoprotein (LETS protein) at the surface of mouse cells.[356] Only 20% of the sialoglycoproteins in rat-embryo fibroblasts was affected by the actions of trypsin and neuraminidase.[357] The low affinities of membrane glycoproteins from mouse cells for a D-galactose-binding lectin has been attributed to the high proportion of sialic acid residues in the carbohydrate components of the glycoproteins.[358] The synthesis and the excretion of a glycoprotein on the surface of murine palatal cells were inhibited by diazo-oxonorleucine.[359] Labelling experiments have indicated that both glycolipid- and glycoprotein-bound sialic acids in the synaptic membrane of rats are released *in situ* by the action of an intrinsic sialidase, whose function may be to modulate the physical properties of the membrane.[360]

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Tunicamycin (an inhibitor of glycosylation) caused the death of mouse and human cells transformed with simian virus 40, whereas it inhibited the growth and altered the morphology of normal cells.[361] These differences were ascribed to changes in the compositions of membrane-bound glycoproteins. Tryptic peptide mapping has revealed that the structures of H-2K glycoproteins from the principal histocompatibility complex of each of two mouse mutants differ from those of the parent strain.[362] Glycopeptides released by exhaustive digestion of a number of H-2K, H-2D, and Ia glycoproteins with pronase had similar molecular weights (*ca.* 3.25×10^3) and carbohydrate chains of roughly the same size, which contained a variable number of sialic acid residues that were readily removed by neuraminidase.[363] Little change in the pattern of cell-surface glycoproteins, a phenotype observed in many transformed cells, was observed between 32 and 39° C with a temperature-sensitive mutant of murine 3T3 cells transformed with simian virus 40[364] (*cf.* refs. 365 and 366). The low adhesion to substratum, *etc.*, of mutant 3T3 Balb/c cells, which were unable to *N*-acetylate 2-amino-2-deoxy-D-glucose 6-phosphate, is most likely due to changes in the carbohydrate components of cell-surface glycoproteins, although the cells grow normally.[365] Alterations to glycoproteins of the plasma membrane and in the level of sialyltransferase activity were shown to be temperature dependent in mouse cells transformed by a temperature-sensitive mutant of simian virus 40 belonging to complementation group A.[366] It was suggested that the viral gene A product somehow affects the level of sialyltransferase activity in cells growing at the restrictive temperature (39.5 °C), resulting in alterations to cell-surface glycoproteins that may have a crucial role in regulating the growth of the cell. The immunological properties of the principal surface glycoprotein (mol. wt. 5.2×10^4) from murine mammary tumour virus have been examined.[367]

Work has continued on the identification of glycoproteins and glycosaminoglycans from the plasma membranes of murine ascites hepatomas: strains AH 130FN and AH 66 are currently under investigation.[368, 369] The principal glycoprotein from strain AH 66 has a molecular weight of 1.6×10^5 and contains 44% of carbohydrate consisting of residues of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, D-galactose, D-mannose, L-fucose, and sialic acid.[369] The oligosaccharide chains appear to be linked to the polypeptide backbone by *N*- and *O*-glycosidic linkages. The synthesis of glycoproteins by rat ascites hepatoma cells was stimulated by a mitogenic lectin from *Robinia pseudoacacia*, whereas growth of the cells was inhibited and no morphological changes were induced.[370]

The change in cell-surface adhesiveness that occurs during development of the embryonic neural tube (epithelium) and neural crest (mesenchymal outgrowth) of Japanese quails may be related to changes in the principal cell-surface glyco-

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proteins (two in the neural crest and four in the neural tube).[371] The apparent weakness of the interaction on the lipid bilayer between the molecules of a principal cell-surface glycoprotein in chicken-embryo fibroblasts suggested that the assembly of fibrils of this glycoprotein is nucleated by other cell-surface components.[372] A portion of the monomeric form of fibronectin, a major glycoprotein on the cell-surface of chicken-embryo fibroblasts, was converted into polymeric complexes of high molecular weight under conventional conditions of culturing.[373]

Glycopeptides derived by chemical and enzymic degradations of the intestinal basement membrane of *Ascaris suum* contain two types of carbohydrate component: one consists of residues of 2-*O*-α-D-glucopyranosyl-D-galactopyranose β-linked to hydroxylysyl residues, whereas the other is composed of residues of L-fucose, D-galactose, D-mannose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose in the molar proportions 1:1:1:2:1.[374] Receptors for concanavalin A on the plasma membrane of cultured muscle cells from *Xenopus laevis* embryos were grossly redistributed when the cells were exposed to an applied electric field.[375] A crude estimate of the mobilities of the receptors can be obtained from data on the time and the course of redistribution.

Additional evidence has been presented to show that pallidin, a lectin from cohesive cells of *Polysphondylium pallidum*, is located on the surface of the cells, where receptors for pallidin are also found.[376]

The effects of diverse detergents on the haemagglutinating activities of concanavalin A and agglutinins from *Ricinis communis*, wheat germ, soybean, and *Limulus polyphemus*, and the binding of glycoproteins by immobilized lectins have been examined.[377] Cells fixed with glutaraldehyde and immobilized on Sephadex have been used in the affinity chromatography of immunoglobulins that interact specifically with cell-surface glycoproteins.[378]

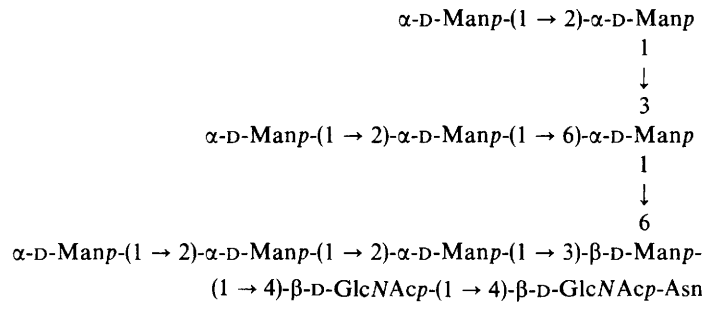
Hormonal Glycoproteins

The chemistry of the gonadotrophins, particularly the biological roles of sialylation and desialylation and the excretion of subunits from placental and pituitary tissues, has been reviewed.[379] Aspects of the chemistry, physiology, and assay of prolactin have been discussed.[380]

A degree of homology has been established among the sequences of amino-acids located at the NH_2-terminus of the β-subunit of cholera toxin and those in human chorionic gonadotrophin, thyrotrophin, and luteinizing and follicle-stimulating hormones.[381]

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A disaccharide repeating-unit consisting of residues of D-glucuronic acid and 2-amino-2-deoxy-D-galactose has been identified in one of the carbohydrate components of human thyroglobulin.[382] This component, like those in proteoglycans, appears to be attached through a (D-galactopyranosyl)$_2$-D-xylopyranosyl unit to a seryl residue in the protein chain. This represents the fourth distinct type of carbohydrate unit that has been detected in human thyroglobulin. Removal of the sialic acid residues from either human or porcine thyroglobulin markedly decreased the solubility of the hormone.[383] The structure (3) has been assigned to a carbohydrate component of calf thyroglobulin.[384] Guinea-pig thyroglobulin contains D-galactose (50), D-mannose (104), L-fucose (15), 2-amino-2-deoxy-D-glucose (97), 2-amino-2-deoxy-D-galactose (25), and sialic acid (38 residues for each molecule of protein).[385]



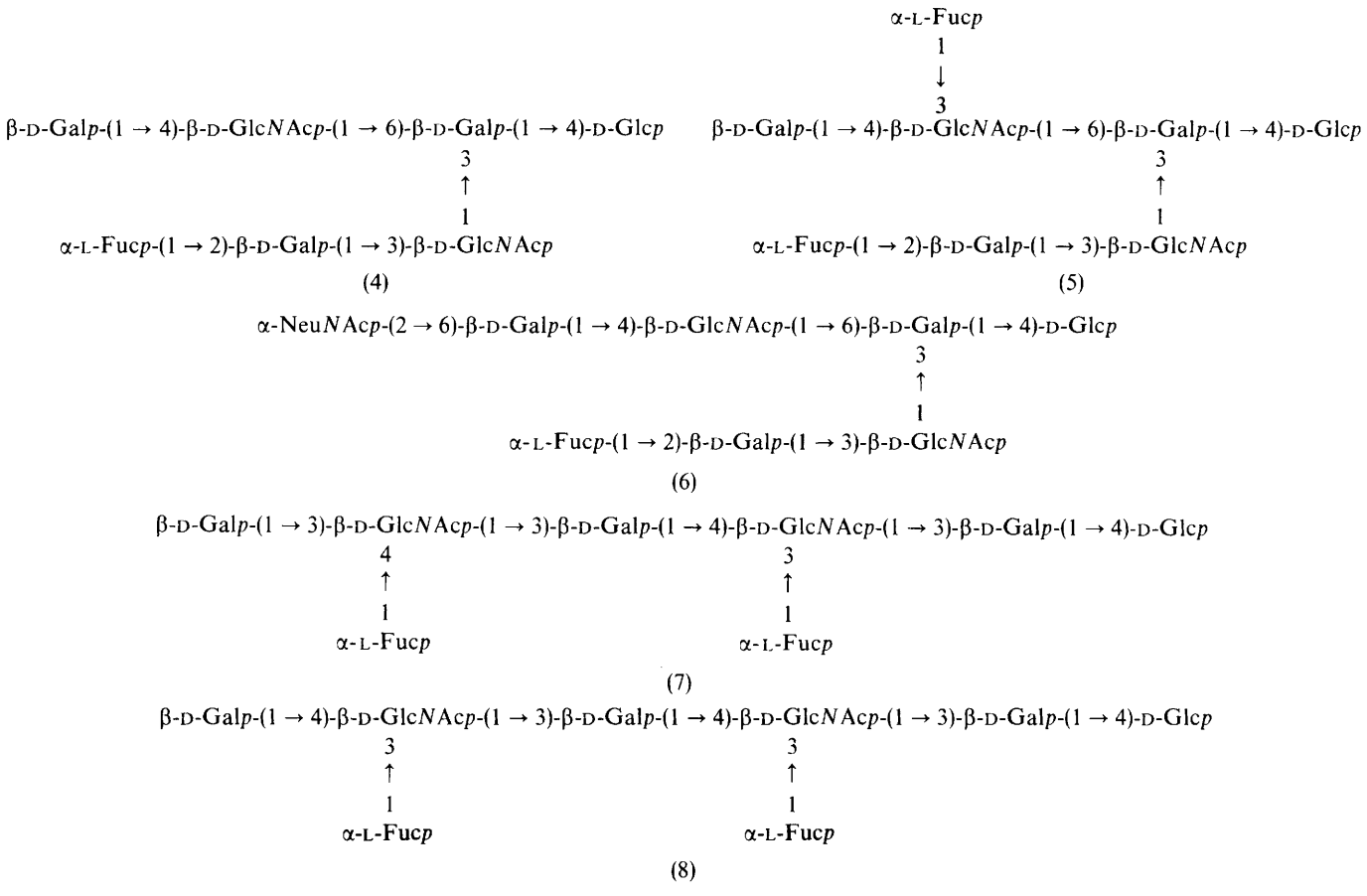
(3)

Five active components obtained from human pituitary thyrotrophin by polyacrylamide gel electrophoresis have similar amino-acid and carbohydrate compositions; all of them contain residues of L-fucose, D-mannose, D-galactose, 2-amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-glucose, and sialic acid.[386] Bovine and whale thyrotrophins have been purified by affinity chromatography on immobilized concanavalin A.[387]

Elucidation of the sequence of amino-acids in a fragment (residues 115—145) from the CO_2H-terminus of the β-subunit of human chorionic gonadotrophin showed that carbohydrate components are attached to serine-121, -127, -132, and -138.[388] The sequence of the amino-acids 109—145 has been determined independently.[389]

Affinity chromatography on agarose–concanavalin A has shown that a significant fraction of the high-molecular-weight form of adrenocorticotrophic hormone from human pituitaries is a glycoprotein.[390]

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α-L-Fucp-(1 → 2)-β-D-Galp-(1 → 3)-β-D-GlcNAcp
(4)
α-L-Fucp
1
↓
3
β-D-Galp-(1 → 4)-β-D-GlcNAcp-(1 → 6)-β-D-Galp-(1 → 4)-D-Glcp
3
↑
1
α-L-Fucp-(1 → 2)-β-D-Galp-(1 → 3)-β-D-GlcNAcp
(5)
α-Neu5Ac-(2 → 6)-β-D-Galp-(1 → 4)-β-D-GlcNAcp-(1 → 6)-β-D-Galp-(1 → 4)-D-Glcp
3
↑
1
α-L-Fucp-(1 → 2)-β-D-Galp-(1 → 3)-β-D-GlcNAcp
(6)
β-D-Galp-(1 → 3)-β-D-GlcNAcp-(1 → 3)-β-D-Galp-(1 → 4)-β-D-GlcNAcp-(1 → 3)-β-D-Galp-(1 → 4)-D-Glcp
4
↑
1
α-L-Fucp
3
↑
1
α-L-Fucp
(7)
β-D-Galp-(1 → 4)-β-D-GlcNAcp-(1 → 3)-β-D-Galp-(1 → 4)-β-D-GlcNAcp-(1 → 3)-β-D-Galp-(1 → 4)-D-Glcp
3
↑
1
α-L-Fucp
3
↑
1
α-L-Fucp
(8)

The binding of rat luteinizing hormone (labelled with 2-amino-2-deoxy-D-[^{3}H]-glucose) to Leydig cells has been examined.[391] Glycosylation of rat luteinizing hormones was stimulated by the gonadotrophin-releasing hormone.[392]

The synthesis of erythropoietin in regenerating rat liver is controlled, in part, by the level of hypoxia.[393]

Milk Glycoproteins

Three oligosaccharides (4)—(6) containing a core of lacto-*N*-hexaose have been isolated from the milk of human secretors and identified.[394] They are not found in the milk of female non-secretors. However, two new octasaccharides (7) and (8) have been isolated from the milk of female non-secretors of the Lewis a^+b^- phenotype.[395] A chemical synthesis of lacto-*N*-triose I [β-D-Gal*p*-(1 → 3)-β-D-Glc*NAc*p-(1 → 3)-D-Gal*p*], the core trisaccharide of blood-group substances in human milk, has been described.[396] A purified (> 95%) sialoglycoprotein from the membrane surrounding the fat globules in cows' milk contains 50% of carbohydrate consisting mainly of residues of sialic acid (30.5%), 2-acetamido-2-deoxy-D-glucose (22.3%), D-galactose (15.9%), 2-acetamido-2-deoxy-D-galactose (14%), D-mannose (11.1%), and L-fucose (5.8%); leucine, glycine, and glutamic acid are the principal amino-acids.[397] A soluble glycoprotein isolated from the delipidized membrane of bovine milk-fat globules was dissociated into, at least, eight polypeptides (mol. wts. 1.0—8.8 × 10^4) on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate.[398]

In contrast to most other α-lactalbumins, the α-lactalbumin isolated from the milk of rats is a glycoprotein (mol. wt. 1.54 × 10^4) containing D-mannose (3), D-galactose (3), 2-amino-2-deoxy-D-glucose (3), L-fucose (1), and sialic acid (2 residues for each molecule of protein).[399] Rat α-lactalbumin can be fractionated into, at least, three principal molecular species, which probably contain different proportions of sialic acid residues.

Lactoferrin from the milk of mice has a molecular weight of 7.5 × 10^4 and contains 8% of carbohydrate, part of which is 2-amino-2-deoxy-D-glucose (5 residues for each molecule of protein).[400]

Serum Glycoproteins

The isolation and chemical compositions of α_1-acid glycoproteins from the plasma of humans, rabbits, pigs, rats, and chickens have been reported.[401] The α_1-acid glycoproteins from the plasmas of chickens, humans, and rabbits contain almost identical amounts of carbohydrate, whereas those from porcine and murine plasmas contain less carbohydrate. The concentrations of orosomucoid, α_1-anti-

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trypsin, haptoglobulin, α_2-macroglobulin, and haemopexin in pleural effusions from neoplastic tissues are usually higher than those normally found.[402]

The heterogeneity of each of the five carbohydrate components of human-serum α_1-glycoprotein has been examined.[403] A function of this glycoprotein might be to inhibit certain lymphocyte responses, including blast transformation induced by mitogens and allogeneic cells.[404]

Chemical modification (*e.g.* periodate oxidation and then reduction) or loss of the terminal *N*-acetylneuraminic acid residues of human-plasma α_1-antitrypsin did not destroy its ability to inhibit either trypsin or chymotrypsin; neither did oxidation of the exposed D-galactopyranosyl residues in the desialylated glycoprotein with D-galactose oxidase.[405] However, enzymic oxidation of the D-galactopyranosyl residues increased the survival time of the modified glycoprotein in plasma towards that exhibited by fully sialylated α_1-antitrypsin, whereas the desialylated glycoprotein was rapidly cleared following injection into rats. Contrary to previous evidence, there appears to be little or no difference between the carbohydrate compositions of the M and Z variants of human-plasma α_1-antitrypsin.[406]

A leucine-rich α_2-glycoprotein (mol. wt. 4.96×10^4) in human serum (2.1 mg per 100 ml of serum) appears to consist of a single polypeptide chain and 23% of carbohydrate.[407] Human-plasma α_2-SH glycoprotein has been freed from zinc α_2-glycoprotein—which possesses a similar molecular weight and a similar isoelectric point—by affinity chromatography on a gel-bound zinc chelate.[408]

The extents of sialylation of eleven plasma hydrolases have been examined.[409] Most plasma hydrolases (*e.g.* α-L-fucosidase and α-D-mannosidase) were eluted from DEAE-cellulose at a lower salt concentration after treatment with neuraminidase, although the elution profiles of β-D-glucosidase, β-D-xylosidase, and acid phosphatases were unaffected, indicating that they are less susceptible to the action of neuraminidase. The structure (9) assigned (G. Spick, B. Bayard, B. Fournet, and G. Strecker, *F.E.B.S. Letters*, 1975, **50**, 296) to the carbohydrate component of human serotransferrin has been confirmed by high-resolution ^{1}H n.m.r. spectroscopy.[410] Electrophoresis separated human serotransferrin into four molecular forms that contain different proportions of bound iron.[411] The nature of the interaction of renins from human and rabbit kidneys with immobilized concanavalin A, and their subsequent desorption with methyl α-D-mannopyranoside, suggested that both proteins are glycosylated.[412]

Plasma kallikrein acted on the high-molecular-weight form of bovine plasma kininogen with the release of a glycopeptide containing oligosaccharide chains

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α-Neu*N*Ac*p*-(2→6)-β-D-Gal*p*-(1→4)-β-D-Glc*N*Ac*p*-(1→2)-α-D-Man*p*
1
↓
6
α-Neu*N*Ac*p*-(2→6)-β-D-Gal*p*-(1→4)-β-D-Glc*N*Ac*p*-(1→2)-α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*N*Ac*p*-(1→4)-β-D-Glc*N*Ac*p*-Asn

(9)

that consist mainly of the trisaccharide α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 3)-D-Gal*N*Ac*p* and some of the tetrasaccharide α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 3)-[α-Neu*N*Ac*p*-(2 → 6)]-D-Gal*N*Ac*p*.[413] Highly purified bovine kininogen (mol. wt. 7.6×10^4) has been shown to act as a corrective factor in human plasmas which are deficient in a kininogen of high molecular weight (Flaujeac factor) that participates in coagulation and fibrinolysis.[414] Bovine ribonuclease B has been shown to be more resistant to proteolysis than the non-glycosylated A isoenzyme.[415]

Porcine haptoglobin has been purified by affinity chromatography on Sepharose–concanavalin A.[416]

Two ribonucleases from the pancreas of guinea-pigs differ not only in their amino-acid sequences but also in the extents of glycosylation of asparagine-21 and -34.[417]

Murine asialofetuin is taken up by hepatocytes by way of the microsomes and lysosomes.[418]

No significant differences were found in the carbohydrate compositions of glycopeptide fractions derived from the principal apoprotein of the low-density lipoprotein from normal individuals and from patients with hyperlipoproteinaemia type IIa.[419] The carbohydrate compositions of the apoproteins of low- and high-density lipoproteins from the sera of chimpanzees, patas, baboons, and rhesus and spider monkeys have been compared.[420] The proportions of sialic acid and L-fucose in the apolipoproteins may be related to lipoprotein metabolism and to the susceptibility (or the resistance) of the species to either spontaneous or diet-induced atherosclerosis. Analyses of three glycopeptides from a pronasic digest of porcine low-density lipoprotein showed that one (mol. wt. 2.3×10^3) of them contains residues of D-mannose and 2-acetamido-2-deoxy-D-glucose in the molar proportions 6:2, whereas the others (mol. wts. 2.1×10^3 and 3.1×10^3) contain residues of sialic acid, D-mannose, D-galactose, L-fucose, and 2-acetamido-2-deoxy-D-glucose in the molar proportions 1:4:2:1:3 and 2:4:3:1:3, respectively.[421]

The preparation and the assay of the components of fibrinolysis and thrombolysis have been reviewed.[422] The amino-acid sequences at the NH_2-terminus, molecular weights, and amino-acid compositions of human prothrombin, factors IX and X, and a new plasma protein have been compared.[423] Desialylation of human plasma factor VIII decreased its ristocetin-induced platelet-aggregation activity and facilitated its clearance by hepatocytes, but its procoagulant activity was not affected.[424] Experiments *in vivo* have confirmed the view that the inhibi-

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tion of activated factor IX by antithrombin III in the presence of heparin is important in preventing thrombosis.[425] An improved method for isolating factor IX has been reported.[426] Factor IX has a molecular weight of *ca.* 6.6×10^4 (determined by sedimentation or by gel electrophoresis in the presence of sodium dodecyl sulphate) and contains residues of D-mannose (1.70%), D-galactose (1.62%), D-glucose (0.92%), L-fucose (0.45%), 2-acetamido-2-deoxy-D-hexose (6.80%), and sialic acid (6.0%). The interaction between activated factor X(Xa) and its natural inhibitor (XaI) was accelerated *in vitro* by sodium heparinate and also by a heparinoid from pig duodenum, which was three times less effective.[427] However, the heparinoid did not enhance the plasma inhibitor activity *in vivo*, so that heparinoids should not be considered as an alternative to heparin in preventing thromboembolism. A substantial purification of factor XI from bovine plasma has been achieved; the purified factor has a molecular weight of 1.24×10^5 and consists of two similar polypeptide chains and residues of hexose (5.4%), 2-acetamido-2-deoxy-D-hexose (4.7%), and sialic acid (1.0%).[428] Bovine factor XII (purified 1.4×10^4-fold) has a molecular weight of 7.4×10^4 and contains 13% of carbohydrate, including residues of hexose (3.4%), 2-acetamido-2-deoxy-D-hexose (4.7%), and *N*-acetylneuraminic acid (5.4%).[429] The interactions of two forms of human-plasma factor XII, possessing widely different physical properties and enzymic activities, with the antithrombin–heparin cofactor have been examined.[430] Both forms were progressively neutralized by the antithrombin–heparin cofactor, and heparin markedly accelerated the rate of each of the reactions. The speed and selectivity of this inhibitory mechanism may be important in modulating the activities of other enzymes (serine proteases) of the haemostatic system.

Activation of human plasminogen by streptokinase requires the formation of a 1:1-complex, and it has been shown that the peptide at the NH_2-terminus of plasminogen is not required to be released before cleavage of the arginyl–valine linkage that results in the formation of a two-chain plasmin molecule.[431] This peptide is cleaved from native plasminogen or from the heavy-chain of plasmin in the streptokinase complex by free plasmin rather than by streptokinase-associated plasmin.

Asialofibrinogen formed aggregates faster than the sialylated protein, but its ability to form cross-linked fibrin is not impaired and its metabolic properties do not differ significantly from those of normal fibrinogen.[432] A sequence of 21 amino-acids, which includes the asparagyl residue carrying the carbohydrate component, in the β-chain of human fibrin has been established.[433]

The purification, crystallization, and preliminary *X*-ray analysis (2.5 Å resolution) of a fragment (corresponding to residues 1—156 and carrying two of the three oligosaccharide chains) of bovine prothrombin have been reported.[434] Highly

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purified ovine prothrombin contains 5.6% of neutral sugars and has a molecular weight of 7.7×10^4.[435]

High concentrations of α-foetoprotein appear to alter the receptors on the surface of lymphocytes in a differential manner, inhibiting lymphocyte transformation by phytohaemagglutinin *in vitro*.[436] Activities varying over three orders of magnitude have been found for three molecular species of α-foetoprotein isolated from the sera and ascitic fluids of five patients with hepatomas and from foetal livers.[437] Treatment of these species with neuraminidase did not alter their biological potencies, but it did reduce their number to two. Nine molecular species of α-foetoprotein in rat amniotic fluid have been characterized following separation by gel electrophoresis and affinity chromatography on concanavalin A and a lectin from *Ricinus communis*.[438, 439] The polypeptide and carbohydrate chains are heterogeneous[440] and increasing amounts of sialic acid appear to be added to α-foetoprotein during development of the foetus.[441] Small quantities of α-foetoprotein can be isolated from rat amniotic fluid by gel filtration.[442] The α-foetoprotein (mol. wt. 7.2×10^4) from a mouse hepatoma (BW 7756) contains D-mannose (4.6), D-glucose (3.1), D-galactose (1.2), 2-amino-2-deoxy-D-galactose (5.1), 2-amino-2-deoxy-D-glucose (1.4), and sialic acid (2.9 residues for each molecule of protein).[443] Residues of sialic acid appear to be essential for the immunosuppressive activity of α-foetoprotein.[444]

Possible uses of the heterogeneity of carcinoembryonic antigen as a tumour marker have been reviewed.[445] Part of the heterogeneity resides in the composition of the carbohydrate components, which can be monitored by a simplified Farr radioimmunoassay using concanavalin A and wheat-germ agglutinin.[446] Immunoadsorbent columns have been used to obtain small amounts of purified carcinoembryonic antigen from normal adult colon mucosa.[447] The immunological and physicochemical properties of the purified antigen are indistinguishable from those of carcinoembryonic antigen found in larger amounts in colon and rectum carcinomas.

The elevated level of sialyltransferase activity in the sera of rats bearing mammary tumours correlates with the high level of sialylated proteins in their sera, and may account for the increased half-lives of tumour-specific glycoproteins.[448]

Erythropoietin from patients with aplastic anaemia is heterogeneous, owing to variable proportions of sialic acid residues in the molecules.[449]

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β-D-Galp-(1→4)-β-D-GlcNAcp-(1→2)-α-D-Manp-(1→3)-β-D-Manp-(1→4)-β-D-GlcNAcp-(1→4)-β-D-GlcNAcp-Asn
6
↑
1
(α-Neu5Ac)0 or 1-(2→6)-β-D-Galp-(1→4)-β-D-GlcNAcp-(1→2)-β-D-Manp
(α-L-Fucp)0 or 1

(10)

Immunoglobulins

The location, number, and types of oligosaccharide chains in human immunoglobulins IgA1 and IgA2 have been determined by analysis of the amino-acid sequences of the α-heavy-chains.[450] Both A2m allotypes of the α2-chain of immunoglobulin IgA2 have two oligosaccharide chains containing 2-amino-2-deoxy-D-glucose that are absent from the α1-chain, but they do not contain 2-amino-2-deoxy-D-galactose. The A2m(2) allotype has a fifth oligosaccharide chain that contains 2-amino-2-deoxy-D-glucose. The evolutionary retention of the tripeptide acceptor sequence for 2-amino-2-deoxy-D-glucose suggests that the oligosaccharide components of immunoglobulins have structural and biological roles. The secretion and the glycosylation of immunoglobulins IgA and IgE by MOPC 315 mouse and IR162 rat plasma cells, respectively, were inhibited by tunicamycin.[341] However, it had little effect on the secretion of normally non-glycosylated λ-light-chains or on cell-free protein synthesis, demonstrating that tunicamycin is neither a general inhibitor of protein synthesis nor a non-specific inhibitor of protein secretion. Efforts have been made to map the genes coding for the transferases responsible for the glycosylation of mouse immunoglobulins.[452]

Partial hydrolysis with acid, oxidation with periodate, methylation analysis, and enzymic degradations, *etc.*, have shown that immunoglobulin IgG from bovine colostral whey contains carbohydrate components having the structure (10).[453] An immunoglobulin IgM associated with human myeloma contains a carbohydrate component composed of 2-acetamido-2-deoxy-D-glucose (2) and D-mannose (10 residues for each molecule of protein); the high content of D-mannose is attributed to a hyperactive D-mannosyltransferase in the transformed cell.[454]

Affinity chromatography on immunoadsorbents prepared from cellulose *trans*-2,3-carbonate has been used to separate rabbit immunoglobulin IgG λ- and κ-light-chains.[455] Structural studies on the heavy-chain of HLA antigens have provided further evidence that HLA antigens and immunoglobulins have a common evolutionary origin.[456] The structures and the functions of HLA antigens have been reviewed.[457]

Erythrocyte Glycoproteins

The structure 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactopyranose is found in all glycoproteins extracted with phenol from equine, ovine, bovine, porcine, and human erythrocyte membranes and from human fat-globule membranes.[458] Structures containing a tetrasaccharide, which consists of the foregoing disaccharide sialylated at O-3′ and O-6, are also present. The proportion of carbohydrate residues in human-erythrocyte glycoproteins decreases with age, while the resistance of the glycoproteins to the actions of neuraminidase and pronase

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increases.[459] The binding of four ^{125}I-labelled lectins to normal and neuraminidase-treated human erythrocytes has been studied in detail.[460] Quantitative inhibition of the binding of these lectins was also performed with diverse glycoproteins and glycopeptides as inhibitors. The inhibition constants and the association constants for binding the lectins to the cells were used to assess the relative receptor activities of cell-surface glycoproteins for the lectins. The structures and occurrence of receptors for lectins, particularly those lectins that bind to the antigenic structure 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose (a component of the MN blood-group antigen), on glycoproteins in the plasma membranes of human and bovine erythrocytes, milk-fat globules, and lymphocytes have been examined.[461]

The topography of the external surface of human red-cell membranes has been studied using the small, non-penetrating probe diazodi[^{125}I]iodosulphanilic acid, whose reaction with the membranes differs quantitatively and qualitatively from those of other probes in common use.[462] The work emphasizes the need to take into account the properties of the probe in interpreting information about the surface of cells. Two of the sialoglycoproteins (mol. wts. 2.7×10^4 and 3.5×10^4) present in the membrane of human erythrocytes did not appear to be labelled by lactoperoxidase-catalysed iodination—a reaction specific for components on the external surface of cells.[463] Liposomes into which the receptor for concanavalin A on red-cell membranes had been incorporated were agglutinated by concanavalin A, where human erythrocytes are not normally considered to be agglutinable by the lectin.[464] Liposomes incorporating glycophorin A were also readily agglutinated. A study of the freeze-fracture patterns of model membranes deposited on mica has supported the view that sialoglycoprotein fractions represent the particles seen in human red-cell membranes.[465] Increased proportions of sialic acid and neutral hexose residues, and decreased proportions of L-fucose and hexosamine residues, are characteristically found in the erythrocyte membranes of patients with cirrhosis of the liver.[466]

Immunochemical evidence has confirmed that glycophorin A traverses the membrane of human red-blood cells.[467] ^{31}P N.m.r.-spectroscopic analysis of glycophorin A and of the peptides released from glycophorin A by trypsinolysis revealed that diphospho-inositide is closely associated with the hydrophobic region of the protein, suggesting that there is a specific interaction between the phospholipid and the intramembranous portion of glycophorin A.[468] The lectin activity found in ghost membranes from human erythrocytes is associated with glycophorin A, and it was proposed that endogenous lectin–receptor interactions may be important in controlling the lateral mobility of membrane components.[469]

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Characterization of the principal glycoprotein from glycophorin A has revealed that carbohydrate components are attached to three sequences of amino-acid residues, which are mainly located at the CO_2H-terminus. This region also contains the receptors for concanavalin A and for the lectins from *Ricinis communis* and *Phaseolus vulgaris*.[470,471] The glycoproteins PAS-1, -2, and -3 (no sulphydryl groups) were rapidly and quantitatively separated from other red-cell membrane proteins by passage through an organomercurial affinity gel, which retained the other proteins.[472] A study of erythrocyte membranes from donors of the Ss^- phenotype (*i.e.* who lack Ss blood-group antigens located on the minor PAS-3 glycoprotein) has revealed that the PAS-3 glycoprotein does not contain some of the carbohydrate components which are present in the corresponding glycoprotein from normal erythrocytes.[473] The use of anti-glycolipid antibodies has revealed the presence on the erythrocyte surface of glycoproteins that carry carbohydrate chains identical to those found in such glycolipids as globoside and ganglioside G_{M_1}.[474]

The principal 'intrinsic' membrane protein and MN glycoprotein from human erythrocytes have been separated by isoelectric focusing in polyacrylamide gels containing Triton X-100.[475] Quantitative immunoelectrophoresis indicated that the 'intrinsic' glycoprotein traverses the membrane, whereas all determinants of the MN glycoprotein are found only on the external surface of the membrane. A property of the external surface of human erythrocytes can be transferred to a synthetic substrate.[476] Thus, polystyrene latex spheres adsorbed purified MM glycoprotein, which retained its antigenic reactivity upon adsorption. Differences have been found between the amino-acid compositions of the M and N glycoproteins from human erythrocytes.[477]

Extraction of human-platelet membranes with a 1 % solution of sodium deoxycholate and affinity chromatography of the extract on lectins from *Lens culinaris*, wheat germ, and *Abrus precatorius* afforded, at least, seven glycoproteins.[478] A glycoprotein from human platelets that is sensitive to proteolysis by thrombin has a molecular weight of 1.5×10^5.[479] Since the sialic acid residues of a glycoprotein (glycocalicin) of high molecular weight were removed when suspensions of human platelets were treated with neuraminidase, it is probably located at the surface of the platelets.[480] Two unrelated patients with clinically evident haemostatic problems showed an abnormal polymorphic pattern of glycoproteins within their platelet membranes, and the ability of the intact platelets to bind thrombin was reduced.[481]

1-Deoxy-1-(*N*-valyl)-D-fructose has been identified as the NH_2-terminal unit

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of the β-chain of haemoglobin A_{1c}, a minor component of normal adult erythrocytes.[482] Phosphorylated hexoses or trioses containing a free aldehydo- or keto-group are able to glycosylate haemoglobin A in a non-enzymic reaction that affords an adduct indistinguishable from haemoglobin A_{1b} (as judged by ion-exchange chromatography and isoelectric focusing).[483] The total level of glycosylated haemoglobins in blood appears to be a useful index for evaluating the long-term control of blood D-glucose by diabetic patients.[484]

The surface components of rabbit reticulocytes and erythrocytes appear to be identical, whereas nucleated erythroid cells bound to the bone marrow have an entirely different complement of surface components.[485] Stimulation of rabbit reticulocytes with phenylhydrazine caused, among others, a change in the content of sialic acid residues in the membrane.[486]

Sialidase-treated erythrocytes from rats, unlike the normal erythrocytes, preferentially adhered to mononuclear spleen cells and Kupffer cells of the liver.[487] This recognition manifests itself, both in autologous and homologous systems, by the adhesion of complementary cells in the form of rosettes. This process may be the normal physiological mechanism for removing circulating erythrocytes that have a decreasing proportion of sialic acid residues.

Primitive erythroid cells from 5-day-old chicken embryos do not contain a glycoprotein (mol. wt. 4×10^4) that is present in definitive erythrocytes from 17-day-old embryos and from adult chickens; erythrocytes from young chickens contain less of a glycoprotein of molecular weight 5×10^4.[488] Lactoperoxidase-catalysed iodination revealed the presence of three glycoproteins on the external surface of the primitive erythroid cells. A systematic examination of the protein composition of plasma membranes from embryonic chicken erythroid cells at various stages of maturation has been reported.[489] Significant changes in the types and the amounts of membrane proteins occur during the early stages of differentiation of embryonic erythroid cells. The rate of turnover of each component seems to have stabilized by the late polychromatophilic erythroblast stage.

Salivary and Mucous Glycoproteins

Differences in the rheological properties of mucociliary epithelial secretions from bovine cervix and human lung and middle ear have been ascribed to differences in the extents of cross-linking of glycoprotein units, rather than to differences in their chemical compositions and structures.[490] The glycoproteins in mucociliary epithelial secretions from frogs' palates have different chemical compositions from those in mammalian secretions, in keeping with their markedly different rheological properties.

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Glycoproteins in the sputum of patients with chronic bronchitis have been solubilized and then fractionated on Sepharose 4B into components of high and low molecular weight.[491] The sputum of bronchial patients contains more components of low molecular weight. Mucins from macroscopically healthy areas of the bronchial mucosa of six adults are anionic and contain residues of sialic acid and sulphate.[492] Affinity chromatography on immobilized *Ricinus communis* lectin has enabled the mucins and other glycoproteins secreted by human bronchia to be characterized.[493]

Only slight differences were found in the glycoprotein contents of secretions from patients with chronic bronchitis and from those with cystic fibrosis, although some of the glycoproteins in the latter secretions appear to be more highly sulphated.[494] Neither is heterogeneity of the carbohydrate components a characteristic feature of glycoproteins in the secretions of patients with cystic fibrosis, since those of glycoproteins in the secretions of patients with chronic bronchitis are just as disperse.[495]

The c.d. spectra of proline-rich glycopeptides (mol. wts. 6—12 $\times$ 10^3) from human parotid saliva have been examined, and it was suggested that these glycopeptides are the precursors of glycoproteins of higher molecular weight.[496]

Similarities have been found among the proline-rich glycoproteins isolated from the submandibular glands of humans and monkeys (*Cercopithecus aethiops*).[497] A glycoprotein of high molecular weight composed of 1800 amino-acid residues and roughly 400 oligosaccharide chains, each containing approximately nine sugar residues, has been isolated from the cervical mucus of the bonnet monkey (*Macaca radiata*).[498] The oligosaccharide chains consist of residues of D-galactose, 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, L-fucose, sialic acid, and sulphate, and they are attached, *via* 2-acetamido-2-deoxy-D-galactose, to residues of serine or threonine in the polypeptide chain. Enzymic degradation and methylation studies showed that the tetrasaccharide α-D-Gal*p*-(1 → 3)-[α-L-Fuc*p*-(1 → 2)]-D-Gal*p*-(1 → 4)-D-Glc*N*Ac*p* is linked to a core component containing residues of 2-acetamido-2-deoxy-D-galactose.

A glycoprotein composed of a single polypeptide chain and containing more than 150 D-galactosyl-2-acetamido-2-deoxy-D-galactosyl side-chains has been isolated from the synovial fluid obtained from metacarpal–phalangeal joints of adult cattle.[499] The disaccharide side-chains are distributed along the length of the polypeptide chain and sialic acid residues are attached to the non-reducing end of approximately two-thirds of them. The regions carrying carbohydrate

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components and those containing the disulphide bridges that join together the four subunits appear to be distinct in a glycoprotein (mol. wt. 2×10^6) from porcine gastric mucus.[500]

A purified glycoprotein (mol. wt. 5.83×10^4) from ovine submaxillary mucin contains residues of 2-acetamido-2-deoxy-D-galactopyranose linked to either seryl or threonyl residues; 86% of the sugar residues are sialylated.[501] Sequencing of 106 of the 650 amino-acid residues indicated that the protein chain of this glycoprotein has a unique primary structure.[502] The amino-acid sequences carrying 28 of the carbohydrate components do not appear to be homologous and no repeating sequences were found. Mucins from the intestinal mucosa of sheep suffering from a deficiency of zinc appear to differ from those in healthy animals.[503]

Urinary Glycoproteins

The hexasaccharide (11) found in the urine of pregnant women is also present in their milk.[504] One (12) of nine hexasaccharides isolated from the urine of an O(H)/Le(a + b−) non-secretor during pregnancy and lactation has not been found previously,[505] neither have three [(13)—(15)] of a number of oligosaccharides isolated from the urine of an A/Le(a−b+) female secretor.[506] Three new oligosaccharides (16)—(18) containing *myo*-inositol[506] and an octasaccharide containing D-galactose, D-glucose, L-fucose, 2-acetamido-2-deoxy-D-glucose, and sialic acid in the molar proportions 3:1:1:2:1,[507] which are found in the urine of women during pregnancy, have also been isolated and partially characterized. The rates of excretion of these and other urinary oligosaccharides have been monitored during pregnancy and lactation.[508]

The structures of sialylated oligosaccharides in the urine of patients with mucolipidosis I[509] or II[510] have been determined.

Patients with severe macroglobulinaemia IgM secrete an acidic glycoprotein (containing 13.2% of carbohydrate) that binds Ca^{2+} ions.[511] This glycoprotein has a molecular weight of 6.2×10^4, which decreases to 2.1×10^4 under dissociative conditions. A new glycoprotein of low molecular weight (3.1×10^4) recovered from the urine of ureamic patients contains 19% of carbohydrate consisting of residues of D-mannose (3.3%), D-galactose (3.0%), 2-acetamido-2-deoxy-D-glucose (6.1%), 2-acetamido-2-deoxy-D-galactose (1.2%), and sialic acid (5.7%).[512] The amounts of a urinary glycoprotein, which originates from the glomerular basement

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[501] H. D. Hill, J. A. Reynolds, and R. L. Hill, *J. Biol. Chem.*, 1977, **252**, 3791.
[502] H. D. Hill, M. Schwyzer, H. M. Steinman, and R. L. Hill, *J. Biol. Chem.*, 1977, **252**, 3799.
[503] J. Quarterman, F. A. Jackson, and J. N. Morrison, *Life Sciences*, 1976, **19**, 979.
[504] M. Lemonnier and R. Bourillon, *Carbohydrate Res.*, 1976, **51**, 99.
[505] P. Hallgren and A. Lundblad, *J. Biol. Chem.*, 1977, **252**, 1014.
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[509] J.-C. Michalski, G. Strecker, B. Fournet, M. Cantz, and J. Spranger, *F.E.B.S. Letters*, 1977, **79**, 101.
[510] G. Strecker, M. Peers, J.-C. Michalski, T. Hondi-Assah, B. Fournet, G. Spik, J. Montreuil, J. Farriaux, P. Maroteaux, and P. Durand, *European J. Biochem.*, 1977, **75**, 391.
[511] K. Bobrzecka, L. Konieczny, R. Rybarska, and E. Krzemińska, *Biochim. Biophys. Acta*, 1977, **497**, 377.
[512] L. Svensson and U. Ravnskov, *Clinica Chim. Acta*, 1976, **73**, 415.

α-Neu*N*Ac*p*-(2→3)-β-D-Gal*p*-(1→3)-β-D-Glc*N*Ac*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*
6
↑
2
α-Neu*N*Ac*p*

(11)

β-D-Gal*p*-(1→4)-β-D-Glc*N*Ac*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*
3 3
↑ ↑
1 1
α-L-Fuc*p* α-L-Fuc*p*

(12)

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*N*Ac*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*
3 2
↑ ↑
1 1
α-L-Fuc*p* α-L-Fuc*p*

(13)

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*N*Ac*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*
3
↑
1
α-L-Fuc*p*

(14)

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Glc*N*Ac*p*-(1→3)-β-D-Gal*p*-(1→4)-D-Glc*p*
2
↑
1
α-L-Fuc*p*

(15)

membrane, have been used to assess the extent of damage to the glomerular basement membrane in cases of systemic lupus erythematosus (membranous type) and membranous glomerulonephritis.[513]

Affinity chromatography on a lectin from *Phaseolus vulgaris* coupled to agarose has been used to purify human urinary erythropoietin.[514] The structures (19)—(24) have been reported for glycosylated asparagines isolated from the urine of patients

[513] S. Shibata and T. Nagasawa, *J. Lab. Clin. Med.*, 1977, **89**, 207.
[514] F. Sieber, *Biochim. Biophys. Acta*, 1977, **496**, 146.

α-D-Gal*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → ?)-*myo*-inositol
2 ?
↑ ↑
1 1
α-L-Fuc*p* α-L-Fuc*p*

(16)

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → ?)-*myo*-inositol
?
↑
1
α-L-Fuc*p*

(17)

α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → ?)-*myo*-inositol

(18)

β-D-Glc*N*Ac*p*-Asn

(19)

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

(20)

α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-Asn

(21)

α-Neu*N*Ac*p*-(2 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-Asn

(22)

with aspartylglycosylaminuria.[515] A method for the routine determination of the Tamm–Horsfall glycoprotein in human urine is based on quantitative electro-immunoassay.[516] The glycoprotein was solubilized (0.3% sodium dodecyl sulphate at pH 5.5 and 37 °C) and maintained in solution before assay.

There is considerably more of a non-dialysable glycoprotein in the urine of germ-free rats than in the urine of normal animals.[517] This mucin-like material contains residues of L-fucose (10.1%), D-galactose (12.2%), 2-acetamido-2-deoxy-D-glucose (16.6%), 2-acetamido-2-deoxy-D-galactose (19.8%), sialic acid (11.9%), and sulphate (0.7%).

[515] K. Sugahara, M. Akasaki, I. Funakoshi, P. Aula, and I. Yamashina, *F.E.B.S. Letters*, 1977, **78**, 284.
[516] J. Wieslander, P. Bygren, and D. Heinegård, *Clinica Chim. Acta*, 1977, **78**, 391.
[517] J. K. Wold and T. Midtvedt, *Biochim. Biophys. Acta*, 1977, **490**, 395.

α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-Asn

(23)

α-Neu*N*Ac*p*-(2 → 6)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-Asn

(24)

Avian Glycoproteins

Purified ovotransferrin has been separated into three components by affinity chromatography on Sepharose–concanavalin A; the components have different amino-acid compositions as well as different carbohydrate compositions.[518] Three glycopeptides containing mainly four amino-acids in the sequence Asn-Leu-Thr-Ser, with small amounts of other amino-acid residues, have been isolated from enzymic hydrolysates of hen egg-white albumin.[519] The results of acid–base titrations on these glycopeptides did not support the existence of a hydrogen bond between the hydroxy-group of threonyl residues and the β-oxygen atom of substituted asparagyl residues in hen egg-white albumin, although the transition state for the glycosylation of asparagyl residues might still involve assistance by the hydroxy-group of relevant serine or threonine residues.

Miscellaneous Glycoproteins and Chitin

One of the latest volumes in the series 'International Review of Science' covers the isolation and structures of glycoproteins from ovarian cysts, gastric mucosa, submaxilliary glands, and erythrocytes.[520] Physical and chemical data have been compiled for glycoproteins.[521] Other reviews have discussed the chemistry, occurrence, and metabolism of sialic acids, with reference to cell-surface neuraminidases and the metabolism of molecules containing residues of sialic acid by cells transformed with tumourigenic viruses,[522] and the effects of neuraminidases on intermolecular and intercellular forces.[523]

Expressions have been derived for the distribution, at density-gradient equilibrium, of macromolecules whose densities are either close to the values characterizing the solution limits or outside the limits of the gradient.[524] The distribution equations were applied to hypothetical mixtures of proteins and glycoproteins in commonly used density-gradient media (*e.g.* cesium chloride or bromide solutions) and they should be helpful in deciding on experimental conditions for the separation of such macromolecules. The c.d. spectra of glycopeptide fragments derived from α_1-acid glycoprotein, thyroglobulin, and ovalbumin are reasonably additive when compared with those of monomeric sialic acid and the corresponding asialoglycopeptides, implying that the covalent attachment of sialic acid residues to the ends of oligosaccharide chains does not lead to interactions with other chromophores or to positioning in a particularly asymmetric environment.[525] By contrast, the magnitudes of the ellipticity extrema above 200 nm in the c.d. spectra of the asialoglycopeptides, which have 2-acetamido-2-deoxy-D-glucose as the principal chromophore, are much greater than can be accounted for on the basis of monomeric contributions (*i.e.* free 2-acetamido-2-deoxy-D-glucose)

[518] H. Iwase and K. Hotta, *J. Biol. Chem.*, 1977, **252**, 5437.
[519] B. M. Austen and R. D. Marshall, *Biochem. J.*, 1977, **163**, 31.
[520] K. O. Lloyd, in ref. 157, p. 251.
[521] 'Handbook of Biochemistry and Molecular Biology, Section C', vol. 1, 3rd edn., CRC Press Inc., Cleveland, Ohio, 1975.
[522] 'Biological Roles of Sialic Acid', ed. A. Rosenberg and C. Schengrund, Plenum Press, New York and London, 1976.
[523] D. W. Hutchinson and J. P. Kabayo, *Trends in Biochemical Sciences*, 1977, **2**, 1.
[524] J. M. Creeth and J. R. Horton, *Biochem. J.*, 1977, **161**, 449.
[525] D. Puett, L. A. Holladay, J. D. Ford, and L. W. Cunningham, *Biochim. Biophys. Acta*, 1977, **491**, 129.

and suggest the possibility of secondary structure in the carbohydrate component of glycoproteins. An attempt has been made to relate the sites of attachment of the glycan chains in glycoproteins of known primary structure (*e.g.* human orosomucoid and luteinizing hormone) to the nature of the secondary structure (β-coil, disordered regions, *etc.*) in which glycosylated asparagyl and seryl residues are located.[526]

The amount of bound water (studied by ^{1}H n.m.r. spectroscopy) and the extended molecular conformation appear to be responsible for the anti-freeze properties of a glycoprotein from the Antarctic fish *Dissostichus mawsoni*.[527] Chemical modification has shown that the carbohydrate moiety of the glycoprotein component of the $(Na^+ + K^+)$ATPase in membrane preparations of the salt glands from dog fish does not contribute to the catalytic activity of the enzyme.[528] One of two glycoproteins extracted from squids (*Sepia officinalis*) resembles collagen.[529]

A protease from the venom of Russell's viper, which is able to activate the blood-clotting factors X, IX, and protein C, contains 13% of carbohydrate, consisting of residues of hexose (6%), sialic acid (1.7%), and 2-amino-2-deoxy-D-galactose (5.3%), that is distributed between two polypeptide chains comprising the glycoprotein.[530] An *exo*-β-D-2-acetamido-2-deoxyglucosidase from the silkworm (*Bombyx mori*) is composed of two identical subunits (mol. wt. 6.1×10^4) and contains residues of neutral sugars (4%) and 2-amino-2-deoxy-D-glucose (1.3%).[531]

As well as residues of L-fucose, D-xylose, D-mannose, D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and 2-acetamido-2-deoxy-D-galactose, the carbohydrate component of *Helix pomatia* haemocyanin contains residues of 3-*O*-methyl-D-galactose and that from *Lymnaea stagnalis* haemocyanin contains residues of 3-*O*-methyl-D-galactose and 3-*O*-methyl-D-mannose.[532] When the D-galactan from the albumin glands of the snail *Strophocheilus oblongus* was submitted to three cycles of Smith degradation, the degraded polysaccharide (6% yield) was more linear.[533] Methylation analyses of the original and the Smith-degraded polysaccharides indicated that oxidation with periodate proceeds by way of non-reducing end-groups and through some interior residues that are exposed during the oxidation. Galactogen in perivitelline fluids secreted by the albumin glands of the freshwater snails *Bulimnae megasoma* and *Lymnaea stagnalis* is metabolized by the embryos.[534]

Proteins can be attached to Sephadex or Sepharose or cellulose by reaction with the oxidized polysaccharide (aqueous bromine at pH 7.0) and reduction of the resulting Schiff base with sodium cyanoborohydride.[535] Cytochemical markers for detecting lectins bound to cell surfaces have been prepared by glycosylation of horseradish peroxidase and ferritin.[536] 8-Methoxycarbonyloctyl glycosides have

[526] M. H. Loucheux-Lefebvre and J. P. Aubert, *Compt. rend.*, 1976, **282**, *D*, 585.
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[533] E. A. D. Segura and J. H. Duarte, *Carbohydrate Res.*, 1976, **52**, 159.
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[535] O. Larm and E. Scholander, *Carbohydrate Res.*, 1977, **58**, 249.
[536] C. Kieda, F. Delmotte, and M. Monsigny, *F.E.B.S. Letters*, 1977, **76**, 257.

been converted into the corresponding 8-azidocarbonyloctyl glycosides, which can be coupled to bovine serum albumin to produce artificial antigens that possess strongly immunodominant carbohydrate determinants.[537] This method promises to make available a wide range of well-defined antigens and their homologous antibodies.

The chemistry, biochemistry, and industrial and medical uses of chitin have been reviewed.[538] Partially de-*N*-acetylated chitin, which was obtained following treatment of chitin with alkali at 25 °C for two or three days, was reasonably soluble in water at 0 °C.[539] The effect of sonic radiation on the morphology of microfibrils of chitins and celluloses has been correlated with the polarity of the constituent chains.[540] For given conditions, fibrillation of the microfibrils takes place when the polarity of the chains is antiparallel but not when it is parallel. The synthesis of chitin by a wide variety of insect pests is inhibited by diflubenzuron (25).[541]

Analysis and Other Features of Glycoproteins

The di-*N*-acetylchitobiosyl linkage in (D-Man*p*)$_n$ → (D-Glc*N*Ac*p*)$_2$ → Asn (n = 3, 5, or 6) and L-Fuc*p* → (D-Man*p*)$_3$ → (D-Glc*N*Ac*p*)$_2$ → Asn → *X* was hydrolysed by a purified *endo*-β-D-2-acetamido-2-deoxyglucosidase isolated from extracts of hen oviduct.[542] The existence of such an enzyme in animal tissues would account for the accumulation of oligosaccharides containing a 2-acetamido-2-deoxy-D-glucopyranosyl residue at the reducing end in certain lysosomal storage diseases. Little or no modification of the oligosaccharide components of porcine thyroglobulin and pineapple stem bromelain appeared to take place when they were cleaved from the polypeptide chain by hydrazinolysis.[543] Treatment of glycoproteins with anhydrous hydrogen fluoride at 0 °C cleaved all the linkages of neutral and acidic sugars within 1 h and the *O*-glycosidic linkages of amino-sugars within 3 h at 23 °C, leaving intact the peptide linkages and the *N*-glycosidic linkages between 2-acetamido-2-deoxy-D-glucopyranosyl and asparagyl residues.[544] The substitution patterns of 2-amino-2-deoxyhexoses in *O*-glycosylic oligosaccharide chains of rat-brain glycoproteins have been determined following release of the oligosaccharides with alkaline sodium borohydride and methylation analysis.[314] Improved and simple separations of 2-amino-2-deoxy-D-glucose and -galactose in the presence of peptides and tryptophan and of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucitol using an amino-acid analyser have been reported.[545]



(25)

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[542] A. L. Tarentino and F. Maley, *J. Biol. Chem.*, 1976, **251**, 6537.
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[545] T. H. Plummer, *Analyt. Biochem.*, 1976, **73**, 532.

Simultaneous assay of the neutral sugars and hexosamines released from glycoproteins on hydrolysis with an acidic resin has been described.[546] The resin-bound hexosamines were deaminated and the resulting 2,5-anhydroaldoses, together with the neutral sugars, were determined by g.l.c. of the aldononitrile acetates. A procedure for analysing mixtures of glycosaminoglycans from aortas by automated ion-exchange chromatography has been described.[547] A simple method for determining the pattern of *O*-acylation of the sialic acid residues of glycoproteins is based on identification of the product(s) obtained following oxidation of the polyhydroxy side-chain with periodate, reduction, and hydrolysis; glycoproteins containing as little as 230 μg of sialic acid can be analysed in this way.[548] The molecular weights of glycopeptides below 10^4 can be estimated by thin-layer chromatography using Sephadex G-50 gel in 6 M-guanidinium hydrochloride solution.[549] The migrations (R_f) of glycopeptides in the molecular-weight range 1.9—3.3 × 10^3 did not differ significantly from those of peptides within the same molecular-weight range, which can therefore be used as markers. Rocket affinity electrophoresis of lectin-binding glycoproteins in an agarose gel containing lectins (*e.g.* concanavalin A and soybean lectin) can be used for the identification and determination of glycoproteins, irrespective of the extent of denaturation of the protein component.[550] Glycoproteins in polyacrylamide gels can be located on binding a [^{125}I]lectin and autoradiography.[551] Membrane proteins and glycoproteins can be separated by a novel technique that uses discontinuous electrophoresis in two directions.[552] After electrophoresis in the first direction in the presence of chloral hydrate, electrophoresis in the second direction is conducted using the dodecyl sulphates of Tris and 4-picoline to stack and to separate further the glycoproteins. Antibodies raised against sugar-azoproteins have been purified by affinity chromatography.[553] They have a high specificity for monosaccharides and react with terminal, non-reducing sugars on the surface of human erythrocytes. A histochemical method, which is based on the detection of polyaldehydes (*i.e.* periodate-oxidized carbohydrates) in tissue sections with 4-hydrazinoacridine, has been adapted for the detection of glycoproteins in polyacrylamide gels.[554] Other cytochemical techniques have been modified to enable them to detect carbohydrates and glycoproteins in mitochondria.[555] Sialic acid residues exposed on the surface of cells can be labelled by oxidation with sodium periodate at 0 °C and reduction of the resulting aldehydes with sodium borotritide.[556] Since the periodate ion does not enter the cell at this temperature, only those sialic acid residues on the external surface of the cell are oxidized.

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Biosynthesis of Glycoproteins

The *de novo* synthesis and subsequent glycosylation of the MOPC-46B mouse immunoglobulin light-chain in an Ehrlich ascites cell-free extract has been reported.[557] The cell-free product was glycosylated after translation, apparently at the same site glycosylated by intact cells. The presence of the tripeptide sequence Asn-*X*-Thr(Ser) (where *X* is a variable amino-acid residue) is necessary, but not sufficient, for the transfer of the oligosaccharide chain from an oligosaccharyl-lipid to denatured forms of ovalbumin, α-lactalbumin, and ribonuclease A by a membrane fraction from hen oviduct.[558] Partial unfolding of the polypeptide chain must also occur before glycosylation can take place. Studies of the glycosylation of nascent chains of ovalbumin have revealed that, although the oligosaccharide component is attached to an asparagyl residue near the NH_2-terminus, the sugar residues are attached mainly or entirely to those polypeptide chains that are essentially complete.[559] Similar conclusions have been reached using rat-liver cells stimulated to produce an excess of α_1-acid glycoprotein.[560] A survey of 31 glycosylated sites in glycoproteins has shown that 30 of them occur in sequences favouring turn or loop structures and that 22 of the glycosylated asparagyl residues occur in tetrapeptide sequences that are predicted to have a β-turn conformation.[561]

Lipid-linked oligosaccharides in hen oviduct participate in the synthesis of the core oligosaccharides of membrane glycoproteins, as well as in that of the carbohydrate component of the principal secretory protein.[562] The main effect of 2-deoxy-D-*arabino*-hexose on chicken-embryo cells infected with Semliki forest virus was to inhibit the glycosylation of virus-specific glycoproteins; this blocks the synthesis of the infectious virus, possibly by GDP-2-deoxy-D-*arabino*-hexose competing with GDP-D-mannose as a glycosyl donor in the glycosylation either of viral glycoprotein acceptors or of dolichol derivatives.[563] Glycosylation of the polypeptide κ-type immunoglobulin light-chain (K-46) of murine myeloma tumour does not appear to be essential for its secretion, since 2-deoxy-D-*arabino*-hexose inhibited the attachment of carbohydrate chains without affecting the intracellular transport or the secretion of the non-glycosylated protein.[564]

The growth of *Halobacterium salinarium* is inhibited by bacitracin, suggesting that glycosylation of the principal component (a glycoprotein) of the cell envelope occurs by a pathway involving lipid intermediates.[565] Enzymes in cell homogenates of *H. salinarium* were shown to catalyse the transfer of sugars from UDP-D-glucose, GDP-D-mannose, and UDP-2-acetamido-2-deoxy-D-glucose to endogenous lipid acceptors. The glycosylation of apoproteins of very-low-density lipoproteins in rat liver continues during passage through the Golgi apparatus and into the secretory vesicles.[566] Oligosaccharide and protein are synthe-

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sized independently from different pools in the biosynthesis of a membrane glycoprotein of *Tetrahymena pyriformis*.[567]

The addition of very small amounts of UDP-D-glucose can modify the nature of lipid-linked oligosaccharides synthesized from GDP-D-mannose by cell-free systems from cultured fibroblasts (NIL-8).[568] As well as being incorporated into lipid-linked oligosaccharides, D-glucosyl residues were also incorporated into endogenous glycoproteins. The lipid-linked oligosaccharides synthesized from GDP-D-[^{14}C]mannose and dolichyl D-mannosyl phosphate by microsomes of calf pancreas have been partially characterized.[569] Evidence was presented for the presence in calf pancreas of a mixture of acidic glycolipids containing 8—15 residues of 2-acetamido-2-deoxy-D-glucose, D-glucose, and D-mannose in oligosaccharide chains that are attached to the lipid (probably dolichol) by way of a pyrophosphate linkage. Diethylstilbestrol produced a marked increase in the incorporation of D-mannosyl residues from GDP-D-mannose into dolichyl D-mannosyl phosphate, D-manno-oligosaccharyl dolichol, and D-manno-proteins in hen's oviduct, due, probably, to increased levels of endogenous dolichyl phosphate rather than to increases in the D-mannosyltransferase activities.[570] Membranes prepared from mutant, concanavalin A-resistant ovary cells of Chinese hamsters synthesized less D-manno-oligosaccharyl lipids than those from the wild-type cells.[571] D-Mannosylation of proteins *in vitro* was also reduced. Although tunicamycin reduced the synthesis of oligosaccharyl lipid by membranes of the wild-type cells, it had little effect on that by the mutant cells. Most of the D-mannosyltransferase activity in bovine retina occurs in membranes other than those from the rod outer segments.[572] An *in vitro* study of glycosylations catalysed by a particulate preparation from the intimal layer of porcine aorta indicated that, whereas some D-mannosyl residues are donated by GDP-D-mannose, others are donated by lipid-linked D-mannose intermediates.[573] The D-mannosyltransferases from the retina of chicken embryos catalysed the transfer of D-mannosyl residues from GDP-D-mannose to endogenous protein by way of lipid-linked mono- and oligo-saccharides.[574] An enzyme preparation from pea (*Pisum sativum* L.) seedlings has been shown to use endogenous acceptors to form lipid-linked mono-, di-, and oligo-saccharides containing D-mannose or 2-acetamido-2-deoxy-D-glucose or both from the appropriate nucleotide-linked sugars.[575] The endogenous acceptors in peas are α-saturated polyprenols, possibly dolichyl phosphate. Similar findings were reported using enzyme preparations from *Phaseolus vulgaris*.[576] Oligosaccharyl-lipid intermediates also participate in D-mannosylation of the envelope glycoprotein of Sindibis virus infecting chicken-embryo fibroblasts—a process that is inhibited by tunicamycin.[577] Whereas both D-mannosyl

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[568] P. W. Robbins, S. S. Krag, and T. Liu, *J. Biol. Chem.*, 1977, **252**, 1780.
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retinyl phosphate and dolichyl D-mannosyl phosphate are formed from GDP-D-mannose by membranes of isolated human platelets, only dolichyl D-glucosyl phosphate is formed from UDP-D-glucose.[578] A D-mannosyltransferase specific for each D-mannosyl polyprenyl phosphate appears to be present in human platelets, and the addition of retinyl phosphate specifically stimulated the synthesis of D-mannosyl retinyl phosphate. A preparation from rat liver catalysed the transfer of D-mannosyl residues from D-mannosyl retinyl phosphate, but not from dolichyl D-mannosyl phosphate, to endogenous protein.[579]

Incubation of UDP-D-[^{14}C]glucose with microsomes from calf pancreas afforded a labelled glycolipid (probably dolichyl D-glucosyl phosphate) and lipid-linked D-[^{14}C]gluco-oligosaccharides having slightly longer carbohydrate chains than those of the corresponding D-manno-oligosaccharides synthesized by the same system.[569, 580] D-Glucose was transferred to lipids consisting of 15 or 16 isoprenyl residues when UDP-D-[^{14}C]glucose was incubated with a particulate fraction from bakers' yeast.[581] Incubation of dolichyl D-glucosyl phosphate with this yeast particulate fraction resulted in the transfer of D-glucosyl residues to an endogenous acceptor that also contains two hexosamine residues and several D-mannosyl residues in an oligosaccharide chain joined to yeast dolichol by way of a pyrophosphate group. A soluble D-galactosyltransferase (mol. wt. 5.7×10^4) isolated from the mesentary lymph nodes of pigs catalysed the formation of a β-(1 → 4)-linkage between D-galactopyranose and the terminal 2-acetamido-2-deoxy-D-glucopyranosyl residues of immunoglobulin IgG, fetuin, ovalbumin, and ovomucoid.[582] An endogenous glycoprotein (identified as an incomplete heavy-chain of immunoglobulin IgG) present in particulate subcellular fractions of the mesentary lymph nodes was shown to be a very good substrate for the enzyme, which is probably involved in the synthesis of the oligosaccharide chain of immunoglobulin IgG. Removal of residues of sialic acid and D-galactose from the D-galactosyltransferase in bovine skim milk did not impair the catalytic activity.[583] The specific activity of the cell-surface D-galactosyltransferase in fibroblasts from baby-hamster kidneys increased markedly when growth was stimulated by foetal calf serum.[584] Dividing, but not resting, fibroblasts released a soluble form of D-galactosyltransferase into the culture medium, whereas sialyl- and L-fucosyl-transferases were retained. More D-galactosyltransferase was released from virus-transformed fibroblasts than from non-transformed fibroblasts. The glycoprotein: D-galactosyltransferase activity associated with membrane fractions from the Golgi complex of rat pancreas participates in the glycosylation of proteins in the membrane of zymogen granules, although lipid intermediates do not appear to be involved in this process.[585] YC8 Lymphocytes from Balb/c mice exhibit D-galactosyltransferase activity whereas the normal lymphocytes do not.[586, 587] D-Galactosyltrans-

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[587] E. Morel, G. Spik, and J. Montreuil, *Compt. rend.*, 1976, **282**, *D*, 317.

ferases have been found in the surface mucosal cells of rat stomach[588] and in the membranes[589] and microsomes[590, 591] of rat liver. The enzyme from rat-liver microsomes transferred D-galactosyl residues to glycoproteins (*e.g.* from the Antarctic fish *Trematomus borchgrevinki*) from which D-galactosyl residues had been removed.[591] An investigation of the role of glycoproteins in regulating fertility showed that the D-galactosyltransferase activity starts to increase in the endometrium of rat uteri at dioestrus and reaches a maximum on the afternoon of pro-oestrus.[592] The sialyltransferase activity is low during metoestrus and dioestrus but starts to increase on the morning of pro-oestrus and reaches a maximum on the morning of oestrus. A decrease in UDP-D-galactose pyrophosphatase activity accompanies the increase in D-galactosyltransferase activity and this should result in maximal production of glycoproteins about the time of ovulation. Acute ethanol intoxication produced an increase in the level of D-galactosyltransferase activity, and a decrease in the level of sialyltransferase activity, in the Golgi complex of rat liver.[593] The synthesis of D-galactosylated glycoproteins has been studied in hamster cells; although dolichyl phosphate stimulated the incorporation of D-galactosyl residues into glycoproteins, no D-galactosyl polyisoprenyl phosphate could be detected.[594]

Two pools of L-fucosylated glycoproteins appear to be present in HeLa cells. From one of them is derived plasma-membrane L-fucose, which eventually enters the growth medium (mostly as free L-fucose), whereas macromolecular L-fucose by-passes the cell surface to enter the growth medium directly from the other.[595] The L-fucosyl- and D-galactosyl-transferases in scrapings from murine gastric epithelia have been characterized.[596]

Guanosine triphosphate has been found to enhance the glycosylation of various endogenous acceptors by rough microsomes from rat liver previously treated with sodium pyrophosphate.[597] Partial hepatectomy stimulated the level of 2-acetamido-2-deoxy-D-glucosyltransferase activity in the microsomes of rats, a $\leqslant$ 20-fold increase being observed in regenerating liver within 48 h of the operation.[598] The incorporation of 2-amino-2-deoxy-D-[^{14}C]glucose into brush-border membranes by human small intestinal mucosa in organ culture has been investigated.[599] The radiolabel was incorporated into brush-border enzymes exhibiting maltase, glucoamylase, lactase, sucrase, isomaltase, enterokinase, and alkaline phosphatase activities. The incorporation of 2-amino-2-deoxy-D-[^{14}C]glucose into intact Chang liver cells produced a small amount of P^1-2-acetamido-2-deoxy-D-glucosyl P^2-dolichyl pyrophosphate, which, together with dolichyl D-mannosyl

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phosphate, can also be formed by microsomal preparations from the cells.[600] Related glycolipids were formed when membrane preparations of Chang liver cells were incubated with UDP-2-acetamido-2-deoxy-D-glucose. P^1-2-Acetamido-2-deoxy-D-glucosyl and P^1-di-*N*-acetylchitobiosyl P^2-polyisoprenyl pyrophosphates were obtained when UDP-2-acetamido-2-deoxy-D-glucose was incubated with particulate preparations from porcine aorta.[601] The transfer reaction utilized endogenous lipid and required bivalent (preferably Mn^{2+}) cations. Similar glycosyl transfers, which resulted in the formation of P^1-2-acetamido-2-deoxy-D-glucosyl P^2-dolichyl pyrophosphate and glycoproteins containing 2-acetamido-2-deoxy-D-glucose, were observed with a membrane preparation from the white matter of calf brain.[602] Tunicamycin, although blocking the synthesis of lipid-linked intermediates, did not inhibit the transfer of UDP-linked 2-acetamido-2-deoxy-D-glucopyranosyl residues to non-reducing termini of glycoproteins in the white matter of calf brain. Membrane preparations from hen oviduct have been shown to transfer the saccharide unit from P^1-di-*N*-acetylchitobiosyl P^2-dolichyl pyrophosphate to protein either directly or after elongation of the chain to P^2-dolichyl P^1-oligosaccharyl pyrophosphate.[603] At least two 2-acetamido-2-deoxy-D-glucosyltransferase activities have been identified in the wild-type ovary cells from Chinese hamsters: one acts on acceptors with one or two terminal D-mannopyranosyl residues and the other acts on acceptors with one terminal D-mannopyranosyl residue and one terminal 2-acetamido-2-deoxy-D-glucopyranosyl residue.[604] The first transferase activity is deleted in mutant cells that are resistant to a phytohaemagglutinin from *Phaseolus vulgaris*. The amounts of 2-amino-2-deoxy-D-glucose and L-fucose in glycoproteins extracted from, or excreted by, hamster tracheas in organ culture appear to be very similar.[605] Incubation of a preparation of hen-oviduct membrane with UDP-2-acetamido-2-deoxy-D-[^{14}C]-glucose and bacitracin and then with GDP-D-mannose afforded lipid-linked D-mannosyl-di-*N*-acetylchitobiosyl units, which can be elongated under appropriate conditions to give oligosaccharyl lipids containing 7—9 monosaccharide residues or the trisaccharide unit can be transferred to an endogenous protein acceptor.[606] It is likely that the transfer of 2-acetamido-2-deoxyhexoses from nucleotide donors to glycoproteins controls the synthesis of glycoproteins in regenerating rat liver.[607] Glycosidases acted on purified horseradish peroxidase to give a degraded material that accepted 2-acetamido-2-deoxy-D-glucopyranosyl residues from UDP-2-acetamido-2-deoxy-D-glucose in the presence of a particulate preparation from horseradish roots.[608]

At least two forms of sialyltransferase are present in human liver—one of them is thermolabile and associated with the membrane, whereas the other is thermostable and located elsewhere.[609] Whereas sialyltransferases in the sera of humans

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[601] A. Heifetz and A. D. Elbein, *J. Biol. Chem.*, 1977, **252**, 3057.
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[606] W. W. Chen and W. J. Lennarz, *J. Biol. Chem.*, 1976, **251**, 7802.
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and rats were inhibited by Mg^{2+} and Mn^{2+} cations and nucleoside di- and triphosphates, the sialyltransferase in rat liver required bivalent cations for the expression of optimal activity, and UDP or UTP stimulated the transfer reaction.[610] The sialyltransferase from bovine colostrum has been purified (4.4×10^5-fold) by affinity chromatography on an agarose-bound derivative of cytidine diphosphate.[611] Fractionation of the purified enzyme by gel filtration gave two species of equal specific activity (towards asialo-α_1-acid glycoprotein) that appeared to be partially degraded species of the same enzyme. This transferase attaches the *N*-acetylneuraminic acid residue of CMP-*N*-acetylneuraminic acid to the sequence β-D-Gal*p*-(1 → 4)-D-Glc*N*Ac*p*-(1 → in asialoglycoproteins by forming an α-(2 → 6)-linkage with the D-galactopyranosyl residue.[612] Quantitative data have been presented for two pathways that result in glycoprotein-bound *N*-glycolylneuraminic acid in porcine submandibular glands.[613] Hydroxylation of 40% of the *N*-acetylneuraminic acid residues by an hydroxylase occurs before transfer of the neuraminic acid derivatives to growing glycoprotein molecules, whereas 50% of them are hydroxylated after transfer. Increases in the concentrations of hepatic UDP-2-acetamido-2-deoxy-D-glucose 2′-epimerase activity and CMP-*N*-acetylneuraminic acid were observed in rats following laparotomy or partial hepatectomy; these increases were suppressed by either cycloheximide or actinomycin D.[614] An intracellular inhibitor affects the sialylation of endogenous acceptors in rat lymphocytes without inhibiting that of exogenous acceptors.[615] This suggests that the sialyltransferases inside the cell are inhibited but become active (as ectosialyltransferases) on reaching the outer surface of the cell, where there is no inhibitor.

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6
Enzymes

BY J. F. KENNEDY

Introduction

General Works and Nomenclature.—Two books on the enzymes of biological membranes have been published. One of them deals with physical and chemical techniques (including n.m.r. spectroscopy, fluorescent probes, and spin-labelling), as well as with immunochemical techniques that can be used to characterize the enzymes.[1] The other covers the biosynthesis of cell components, emphasizing the participation of membrane-associated enzymes in the biosynthesis of glycoproteins.[2] A book on the chemistry and control of enzymic reactions gives a concise account of the principles of modern enzymology and also covers many aspects of enzyme biochemistry, including the structures and the mechanisms of enzymes involved in metabolic control.[3]

A review of the occurrence, purification, properties, and modes of action of hemicellulases has dealt with L-arabinanases, D-galactanases, D-mannanases, and D-xylanases,[4] while a comprehensive review of pectolytic enzymes has discussed their classification and the assay and properties of pectinesterases and poly-D-galacturonate hydrolases and lyases.[5]

Methods of Assay.—Included in a handbook on methods of enzymic analysis are methods of assay, automated assays, uses of immobilized enzymes, and sources of enzymes and reagents.[6] The latest volume in the series 'Principles of Enzymatic Analysis' introduces a new nomenclature for quantities and units of activity, and discusses in detail prerequisites for the development of new assays.[7]

A review of enzyme analysers has described the automation of fixed-time and continuous-monitoring assays and the uses of partly or completely automated analysers and multi-channel systems.[8] Automated analyses of polysaccharide hydrolases, in soluble or insoluble form, have been based on determination of the liberated reducing sugars with 3,5-dinitrosalicylic acid.[9] A sensitive and specific method for locating glycoside hydrolases (*e.g.* α-D-mannosidases) in polyacryl-

[1] 'The Enzymes of Biological Membranes', vol. 1, ed. A. N. Martonosi, Wiley, Chichester, 1976.
[2] 'The Enzymes of Biological Membranes', vol. 2, ed. A. N. Martonosi, Wiley, Chichester, 1976.
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[9] J. F. Kennedy and I. M. Kay, *Carbohydrate Res.*, 1977, **56**, 211.

amide gels relies on the reaction between Prussian Blue and reducing sugars released from a suitable substrate (*e.g.* yeast D-mannan) incorporated into the gel.[10]

Isolation.—The purification of biologically-active macromolecules, including enzymes, by affinity chromatography has been reviewed.[11] A range of affinity absorbents for glycoside hydrolases has been prepared by coupling mono-, oligo-, and poly-saccharides to macroporous agarose with the aid of butane-1,4-diol diglycidyl ether.[12]

The possibilities of separating biologically-active macromolecules on magnetic supports have been discussed.[13]

Properties.—The latest volume in the series 'Methods in Enzymology' deals with the chemistry and biology of several proteolytic enzymes.[14]

Commercial enzyme preparations (*e.g.* proteases, lipases, and phosphatases) used in the preparation of fungal cell walls have been examined for contaminating glycohydrolase activities, which could result in the removal of cytoplasmic constituents and portions of the cell wall.[15] D-Glucosidase activities were detected in a fungal protease, in a lipase from wheat germ, and in potato phosphatase. Two commercial proteases from *Streptomyces griseus* contained significant amounts of β-1,3-D-glucanase activity, while another contained only traces of this D-glucanase. The protease preparations from *S. griseus* released laminaribiose and traces of D-glucose from the yeast-phase cell walls of *Histoplasma capsulatum* chemotypes I and II. Closer examination of one of the proteases showed that it is most active towards laminarin at pH 5.5 and 50 °C, although it is also highly active at pH 8.0 and 37 °C. A protease preparation from *Aspergillus oryzae* released D-glucose from the yeast-phase cell walls of *H. capsulatum* chemotypes I and II and from the cell walls of *Blastomyces dermatitidis*, suggesting that it contained α- and β-D-glucanases.

Kinetics and Mechanisms of Action.—A book entitled 'Kinetics of Enzyme Mechanisms' has examined the wide-ranging concepts and methods that are used to obtain information on enzyme mechanisms.[16] It covers the essential ideas behind various kinetic methods, with little mathematical detail, and includes sections on inhibitors, activators, co-operative interactions, and transport across membranes.

A general method for the detailed study of enzymic reactions considers the complete enzyme–substrate complex and the surrounding solvent, and evaluates all the quantum-mechanical and classical energy factors that can affect the reaction pathway.[17]

A non-mathematical, steady-state approach to enzyme kinetics has been

[10] L. März, J. Barna, and R. Ebermann, *J. Chromatog.*, 1976, **123**, 495.
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described in a book that also deals with inhibitors, activators, deactivators, one-substrate and non-linear kinetics, and allosteric interactions.[18] The actions of enzymes on water-insoluble substrates in non-aqueous solvents,[19] and the kinetics and mass-transfer effects of immobilized enzymes in packed-bed reactors[20] have been discussed. Recent literature on enzyme mechanisms, including that of glycogen phosphorylase, has been summarized.[21]

Functions.—The clearance of glycoside hydrolases from the circulatory system of rats has been examined.[22]

Applications.—The first volume of a new series entitled 'Topics in Enzyme and Fermentation Biotechnology' reviews areas of current interest and developments in the production of enzymes and other proteins by fermentation techniques.[23] Control of the synthesis of enzymes during continuous culture is given particular attention. A book on the continuous culture of micro-organisms draws attention to areas where continuous culture has distinct advantages over conventional methods of culture, and also discusses the production of proteins from starch and cellulosic wastes.[24] A book on the use of spin-labelling techniques in molecular biology includes sections on the principles of spin-labelling and on the results already obtained with spin-labelled enzymes.[25] The uses of carbohydrate-oriented enzymes have been reviewed.[26]

The first volume of a new series entitled 'Applied Biochemistry and Bioengineering' deals with the principles of immobilized enzymes,[27] which, among other topics, are also discussed in one of the latest volumes of 'Methods in Enzymology'.[28] Recent reviews have covered the uses of immobilized enzymes and immobilized microbial cells in industry,[29] the design of immobilized-enzyme flow reactors,[30] the properties of immobilized enzymes,[31] the reactions used to immobilize enzymes,[32] and the uses of immobilized enzymes in clinical diagnosis, detoxification, and therapy, *etc.*[33] and of immobilized microbial cells.[34]

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[24] 'Continuous Culture: Applications and New Fields', ed. A. C. R. Dean, D. C. Ellwood, C. G. T. Evans, and J. Welling, Wiley, Chichester, 1977.
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[30] W. R. Vieth, K. Venkatasubramanian, A. Constantinides, and B. Davidson, in ref. 27, p. 221.
[31] J.-M. Engasser and C. Horvath, in ref. 27, p. 127.
[32] L. Goldstein and G. Manecke, in ref. 27, p. 23.
[33] 'Biomedical Applications of Immobilized Enzymes and Proteins', vol. 1, ed. T. M. S. Chang, Plenum Press, New York, 1977.
[34] E. J. Vandamme, *Chem. and Ind.*, 1976, 1070.

β-D-Acetamidodeoxygalactosidases, β-D-Acetamidodeoxyglucosidases, and β-D-Acetamidodeoxyhexosidases

The affinity chromatography of β-D-2-acetamido-2-deoxyglucosidase on glycose-substituted agaroses has been investigated.[35]

Proteins of low molecular weight in secondary lysosomes have been shown to activate various sphingolipid hydrolases, including β-D-2-acetamido-2-deoxy-galactosidase.[36] The immunological inter-relationships among the β-D-2-acetamido-2-deoxyglucosidases from a number of mammals have been established.[37] Qualitative immunochemical analyses showed that there are similarities among the enzymes from humans, Rhesus monkeys, Iris macaques, cattle, pigs, goats, and guinea-pigs. The extent of antigenic similarity among the species correlated well with phylogenetic relationships among them.

The levels of β-D-2-acetamido-2-deoxyhexosidases A and B in the tissues and body fluids of normal individuals and Tay–Sachs patients have been measured by sensitive immunoassays specifically developed for this purpose.[38] The results showed that normal tissues contain comparable amounts of both isoenzymes, whereas the tissues of Tay–Sachs patients contain neither the A isoenzyme nor material that carries A-specific antigenic determinants. The use of these assays in the diagnosis of Tay–Sachs disease was discussed.

β-D-2-Acetamido-2-deoxyhexosidases A and B (each of mol. wt. 1.1×10^5) have been purified to homogeneity by affinity chromatography on immobilized concanavalin A and ion-exchange chromatography on diethyl(2-hydroxypropyl)-aminoethyl-Sephadex.[39] Both enzymes contain neutral sugars (2.5 and 5.4%) and 2-acetamido-2-deoxy-D-glucose (2.5 and 3.4%, respectively), but they do not appear to contain sialic acid.

Characterization of the β-D-2-acetamido-2-deoxyhexosidases C and S from the fibroblasts of controls and patients with Tay–Sachs disease did not provide any evidence for a structural relationship between β-D-2-acetamido-2-deoxyhexosidases A and C, so that a new interpretation of the results of man–rodent hybrids is favoured.[40]

The β-D-2-acetamido-2-deoxyhexosidase A from human kidneys is a heteropolymer composed of two immunologically distinct α and β subunits, whereas the corresponding B enzyme is a homopolymer composed of β subunits only.[41] The physicochemical properties of the species obtained on dissociation of the enzymes with 4-hydroxymercuribenzoate have been studied, and attempts have been made to obtain hybrid species. It was concluded that the A form, $(\alpha\beta)_3$, dissociates into its subunits on reaction with the reagent and that the β subunits recombine to form the relatively stable B enzyme, $(\beta\beta)_3$, whereas the α subunits recombine to form the dimer α_2 and the polymer α_8.

[35] T. Mega and Y. Matsuchima, *J. Biochem. (Japan)*, 1977, **81**, 571.
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[39] J. E. S. Lee and A. Yoshida, *Biochem. J.*, 1976, **159**, 535.
[40] A. J. J. Reuser and H. Galjaard, *F.E.B.S. Letters*, 1976, **71**, 1.
[41] S. K. Srivastava, J. E. Wiktorowicz, and Y. C. Awasthi, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 2833.

Separation of the β-D-2-acetamido-2-deoxyhexosidase A and B isoenzymes from human liver by ion-exchange chromatography resulted in the recovery of >80% of the activity in the crude extracts, when synthetic substrates were used to assay the enzymic activity.[42] Only 15% of the β-D-2-acetamido-2-deoxyhexosidase activity towards ganglioside G_{M_2} {*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1 → 4)-[*O*-α-*N*-acetylneuraminyl-(2 → 3)]-*O*-β-D-galactopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 1)-ceramide} was recovered and all of this activity was associated with a fraction containing β-D-2-acetamido-2-deoxyhexosidase A. The level of G_{M_2}-ganglioside hydrolase activity in this fraction was enhanced on incubation of the fraction with those containing the B isoenzyme. The activating factor, which was partially purified by gel filtration, is a heat-stable protein of molecular weight 3.6×10^4 that showed no activity towards substrates for β-D-2-acetamido-2-deoxyhexosidase A. Neither highly purified nor crude β-D-2-acetamido-2-deoxyhexosidase A recovered after gel filtration on Sephadex G-100 possessed G_{M_2}-ganglioside hydrolase activity, but this activity was completely restored on addition of the activating factor, which did not affect the rate of hydrolysis of either synthetic substrates or asialo-G_{M_2}.

β-D-2-Acetamido-2-deoxyhexosidase activity from human livers and kidneys precipitated antisera to the A and B isoenzymes, but the immunologically distinct C isoenzyme was not detected in these tissues.[43] Ion-exchange chromatography of the preparations separated several enzymically active components, some of which migrated towards the anode more strongly than the original enzyme on polyacrylamide disc electrophoresis, possibly as a result of changes in the ratios of the subunits. The residual β-D-2-acetamido-2-deoxyhexosidase activity in the livers and fibroblasts of patients with Sandhoff's disease was similarly resolved.

Antisera to the A and B isoenzymes of β-D-2-acetamido-2-deoxyglucosidase from human foetal liver and placenta have been raised in rabbits.[44] No differences were detected in the antigenicity of the isoenzymes from foetal liver and adult tissues, whereas antisera raised against the placental isoenzymes did not react with the isoenzymes of adult tissues. Antibodies raised against placental and foetal-liver B isoenzymes reduced the level of β-D-2-acetamido-2-deoxyglucosidase activity in the sera of pregnant women (after 38 weeks' gestation) to that normally found. Immunological evidence suggested that the isoenzyme of β-D-2-acetamido-2-deoxyglucosidase found only in the sera of pregnant women originates from the placenta.

2-Acetamido-2-deoxy-β-D-glucopyranosyl isothiocyanate irreversibly inhibited the β-D-2-acetamido-2-deoxyhexosidases from boar epididymis and human placenta, and dialysis did not restore the enzymic activity.[45] 2-Acetamido-2-deoxy-D-glucose (a competitive inhibitor) protected the enzyme from inactivation, showing that the isothiocyanate binds at the active site of the enzyme.

Alkylation and treatment with detergents have been used to examine the

[42] P. Hechtman, *Canad. J. Biochem.*, 1977, **55**, 315.
[43] S. K. Srivastava, J. Wiktorowicz, R. Klebe, and Y. C. Awasthi, *Biochim. Biophys. Acta*, 1975, **397**, 428.
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subunit composition of the β-D-2-acetamido-2-deoxyhexosidase A (mol. wt. 1.10×10^5) from human placenta.[46] Alkylation also converted the A isoenzyme into the S form. Contrary to previous evidence, the results indicated that the A isoenzyme is composed of four subunits, each of molecular weight $2.5—2.7 \times 10^4$, and, together with other data, are consistent with the subunit structures $\alpha_2\beta_2$, β_4, and α_4, respectively, for the A, B, and S isoenzymes.

Reduced levels of β-D-2-acetamido-2-deoxyglucosidase isoenzymes have been found in the retina, tears, and sera of diabetic patients and in streptozotocin diabetic rats.[47] Changes in the levels of the enzyme in the sera of the patients over several months were used to assess the development of microangiopathy. Tears from patients with retinopathy also showed appreciably lower levels of enzymic activity.

A colorimetric method, which uses the corresponding 4-nitrophenyl glycopyranoside as the substrate, has been developed for the assay of β-D-2-acetamido-2-deoxyglucosidase in urine.[48] Interfering substances were removed by gel filtration, and a low-molecular-weight inhibitor of the enzyme was detected. Ways of increasing the speed and precision of the assay were examined, and estimates of the normal level of the enzymic activity in urine were recorded. Practical uses for the assay of urinary β-D-2-acetamido-2-deoxyglucosidase activity have been examined for patients with kidney diseases.[49] Centrifugation and dialysis of urine samples were unnecessary and, when the level of the enzymic activity was related to that of creatinine, random samples could be used. Preservatives, but not mild haemoglobulinuria, interfered with the assay. Use of the level of urinary β-D-2-acetamido-2-deoxyglucosidase activity to diagnose the rejection of transplanted kidneys has been defended.[50] Once again, the need to compare the level of the enzymic activity with that of creatinine, in order to compensate for variations in urine flow, was stressed.

The assay of urinary β-D-2-acetamido-2-deoxyglucosidase for use in the diagnosis of Tay–Sachs disease has been examined.[51] Whereas assay of the isoenzyme A in urine will distinguish between homozygotes (patients) and heterozygotes (carriers), it could not replace assay of this isoenzyme in sera and leucocytes for distinguishing between heterozygotes and normal individuals in the Ashkenazic Jewish population.

β-D-2-Acetamido-2-deoxyhexosidase from bull epididymis has been purified by affinity chromatography on a benzidine derivative of agarose to which monosaccharide lactones had been covalently attached.[52] The use of 2-acetamido-2-deoxy-D-mannono-1,5-lactone is advantageous, and the enzyme fraction obtained could be separated into two bands, one of which was enzymically active, by electrophoresis on polyacrylamide gel.

The 'B' variant of the β-D-2-acetamido-2-deoxyhexosidase from pig epididymis has been separated into two isoenzymes (B_α and B_β) for the first time.[53] Like those

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[47] H. Fushimi and S. Tarui, *Clinica Chim. Acta*, 1976, **71**, 1.
[48] D. Marvan, *Clinica Chim. Acta*, 1976, **73**, 453.
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[50] A. E. Thompson, S. M. Tucker, and R. G. Price, *Clinical Chem.*, 1977, **23**, 301.
[51] A. Saifer, J. Amoroso, and G. Perle, *Clinica Chim. Acta*, 1976, **67**, 315.
[52] M. Pokorny and C. P. J. Glaudemans, *F.E.B.S. Letters*, 1975, **50**, 66.
[53] D. H. Leaback and H. K. Robinson, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 248.

of the 'B' variant of the arylsulphatase from bovine liver, the isoenzymes could not be separated by isoelectric focusing, although they were separated by ampholyte-displacement chromatography. This is a new technique in which proteins are eluted from an ion-exchange absorbent by means of amphoteric substances with similar isoelectric points.

In common with other lysosomal enzymes in canine gastric mucosa, the β-D-2-acetamido-2-deoxy-galactosidase and -glucosidase activities are bound to particles.[54] In this form, the enzymes exhibit high degrees of latency.

The β-D-2-acetamido-2-deoxyglucosidase (see also *endo*-β-D-2-acetamido-2-deoxyglucosidase) from rabbit sera has been purified by affinity chromatography on 2-acetamido-2-deoxy-β-D-glucopyranose immobilized on an agarose support.[55] The enzyme hydrolysed synthetic glycopyranosides and 2-acetamido-2-deoxy-β-D-glucopyranosyl linkages within oligosaccharide chains.

Labelling experiments with tritiated amino-acids and neuronal cell-bodies from rat cerebral cortex have provided evidence on how β-D-2-acetamido-2-deoxyglucosidase is synthesized *in vitro*.[56] Ion-exchange chromatography revealed the presence of a principal and two minor components of β-D-2-acetamido-2-2-deoxyglucosidase, but only the principal component reacted with concanavalin A. Interconversion between the principal component and one of the minor components was possible under certain conditions.

The clearance of β-D-2-acetamido-2-deoxyglucosidase from the circulatory system and the clearance induced by modified glycoproteins have been studied in rats.[22] Intravenous infusion of a purified β-D-2-acetamido-2-deoxyglucosidase from rat epididymis showed that the enzyme is cleared comparatively slowly.[57] Kinetic and competitive experiments with β-D-2-acetamido-2-deoxyglucosidase and β-D-glucuronidase indicated that the lysosomal compartment of rat liver has an important role in clearing the enzymes.[58]

Affinity chromatography of enzymes from soybean and Takadiastase on glycose-substituted agaroses showed that the β-D-2-acetamido-2-deoxyglucosidases are not adsorbed specifically, although some purification of these enzymes could be achieved.[59]

Eight mutant strains containing $<25\%$ of the level of β-D-2-acetamido-2-deoxyglucosidase present in the wild type have been isolated from a strain of *E. coli*.[60] It appears that a level $<1\%$ of that of the enzyme in the wild type suffices for normal growth and division of the cells. The levels and properties of the β-D-2-acetamido-2-deoxyglucosidase of a *Myxobacter* species have been studied.[61] β-D-2-Acetamido-2-deoxy-galactosidase and -glucosidase activities have been detected in axenic culture media of the myxomycete *Physarum polycephalum*.[62] Electrophoresis revealed multiple forms of these enzymes.

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β-D-Fructofuranosidases

β-D-Fructofuranosidase activity in polyacrylamide gels following electrophoresis has been determined by reaction of the products obtained on hydrolysis of an appropriate substrate with Prussian Blue.[10]

β-D-Fructofuranosidase and leucine β-naphthylamidase are in close proximity on the microvilli membrane of the mucosal cells of rabbit small intestines.[63] It was assumed that where enzymes with, say, A and B antigenicities are in close proximity, the agglutination of vesicles by anti-A antibodies is inhibited by the prior binding of monovalent fragments of anti-B antibodies to enzyme B on the surface.

β-D-Fructofuranosidase activity has been detected in sugar-cane stalks.[64]

Some properties of the β-D-fructofuranosidase from *Candida utilis* have been reported.[65] Purification of the β-D-fructofuranosidase from *Corticium rolfsii* to homogeneity required extensive ion-exchange chromatography.[66] The purified enzyme (mol. wt. 2.2×10^5) is inactive towards phenyl α-D-glucopyranoside and maltose, but released D-fructose from sucrose, raffinose, and inulin. The enzyme is stable between the pH values of 1.5 and 8.0 at 2 °C, is optimally active at pH 4 and 73 °C, and is stable at temperatures $\leqslant 65$ °C. Possible uses of the enzyme in confectionery were discussed.

The β-D-fructofuranosidase from *Neurospora crassa* is stable on storage at low temperatures, but oxidation of the carbohydrate residues with sodium periodate markedly decreased its thermal stability.[67] Some of the carbohydrate residues appear to be attached by *O*-glycosidic linkages to seryl and threonyl residues in the protein, while others are attached by *N*-glycosylaminyl linkages to asparagyl residues. The carbohydrate residues may stabilize the tertiary structure of the enzyme.

The effect of crossing a hyperderepressed strain with a normal strain of *N. crassa* on the endogenous β-D-fructofuranosidase activity has been studied.[68] An increase in the enzymic activity, due to the gene for hyperderepression, was observed.

The effect of D-glucose on the synthesis of β-D-fructofuranosidase by *Saccharomyces carlsbergensis* in batch or continuous culture has been studied.[69] In chemostat cultures of *S. carlsbergensis*, the highest specific β-D-fructofuranosidase activity was observed at an intermediate dilution rate.[70]

Conduritol-B epoxide, a compound structurally related to substrates for yeast (*Saccharomyces* sp.) β-D-fructofuranosidase, is an active-site inhibitor of the enzyme.[71] Inactivation is first-order with respect to time and the concentration of the inhibitor, and does not occur in the presence of substrates. The dependence of inactivation on pH revealed the presence of two dissociable groups (pK_a values 3.05 and 6.8) in the enzyme, one of which, a carboxylic acid group of pK_a 3.05, appears to react with conduritol-B epoxide.

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Infection of *Senecio squalidus* by *Albugo tragopogonis* and of *Tussilago farfara* and *Poa pratensis* by *Puccinia poarum* resulted in an increase in the activity of an acid β-D-fructofuranosidase at the sites of infection.[72] It appears that sucrose from the host is hydrolysed and the hexoses absorbed by the parasite. β-D-Fructofuranosidase also has a key role in providing substrates for the biosynthesis of starch, which accumulates around the pustules of biotrophic fungi acting on host species that store this polysaccharide.

β-D-Fructofuranosidase has been immobilized, with retention of activity, by an ionic reaction with a guanidino derivative of cellulose,[65] by adsorption onto ion-exchange resins[73] and various activated mineral and mineralized supports,[74] by entrapment in gels prepared from dimethylacrylamide or 2-hydroxyethyl acrylate,[75] and by inclusion inside hollow-fibre cartridges.[76] β-D-Fructofuranosidase has also been coupled with succinylated and immobilized concanavalin A; this activated form of the enzyme gave an active, soluble, immobilized conjugate with urease.[77]

α-L-Fucosidases and α-D-Fucosidases

The relationship between the α-D-fucosidase and α-D-galactosidase activities in human and animal tissues, *etc.*, has been investigated.[78]

Two forms of α-D-fucosidase have been separated (by gel filtration) from human and pig kidneys.[79] The elution-profiles of the α-D-fucosidases and α-D-galactosidases on gel filtration and isoelectric focusing were identical. The α-D-fucosidases and α-D-galactosidases from human and pig kidneys possess similar pH optima and thermostabilities and are inhibited to the same extents by D-fucose, D-galactose, and D-galactono-1,4-lactone, suggesting that the hydrolyses of α-D-galactopyranosides and α-D-fucopyranosides are catalysed by the same enzyme, *viz.* α-D-galactoside (α-D-fucoside) hydrolase.

Multiple forms of the α-L-fucosidase from human liver have been revealed by isoelectric focusing in polyacrylamide gel and in thin layers of Sephadex.[80] The aggregation of various forms of the enzyme was investigated.

α-L-Fucosidase was shown to be absent from the white blood-cells of a foetus of identical twins, which was diagnosed as a carrier of fucosidosis.[81] The diagnostic value of α-L-fucosidase activity in amniotic fluids and in cultivated cells from amniotic fluids, and the significance of L-fucose-rich materials in amniotic fluids were discussed.

The α-L-fucosidases from human leucocytes and urine have similar K_m values,

[72] D. E. Long, A. K. Fung, E. E. M. McGee, R. C. Cooke, and D. H. Lewis, *New Phytol.*, 1975, **74**, 173.
[73] J. Boudrant and C. Cheftel, *Biotechnol. and Bioeng.*, 1975, **17**, °27.
[74] D. Thornton, A. Flynn, D. B. Johnson, and P. D. Ryan, *Biotechnol. and Bioeng.*, 1975, **17**, 1679.
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[79] G. Y. Wiederschain and E. M. Beyer, *Arch. Biochem. Biophys.*, 1977, **182**, 335.
[80] R. Thorpe and D. Robinson, *F.E.B.S. Letters*, 1975, **54**, 89.
[81] I. Matsuda, S. Arashima, Y. Oka, T. Mitsuyama, S. Ariga, T. Ikeuchi, and T. Ichida, *Clinica Chim. Acta*, 1975, **63**, 55.

pH optima, electrophoretic patterns, and thermal stabilities, all of which differ from those of an α-L-fucosidase from human sera.[82] The enzyme (mol. wt. 8.0 ± 0·5 × 10^4) from leucocytes was strongly inhibited by Cu^{2+} and Hg^{2+} ions and 4-chloromercuribenzoate. Since this inhibition could be reversed by 2-mercaptoethanol, thiol groups appear to be essential for catalytic activity.

The levels of α-L-fucosidase(s) in two families with fucosidosis have been examined.[83] In one family, the levels of enzymic activity in the leucocytes, urine, sera, and livers of the two patients (one deceased) were appreciably reduced, although those of close relatives were in the heterozygote or normal range. The α-L-fucosidase from the patients exhibited abnormal properties, since its activity increased after heating. The levels of α-L-fucosidase in members of the other family were less depleted.

A study of the distribution of lysosomal enzymes in canine gastric mucosa indicated that the α-L-fucosidase is not bound to particles.[54]

A lysosomal α-L-fucosidase from the cytoplasm of rat liver has been purified (2.7 × 10^4-fold) by affinity chromatography on immobilized 2-amino-2-deoxy-L-fucose.[84] The molecular weight of the enzyme (pH optimum 5.7–5.9, K_m and K_i for 4-nitrophenyl α-L-fucopyranoside 0.19 mmol l^{-1} and 1.6 mmol l^{-1}, respectively) depended on how it was measured; the values obtained were 1.60 × 10^5 by sucrose-density centrifugation, 2.17 × 10^5 by sedimentation equilibration, and 3.0 × 10^5 by gel filtration, although a single molecular weight (5.5 × 10^4) was obtained for the subunits by gel electrophoresis in the presence of sodium dodecyl sulphate. The enzyme contains L-fucose (3—4), D-mannose (32), D-galactose (8), D-glucose (9), 2-amino-2-deoxy-D-glucose (32), and sialic acid (8 residues per tetrameric unit).

The α-L-fucosidase in rat epididymis has been isolated by ion-exchange chromatography on DEAE-Sephadex.[85] The purity of the enzyme was comparable to that of the enzyme isolated by affinity chromatography.

The clearance of α-L-fucosidase from the circulatory system of rats and the effects of modified glycoproteins thereon have been investigated.[22]

α-L-Fucosidase has been isolated from the liver (hepatopancreas) of the marine mollusc *Chamelea gallina* (*Venus gallina*); a contaminating β-D-2-acetamido-2-deoxyglucosidase was removed only by isoelectric focusing.[86] The enzyme (mol. wt. 2 × 10^5) has a broad specificity, is inhibited by Hg^{2+} ions and some sugars, is activated by CN^-, Zn^{2+}, and Ca^{2+} ions and H_4edta, and gave two peaks (p*I* 6.3 and 6.6) on isoelectric focusing.

Almond emulsin contains two α-L-fucosidases that can be separated by gel filtration.[87] One of them hydrolysed 2-acetamido-2-deoxy-3- and -4-*O*-α-L-fucopyranosyl-D-glucopyranose and oligosaccharides from milk, but it did not hydrolyse 2-*O*-α-L-fucopyranosyl-D-galactopyranose and 2-acetamido-2-deoxy-6-*O*-α-L-fucopyranosyl-D-glucopyranose. The other enzyme released L-fucose

[82] J. Troost, M. C. M. van der Heijden, and G. E. J. Staal, *Clinica Chim. Acta*, 1976, **73**, 321.
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[84] D. J. Opheim and O. Touster, *J. Biochem.* (*Japan*), 1977, **252**, 739.
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[87] M. Ogata-Arakawa, T. Muramatsu, and A. Kobata, *Arch. Biochem. Biophys.*, 1977, **181**, 353.

from 2′-*O*-α-L-fucopyranosyl-lactose, but it did not hydrolyse L-fucose in other linkages.

Traces of α-L-fucosidase activity have been found in axenic cultures of the myxomycete *Physarum polycephalum*.[62]

β-D-Galactofuranosidases

A homogeneous (*exo*-)β-D-galactofuranosidase has been obtained from culture filtrates of *Penicillium charlesii* by gel filtration and affinity chromatography on an immobilized peptidophosphogalactomannan.[88] The enzyme hydrolysed methyl and ethyl β-D-galactofuranosides, 6-*O*-β-D-galactofuranosyl-D-galactopyranose, and the (1 → 5)-linked β-D-galactofuranosyl residues in a peptidophosphogalactomannan from *P. charlesii*, but it did not act on methyl α-D-galactofuranoside and methyl α- and β-D-galactopyranosides.

D-Galactosidases, β-D-Galactosyl- and β-D-Galactosyl-D-glucosylceramidases, and β-D-Galactosylsphingosidases

Aspects of the affinity chromatography of β-D-galactosidase on glycose-substituted agaroses have been investigated.[35]

Proteins of low molecular weight in secondary lysosomes activated a variety of sphingolipid hydrolases, including D-galactosylceramidase (E.C. 3.2.1.46).[36] The relationship between the α-D-galactosidase and α-D-fucosidase activities in human and animal tissues, *etc.*, has been investigated.[78]

A simple and inexpensive assay of the β-D-galactocerebrosidase from leucocytes relies on separation of the tritiated sphingolipid (substrate) from the tritiated hydrophobic product (ceramide) by t.l.c. on silica gel impregnated with sodium tetraborate.[89]

D-Galactosyl-D-glucosylceramidase (β-D-galactosidase) activities in the fibroblasts of a patient thought to be suffering from 'lactosyl ceramidosis' have been re-examined by two procedures which appeared to measure two genetically distinct enzymes that can degrade lactosylceramide.[90] Neither enzyme was absent from the patient's fibroblasts.

The presence in the spleen of a factor that stimulates the activity of β-D-galactosidase in Gaucher's disease has been investigated.[91]

A highly specific 'acid' β-D-galactosidase has been obtained from human liver by affinity chromatography on immobilized concanavalin A and immobilized 1-thio-β-D-galactopyranose.[92] The enzyme appears to contain carbohydrate. A new 'acid' β-D-galactosidase from human liver has been separated from other 'acid' β-D-galactosidases.[93] The forms of the enzyme of low molecular weight undergo salt-dependent aggregation, and the form of high molecular weight may

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[89] A. Poulos and A. C. Pollard, *Clinica Chim. Acta*, 1976, **72**, 327.
[90] D. A. Wenger, M. Sattler, C. Clark, H. Tanaka, K. Suzuki, and G. Dawson, *Science*, 1975, **188**, 1310.
[91] S. P. Peters, C. J. Coffee, R. H. Glew, R. E. Lee, D. A. Wenger, S. C. Li, and Y. T. Li, *Arch. Biochem. Biophys.*, 1977, **183**, 290.
[92] A. L. Miller, R. G. Frost, and J. S. O'Brien, *Analyt. Biochem.*, 1976, **74**, 537.
[93] P. S. J. Cheetham and M. E. Dance, *Biochem. J.*, 1976, **157**, 189.

consist of the lower forms bound to a fragment of the cell membrane. The effects of neuraminidase on liver β-D-galactosidases were examined.

4-Nitrophenyl β-D-galactopyranoside has been used as the substrate in a colorimetric assay of the β-D-galactosidase in human urine, after interfering substances had been removed by gel filtration.[48]

Bovine testicular β-D-galactosidase was rapidly taken up by the skin fibroblasts of a patient with generalized gangliosidosis.[94] The enzyme, which contains equimolar amounts of D-mannose and 2-amino-2-deoxy-D-glucose, binds strongly to immobilized concanavalin A. The rate of uptake of this β-D-galactosidase by the fibroblasts was drastically reduced after it had been treated with α-D-mannosidase.

In common with other lysosomal enzymes in canine gastric mucosa, α- and β-D-galactosidases are bound to particles and exhibit high degrees of latency.[54]

The levels of enzymes that cleave lactosylceramide have been examined in dogs afflicted with globoid cell leukodystrophy. Lactosylceramidase I activities were absent from the brains and livers of afflicted dogs and were of an intermediate level in heterozygous carriers, whereas the levels of lactosylceramidase II were normal.[95] Their solubilities and gel-filtration behaviours indicated that, just as in human tissues, lactosylceramidase I is probably identical with D-galactosylceramidase and that lactosylceramidase II is closely associated with the 4-methylumbelliferyl β-D-galactosidase activity. With regard to the levels of lactosylceramidases, globoid cell leukodystrophy in dogs is analogous to that in humans. The D-galactosidase from rabbit brain has been purified.[96] The enzyme catalysed the hydrolysis of synthetic substrates, G_{M_1} ganglioside, lactosylceramide, and acid-treated G_{M_1} ganglioside.[97] D-Galactono-1,5-lactone competitively inhibited the enzyme, which was also inhibited by iodoacetate and 4-chloromercuribenzenesulphonic acid. Small changes in the structures of anionic detergents employed in standard assays were found to affect the hydrolysis of G_{M_1} ganglioside and lactosylceramide by the enzyme.[96]

A partially purified β-D-galactosylcerebrosidase activity from rat brain catalysed the synthesis of labelled D-galactosylceramide from [^{14}C]oleoylsphingosine and a number of β-D-galactopyranosides.[98] This observation, in conjunction with those on the hydrolyses catalysed by the enzyme, suggests that two β-D-galactosidase isoenzymes are involved in the catabolism of G_{M_1} ganglioside or lactosylceramide or both, whereas only one of them is involved in the catabolism of D-galactosylceramide. A selective assay for one of the isoenzymes was developed.

The latency of the lysosomal β-D-galactosidase in rat liver has been examined.[99] The clearance of β-D-galactosidase from the circulatory system of rats and the effect of modified glycoproteins on the clearance have been examined.[22]

β-D-Glucopyranosyl isothiocyanate, (*R*)- and (*S*)-2-β-D-glucopyranosylepoxyethane, (*R*)- and (*S*)-1-β-D-glucopyranosyl-2,3-epoxypropane, and β-D-galacto-

[94] V. Hieber, J. Distler, R. Myerowitz, R. D. Schmickel, and G. W. Jourdian, *Biochem. Biophys. Res. Comm.*, 1976, **73**, 710.
[95] T. W. Kurczynshi, T. F. Fletcher, and K. Suzuki, *J. Neurochem.*, 1977, **29**, 37.
[96] J. W. Callahan and J. Gerrie, *J. Neurochem.*, 1976, **26**, 217.
[97] J. W. Callahan and J. Gerrie, *Canad. J. Biochem.*, 1976, **54**, 803.
[98] T. P. Carter and J. N. Kanfer, *J. Neurochem.*, 1976, **27**, 53.
[99] R. Burton and J. B. Lloyd, *Biochem. J.*, 1976, **160**, 631.

pyranosylepoxyethane irreversibly inhibited sweet-almond β-D-glucosidase.[100] That the formation of a covalent bond (*e.g.* by opening of the oxiran ring) is preceded by binding of the inhibitor at the active site of the enzyme was indicated by the early competitive nature of the inhibition and by protection of the enzyme from inactivation by the competitive inhibitors D-glucose and D-glucono-1,5-lactone. The dependence of inactivation on pH was also investigated.

The α-D-galactosidase from the leaves of *Cucurbita pepo* has been purified by pH and ammonium sulphate fractionation, gel filtration, and ion-exchange chromatography.[101] Three forms of the enzyme were separated, each of which hydrolysed the α-D-galactopyranosyl linkage of raffinose and stachyose. Although the enzymes have the same molecular weight, they differ in their pH optima, reactivities towards metal ions, thermal stabilities, and K_m values for 4-nitrophenyl α-D-galactopyranoside and raffinose. The enzymes appear to be components of a lysosomal system in plant cells.

Inhibition studies have indicated that an endogenous β-D-galactosidase is not involved in auxin- or acid-stimulated growth of oat (*Avena sativa*) coleoptiles.[102]

Affinity chromatography of the enzymes from soybean and Takadiastase on glycose-substituted agaroses has shown that the β-D-galactosidases are not absorbed specifically, although partial purification of the β-D-galactosidases could be achieved.[59]

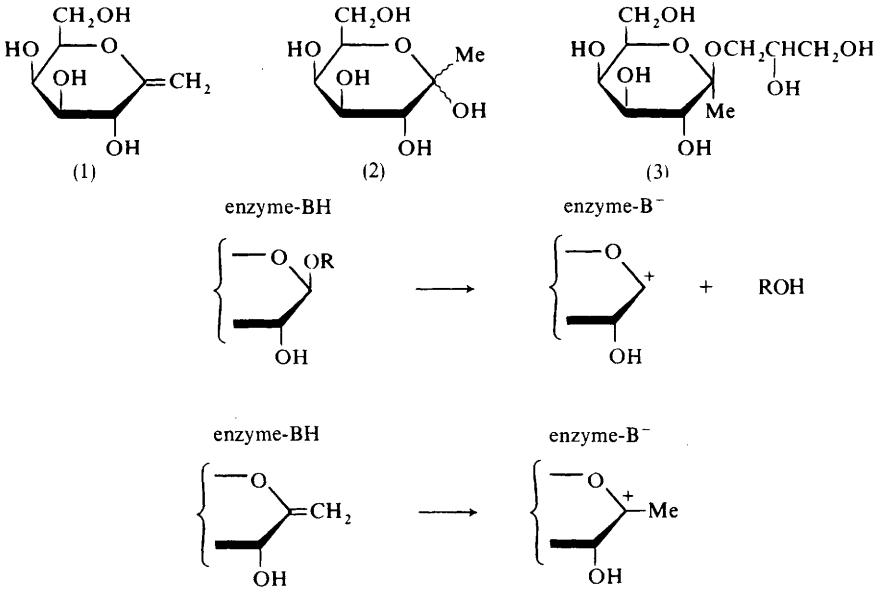
Two α-D-galactosidases, with different pH optima, and traces of β-D-galactosidase have been detected in sugar-cane stalks.[64]

The β-D-galactosidase from wheat germ has been purified by affinity chromatography on a lactose-substituted polyacrylamide gel, which specifically adsorbed the enzyme.[103] Polyacrylamide gel electrophoresis of the purified material revealed two active bands.

α-D-Galactosidase (synthesized by *Aspergillus awamori* growing on wheat bran) in a hollow-fibre reactor has been used to hydrolyse the D-galacto-oligosaccharides in soybean milk.[104] The dependence of the activity of *A. niger* β-D-galactosidase on pH, temperature, and the concentration of the substrate has been investigated.[105] *E. coli* β-D-galactosidase has been purified by affinity chromatography on an agarose-based absorbent to which 1-thio-β-D-galactopyranose was attached.[106] The process was adapted for continuous, pilot-scale operation.

The β-D-galactosidase from *E. coli* catalysed the conversion of 2,6-anhydro-1-deoxy-D-*galacto*-hept-1-enitol (1) into 1-deoxy-D-*galacto*-heptulose (2), and, in the presence of glycerol, into glyceryl 2,6-anhydro-1-deoxy-β-D-*galacto*-heptuloside (3) also.[107] The enzyme-catalysed addition of water or glycerol to (1) was compared to the normal enzymic cleavage of β-D-galactopyranosides in terms of a common triggering process (Scheme 1). The enzyme also catalysed the hydrolysis of (3), which is the first example of the cleavage of a ketoside by an aldosidase.

[100] M. L. Shulman, S. D. Shiyan, and A. Ya. Khorlin, *Biochim. Biophys. Acta*, 1976, **445**, 169.
[101] B. Thomas and J. A. Webb, *Phytochemistry*, 1977, **16**, 203.
[102] M. L. Evans, *Plant Physiol.*, 1974, **54**, 213.
[103] H. Hamazaki and K. Hotta, *F.E.B.S. Letters*, 1977, **76**, 299.
[104] K. L. Smiley, D. E. Hensley, and H. J. Gasdorf, *Appl. Environ. Microbiol.*, 1976, **31**, 615.
[105] V. Krasnobajew, *Chimia* (*Switz.*), 1977, **31**, 110.
[106] P. J. Robinson, M. A. Wheatley, J.-C. Janson, P. Dunnill, and M. D. Lilly, *Biotechnol. and Bioeng.*, 1974, **16**, 1103.
[107] M. Brockhaus and J. Lehmann, *Carbohydrate Res.*, 1974, **53**, 21.



Scheme 1

A severe genetic defect in the mutant M15 of *E. coli* β-D-galactosidase has been repaired.[108] The enzyme, which lacks the amino-acid residues 11—42 at the NH_2-terminus, is a dimer, rather than a tetramer, and an acceptor in α-complementation. Antibody-mediated activation of the defective enzyme was achieved. Intracistronic α-complementation occurred between peptides derived from *E. coli* β-D-galactosidase and the defective β-D-galactosidase from a Z-deletion mutant.[109] The properties of the complemented enzyme and the mechanism of the complementation reaction were investigated; the kinetic data favour a model in which rapid formation of the complex is followed by a slow conformational change.

The inactivation of *E. coli* β-D-galactosidase by specific antibodies occurred by dissociation of the 16*S* enzyme into 10*S* subunits, which are the only species observed after inactivation.[110] Loss of the enzymically active 16*S* species is proportional to the number of 10*S* subunits formed.

E. coli β-D-galactosidase in the form of a conjugate with insulin has been used in an immunoassay of insulin.[111] The kinetic properties of an α-D-galactosidase from *Mortierella vinacea* have been investigated using 4-nitrophenyl α-D-galactopyranoside as the substrate.[112] The enzyme was strongly inhibited by this glycoside and by the D-galactose released, which acted as a mixed inhibitor. Related kinetic

[108] R. S. Accolla and F. Celada, *F.E.B.S. Letters*, 1976, **67**, 299.
[109] K. E. Langley and I. Zabin, *Biochemistry*, 1976, **15**, 4866.
[110] R. A. Roth and B. Rotman, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 1384.
[111] K. Kato, Y. Hamaguchi, H. Fukui, and E. Ishikawa, *J. Biochem. (Japan)*, 1975, **78**, 235.
[112] H. Kobayashi and H. Suzuki, *Biotechnol. and Bioeng.*, 1975, **17**, 1455.

investigations have been carried out with an α-D-galactosidase contained in the spherical pellets formed by *M. vinacea* in submerged cultures.[113]

The levels and properties of the β-D-galactosidase from a *Myxobacter* species have been studied.[61] The α-D-galactosidase from *Penicillium duponti* is moderately thermostable, although its thermal stability is higher in the mycelium.[114] Practical applications of the enzyme in the mycelium were discussed. Both α- and β-D-galactosidases have been detected in the media of axenic cultures of the myxomycete *Physarum polycephalum*.[62] The principal form of the α-D-galactosidase is heterogeneous.

Most of the 0:11 strains of *Pseudomonas aeruginosa* are able to hydrolyse 2-nitrophenyl β-D-galactopyranoside, but they are unable to hydrolyse lactose.[115] Biochemical and ultrastructural studies indicated that the enzyme(s) is located in the periplasm.

Chromatography on Sephadex G-200 has been used to isolate three linkage-specific α-D-galactosidases from extracts of *Trichomonas foetus*.[116] They were identified as α-1,3- and α-1,4-D-galactosidases and an α-1,6-D-galactosidase that also hydrolysed alkyl and aryl α-D-galactopyranosides.

β-D-Galactosidase has been immobilized, with retention of enzymic activity, by inclusion in microcapsules of cellulose nitrate,[117] by reaction with agarose cyclic imidocarbonate,[118] by derivatization with active Bright Orange KH and adsorption onto anion-exchange resins,[119] by coupling with glutaraldehyde to feather-meal protein,[120] by coupling to pumice granules coated with a phenylenediamino–glutaraldehyde complex,[105] by attachment to collagen by a membrane-impregnation process,[121] by reaction with activated nylon tubing,[122] by entrapment in gels formed from dimethylacrylamide or 2-hydroxyethyl acrylate[75] or vinylpyrrolidone,[123] and by inclusion inside hollow-fibre cartridges.[76] Poor retention of the enzymic activity resulted from adsorption of the enzyme onto carbon and cross-linking with glutaraldehyde.[124] β-D-Galactosidase and D-glucose oxidase have been immobilized together, with retention of the enzymic activities, by cross-linking with glutaraldehyde to a derivative of porous glass.[125]

D-Glucosidases and β-D-Glucocerebrosidases

The D-glucosidases contaminating commercial enzymes used in the preparation of fungal cell walls[15] and aspects of the affinity chromatography of β-D-glucosidase

[113] H. Kobayashi and H. Suzuki, *Biotechnol. and Bioeng.*, 1976, **18**, 37.
[114] N. Arnaud, D. A. Bush, and M. Horisberger, *Biotechnol. and Bioeng.*, 1976, **18**, 581.
[115] P. Poindron, A. Bourguignat, F. Scheid, and Y. Lombard, *Canad. J. Microbiol.*, 1977, **23**, 798.
[116] A. D. Yates, W. T. J. Morgan, and W. M. Watkins, *F.E.B.S. Letters*, 1975, **60**, 281.
[117] D. T. Wadiak and R. G. Carbonell, *Biotechnol. and Bioeng.*, 1975, **17**, 1157.
[118] P. Würsch, *Analyt. Biochem.*, 1977, **77**, 265.
[119] V. S. Zolotova, A. S. Tikhomirova, L. V. Kozlov, and V. K. Antonov, *Biochemistry (U.S.S.R.)*, 1976, **41**, 1360.
[120] W. L. Stanley, G. G. Watters, B. G. Chan, and S. H. Kelly, *Biotechnol. and Bioeng.*, 1976, **18**, 1351.
[121] K. Venkatasubramanian, R. Saini, and W. R. Vieth, *J. Ferment. Technol.*, 1974, **52**, 268.
[122] T. T. Ngo, D. Narinesingh, and K. J. Laidler, *Biotechnol. and Bioeng.*, 1976, **18**, 119.
[123] H. Maeda, *Biotechnol. and Bioeng.*, 1975, **17**, 1571.
[124] C. C. Liu, E. J. Lahoda, R. T. Galasco, and L. B. Wingard, jun., *Biotechnol. and Bioeng.*, 1975, **17**, 1695.
[125] L. Björck and C.-G. Rosén, *Biotechnol. and Bioeng.*, 1976, **18**, 1463.

on glycose-substituted agaroses have been examined.[35] The isoenzymes of the β-D-glucosidase from lysosomal membranes have been investigated.[126]

A deficiency of a β-D-glucosidase that hydrolyses steroidal β-D-glucopyranosides has been observed in a particulate fraction from the tissues of patients with Gaucher's disease.[127] There is no reduction in the level of the soluble form of the enzyme in adult tissues, although its level is reduced in the brains and spleens of children with the disease.

The effector and catalytic proteins of the D-glucocerebrosidase from human spleen associated to give the active enzyme in the presence of acidic phospholipids.[128]

Heat-stable glycoproteins that stimulate the β-D-glucocerebrosidase (β-D-glucocerebroside:β-D-glucosidase) from human liver have been isolated from the spleens of five patients with Gaucher's disease [ranging from the acute neuropathic form (type 2) to the chronic non-neuropathic form (type 1)].[91] Each of the glycoproteins gave a coincident peak of protein and activator activity on chromatography on Sephadex G-150 and migrated as a single species (mol. wt. ~ 1.0—1.2×10^4) on disc gel electrophoresis in the presence of sodium dodecyl sulphate. With the exception of one preparation containing 7.1% of carbohydrate, each of the glycoproteins contains approximately 17% of carbohydrate and all of them have similar, but not identical, amino-acid compositions consisting of high proportions of acidic and hydrophobic amino-acids. While this study reveals the presence of a factor that stimulates the D-glucocerebroside:β-D-glucosidase and β-D-galactosidase activities in Gaucher's spleens, it does not reveal any correlation between the concentration or activity of the factor and the onset or extent of the disease; *e.g.* the spleens of patients with severe forms of Gaucher's disease do not contain least simulating factor.

Most of the 'neutral' α-D-glucosidases in human liver are bound to particles.[129] Gel filtration and electrophoresis showed that the soluble 'neutral' α-D-glucosidases exist in, at least, four forms with similar pH optima (pH 6.0—6.6) but with different molecular weights (1.1—3.1×10^5) and substrate specificities.

Although human tissues contain two enzymes that release D-glucose from 4-methylumbelliferyl β-D-glucopyranoside, only one of them, which is absent from the tissues of patients with Gaucher's disease, hydrolyses D-glucocerebroside.[130] The enzymes in human liver differ in their subcellular location, behaviour on ion-exchange chromatography, substrate specificities, and sensitivities to inhibition or activation by sodium taurocholate and phospholipids. Synthetic glycosides can be used as substrates to detect the presence or absence of these enzymes in the diagnosis of Gaucher's disease. The β-D-glucocerebrosidase activity in leucocytes can be assayed by a new and inexpensive method that is based on separation of the tritiated sphingolipid (substrate) from the tritiated ceramide (product) by t.l.c. on silica gel impregnated with sodium tetraborate.[89]

[126] A. A. Pokrovskii, V. A. Tutelian, I. K. Zaburunova, and L. V. Krauchenko, *Doklady Akad. Nauk S.S.S.R.*, 1976, **231**, 1252.
[127] J. N. Kanfer, S. S. Raghavan, R. A. Mumford, R. S. Labow, D. G. Williamson, and D. S. Layne, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 683.
[128] M. W. Ho and M. Rigby, *Biochim. Biophys. Acta*, 1975, **397**, 267.
[129] N. A. Ushakova and I. S. Lukomskaya, *Biochemistry (U.S.S.R.)*, 1975, **41**, 1077.
[130] S. P. Peters, P. Coyle, and R. H. Glen, *Arch. Biochem. Biophys.*, 1976, **175**, 569.

Both the transglycosylating and hydrolase activities of a partially purified β-D-glucosidase from calf brain were stimulated by sodium taurocholate and a 'Gaucher factor'.[131] An activating factor from human liver did not enhance the hydrolysis of D-[^{14}C]glucosylceramide and 4-methylumbelliferyl β-D-glucopyranoside by the enzyme, which was competitively inhibited by conduritol-B epoxide and nojirimycin. Hydrolysis of the glycoside was more sensitive than that of the ceramide to inhibition by 4-chloromercuribenzenesulphonate. The enzyme catalysed the formation of D-[^{14}C]glucosylceramide from *N*-[^{14}C]oleoylsphingosine and a number of β-D-glucopyranosides.

Homogenates of liver and kidney tissues from young steers contain a β-D-glucosidase that acts on 3-*O*-β-D-glucopyranosyl-17α- and -17β-estradiol, 17-*O*-β-D-glucopyranosyl-17α-estradiol, and 21-*O*-β-D-glucopyranosyldeoxycorticosterone.[132] The activities towards the phenolic 3-*O*-β-D-glucopyranosides reside in a soluble fraction, whereas that towards the 17-*O*-β-D-glucopyranoside occurs in the microsomes. The properties of partially purified particle-bound and soluble β-D-glucosidases from porcine kidneys have been compared.[133] The soluble enzyme (p*I* 4.9) possesses a broad pH optimum (5.5—7.0), hydrolyses 4-methylumbelliferyl β-D-glucopyranoside, 3- and 17-*O*-β-D-glucopyranosyl-17α-estradiol, and salicin, but not D-glucosylceramide, and is inhibited by Triton X-100, D-gluconolactam, and *O*-β-D-glucopyranosylestrone. The particulate enzyme (pH optimum 5.0) is stimulated by sodium taurocholate and 'Gaucher's factor' and possesses transglycosylating activity.

Temperature, pH, metal ions, urea, and trehalose have been shown to alter the activities of the 'acid' α-D-glucosidases from porcine spleen towards oligomeric and polymeric substrates containing different types of linkage, supporting the view that the enzyme binds polymers and oligomers at different sites.[134]

The latency of the α-D-glucosidase activity in intact rat-liver lysosomes has been studied using glycogen, maltose, 4-nitrophenyl α-D-glucopyranoside, and α-D-glucopyranosyl fluoride.[99] The results indicated that the lysosomes are impermeable to glycogen and maltose, but are permeable, to some extent, to the glycoside and the fluoride. The latency of lysosomal β-D-glucosidase was also examined.

The secretion of a β-D-glucosidase by the phytoflagellate *Ochromonas danica* was inhibited by cycloheximide, anaerobiosis, and low temperatures.[135]

The ability of glycoside hydrolases to control the anomeric configuration of sugars formed in the enzymic reaction has been demonstrated.[136] Thus, purified *Candido tropicalis* α-D-glucosidase and sweet-almond β-D-glucosidase converted D-glucal initially into 2-deoxy-α- or -β-D-glucopyranose, respectively. Moreover, studies using deuterium oxide showed that the α-D-glucosidase catalysed specific axial deuteriation of D-glucal at C-2 with the formation of 2-deoxy-α-D-[2-$^{2}H_{ax}$]-

[131] R. A. Mumford, S. S. Raghaven, and J. N. Kanfer, *J. Neurochem.*, 1976, **27**, 943.
[132] D. G. Williamson, C. Gwilliam, and D. S. Layne, *Canad. J. Biochem.*, 1977., **55**, 41.
[133] J. N. Kanfer, R. A. Mumford, and S. S. Raghavan, *Canad. J. Biochem.*, 1977, **55**, 140.
[134] A. L. Minakova, M. E. Preobrazhenskaya, and E. L. Rozenfel'd, *Biochemistry* (*U.S.S.R.*), 1975, **40**, 105.
[135] D. H. Meyer, *Arch. Mikrobiol.*, 1976, **109**, 263.
[136] E. J. Hehre, D. S. Genghof, H. Sternlicht, and C. F. Brewer, *Biochemistry*, 1977, **16**, 1780.

glucopyranose, whereas the β-D-glucosidase catalysed equatorial deuteriation at C-2 with the formation of 2-deoxy-β-D-[2-$^2H_{eq}$]glucopyranose (Scheme 2).

A homogeneous α-D-glucosidase has been obtained from buckwheat seeds by fractionation with ammonium sulphate, ion-exchange chromatography, and gel filtration.[137] The ratio of the initial rates of hydrolysis of maltose and starch by the enzyme ($S_{20,w}$ 6.2*S*, mol. wt. 8.0×10^4) is 100:86; these activities were inhibited competitively by tris and erythritol, and were stimulated by Na^+ and K^+ ions.

Inhibition studies have indicated that endogenous β-D-glucosidase is not involved in auxin- or acid-stimulated growth of oat (*Avena sativa*) coleoptiles.[102]

Affinity chromatography of the glycoside hydrolases, including the β-D-glucosidase, from soybean and Takadiastase on glycose-substituted agaroses showed that they are not adsorbed specifically.[59] An α-D-glucosidase that acts on maltose, but not on turanose, has been found in sugar-cane stalks.[64]

A method for the detection of micro-organisms that synthesize extracellular α-D-glucosidases is based on treatment of the colonies on agar with a combination of peroxidase, D-glucose oxidase, and *o*-dianisidine; colonies that secrete α-D-glucosidase are surrounded by orange or orange-red zones.[138]

The synthesis of extracellular α-D-glucosidase (maltase) has been studied with the thermophilic *Bacillus* sp. KP1035, which produces high levels of maltase.[139] The yield of the enzyme was enhanced in the presence of maltose, peptone, and a yeast extract, whereas it was reduced by succinate and fumarate. The enzyme was stable for 24 h at 60 °C over the pH range 5.6—9.0, and it retained 95% of its activity after heating at 70 °C and pH 6.8 for 20 min. Conditions for the maximal synthesis of α-D-glucosidase (maltase) by cultures of *Bacillus subtilis* have been reported.[140] Ion-exchange chromatography and gel filtration freed the enzyme (pH optimum 6.0, temperature optimum 45 °C, p*I* 6.0, mol. wt. 3.3×10^4, K_m values for maltose, maltotriose, and isomaltose 1, 5, and 10 mmol l^{-1}, respectively) from amylase and proteinase activities. The relative rates of hydrolysis of maltose, maltotriose, isomaltose, and maltotetraose by the enzyme are 100:15:14:4. Inhibition of the enzyme by Rose Bengal, together with other evidence, indicated that a histidyl residue is present at the active site.

The maximum rates of hydrolysis of synthetic α-D-glucopyranosides and isomaltose by a purified extracellular α-D-glucosidase (pH optimum 5—6, temperature optimum 75°C) from the thermophile *B. thermoglucosidus* have been compared.[141] Tris, 4-nitrophenyl α-D-xylopyranoside, D-glucose, and D-glucono-1,5-lactone inhibited the hydrolysis of 4-nitrophenyl α-D-glucopyranoside by the enzyme, whose activity was also inhibited by heavy-metal ions but not by H_4edta, 4-chloromercuribenzoate, and iodoacetate.

The α-D-glucosidase ($S_{20,w}$ 6.1*S*, $D_{20,w}$ 4.8×10^{-7} cm^2 sec^{-1}) from *Mucor javanicus* has been crystallized.[142] The enzyme is a glycoprotein containing residues of D-mannose and 2-amino-2-deoxy-D-glucose. Some properties of the

[137] K. Kanaya, S. Chiba, T. Shimomura, and K. Nishi, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1929.
[138] L. H. Wang, S. J. McWethy, and P. A. Hartman, *Analyt. Biochem.*, 1976, **76**, 380.
[139] Y. Suzuki, T. Tsuji, and S. Abe, *Appl. Environ. Microbiol.*, 1976, **32**, 747.
[140] L. H. Wang and P. A. Hartman, *Appl. Environ. Microbiol.*, 1976, **31**, 108.
[141] Y. Suzuki, T. Yuki, T. Kishigami, and S. Abe, *Biochim. Biophys. Acta*, 1976, **445**, 386.
[142] Y. Yamasaki, Y. Suzuki, and J. Ozawa, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1909.

Scheme 2

inducible α-D-glucosidase of *Mucor rouxii* have been investigated.[143] The enzymic activity was induced by growing the cells first on D-glucose and then resuspending them in medium containing maltose; it represented 80—90% of the total enzymic activities present in the cells. Addition of D-glucose before or during the induction period repressed the synthesis of the α-D-glucosidase.

The levels and properties of α- and β-D-glucosidases in a *Myxobacter* sp. have been examined.[61]

An increase in the level of amylase activity was observed on hybridization of a hyperderepressed strain of *Neurospora crassa* with the normal strain.[68] Both α- and β-D-glucosidases have been detected in axenic cultures of the myxomycete *Physarum polycephalum*.[62]

Two distinct forms of β-D-glucosidase have been found in the cells of *Pseudomonas fluorescens*; one is bound to the membrane and the other occurs in the cytosols.[144] The enzymes differ in their molecular weights, kinetic properties, and susceptibilities to various reagents. The enzyme from the cytosols is not a typical β-D-glucosidase and possesses some *exo*-cellulase activity. The β-D-glucosidase activity of a cellulase fraction from *Trichoderma viride* has been studied.[145] The β-D-glucosidase (cellobiase) from the crude cellulase system of *T. viride* has been isolated by gel filtration and ion-exchange chromatography.[146] The steady-state kinetics for the hydrolysis of cellobiose were investigated as a function of the concentrations of D-glucose and cellobiose, pH, temperature, and the dielectric constant of the isopropanol–buffer solution used. Whereas high concentrations of D-glucose inhibited the enzyme, low concentrations of D-glucose stimulated it.

An active immobilized form of α-D-glucosidase has been obtained by reaction of the enzyme with agarose cyclic imidocarbonate.[118] Active immobilized forms of β-D-glucosidase have been prepared by chelation of the enzyme with hydrous oxides of iron(III), tin(II), vanadium(III), and zirconium(IV),[147] and by reaction of the enzyme with diazotized 1,4-diaminobenzene[148] and with agarose cyclic imidocarbonate.[118]

β-D-Glucuronidases

The rate of removal of purified β-D-glucuronidases from rats following intravenous infusion depended on the source of the enzyme.[57] Thus, the β-D-glucuronidase from rat serum was cleared more slowly from circulation than that from rat preputial glands. The lysosomal compartment of rat liver has an important role in the removal of circulating tris and glycoside hydrolases.[58]

The β-D-glucuronidase from the preputial glands of rats crystallized following fractional precipitation, fractionation in ethanol, and gel filtration.[149] The purified enzyme (12*S*, mol. wt. 3.2×10^5) appeared to be homogeneous on examination by polyacrylamide gel electrophoresis, but electrophoresis in the presence

[143] A. P. Sorrentino, J. Zorzópulos, and H. F. Terenzi, *Arch. Biochem. Biophys.*, 1977, **180**, 232.
[144] J.-T. Hwang and H. Suzuki, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 2169.
[145] S. Shikata and K. Nisizawa, *J. Biochem. (Japan)*, 1975, **68**, 479.
[146] R. J. Maguire, *Canad. J. Biochem.*, 1977, **55**, 19.
[147] J. F. Kennedy, S. A. Barker, and J. D. Humphreys, *J.C.S. Perkin I*, 1976, 962.
[148] C. J. Gray and C. M. Livingstone, *Biotechnol. and Bioeng.*, 1977, **19**, 349.
[149] M. Himeno, H. Ohhara, Y. Arakawa, and K. Kato, *J. Biochem. (Japan)*, 1975, **77**, 427.

of sodium dodecyl sulphate revealed that it is a tetramer composed of subunits of molecular weight 7.9×10^4. The enzyme (pH optimum 4.5, K_m for 4-nitrophenyl β-D-glucopyranosyluronic acid 0.53 mmol l^{-1}) contains few sulphur-containing amino-acids and 5.7% of carbohydrate consisting of D-mannose, D-glucose, L-fucose, D-galactose, and 2-amino-2-deoxy-D-glucose (molar proportions 44:9:6:1:41, respectively).

The β-D-glucuronidase in rat-liver microsomes has been isolated by immunoadsorption on agarose-immobilized rabbit immunoglobulin IgG anti-(rat preputial gland β-D-glucuronidase) antibodies.[150] The purified enzyme is a tetramer composed of subunits of molecular weight 7.5×10^4. Although this enzyme can be distinguished from lysosomal β-D-glucuronidase by electrophoresis, they have identical catalytic and immunological properties and molecular dimensions. The microsomal and lysosomal enzymes appear to be charge isomers.

Injection of alkyl phosphates into rats raised the level of β-D-glucuronidase activity in their sera without affecting the levels of lysosomal hydrolases and cholinesterase.[151] Dibutyl and tributyl phosphates were most and equally effective, the levels of β-D-glucuronidase activity increasing 120- and 90-fold after 1 and 2 h, respectively. The increase in enzymic activity elicited with tributyl phosphate correlated with a lowering of the enzymic activity in the liver microsomes, which appear to be the main source of the excess of β-D-glucuronidase activity in the serum.

The removal of β-D-glucuronidase from the circulatory system of rats has been investigated.[22]

Glycoside Hydrolases (Miscellaneous)

An enzyme from *Aeromonas hydrophila* that catalyses the NADH-dependent cleavage of certain anthracycline glycosides has been partially purified.[152] Solutions of this acidic enzyme (mol. wt. 3.5×10^4) are stable, and the enzyme is strongly inhibited by oxygen but by neither CN^- ions nor H_4edta.

α-L-Iduronidases

2,5-Anhydro-4-*O*-(α-L-idopyranosyluronic acid)-D-[1-^{3}H]mannitol is cleaved by α-L-iduronidase to give, as one of the products, 2,5-anhydro-D-[1-^{3}H]mannitol, which can be separated and assayed.[153] This procedure can be used in the diagnosis of mucopolysaccharidosis type I (Hurler's syndrome).

A deficiency of α-L-iduronidase has been reported in a patient with hyperglycosaminoglycanuria, whose phenotypic abnormalities did not resemble either the Hurler or Scheie syndrome.[154]

D-Mannosidases

Aspects of the affinity chromatography of α-D-mannosidase on glycose-substituted agaroses have been investigated.[35] α-D-Mannosidase activity can be detected in

[150] J. W. Owens and P. Stahl, *Biochim. Biophys. Acta*, 1976, **438**, 474.
[151] Y. Suzuki, H. Kikuchi, C. Kato, Y. Horiuchi, K. Tomita, and Y. Hashimoto, *Biochem. Pharmacol.*, 1977, **26**, 881.
[152] M. McCarville and V. Marshall, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 331.
[153] P. di Natale, I. G. Leder, and E. F. Neufeld, *Clinica Chim. Acta*, 1977, **77**, 211.
[154] P. R. Winters, M. J. Garrod, S. A. Molenich-Heetred, J. Kirkpatrick, and R. N. Rosenberg, *Neurology*, 1976, **26**, 1003.

polyacrylamide gels following electrophoresis by reaction of the reducing sugar released from an appropriate substrate with Prussian Blue.[10]

The apparent K_m towards a fluorogenic substrate of the residual acidic α-D-mannosidase activity in extracts of the fibroblasts from four patients with mannosidosis was higher than normal.[155] The enzyme from the patients was also more thermolabile, suggesting that a mutation in the structural gene for the enzyme had occurred. The altered kinetics of the mutant enzyme can result in apparently normal activity towards high concentrations of the fluorogenic substrate, which could lead to errors in the diagnosis of mannosidosis. Radioimmunological evidence has indicated that the fibroblasts from patients with mannosidosis contain a material that reacts with antibodies specifically binding acidic α-D-mannosidases.[156]

Two forms of α-D-mannosidase, each having a neutral pH optimum, have been found in human sera; they can be distinguished by molecular-sieve chromatography, ion-exchange chromatography, or affinity chromatography on immobilized concanavalin A.[157] The properties of the enzymes were compared with those of the acidic α-D-mannosidase.

An α-D-mannosidase (acid pH optimum) that is Zn^{2+}-dependent and three other α-D-mannosidase activities (neutral pH optima) have been found in the tissues of rats.[158] The enzymes most active at a neutral pH value were inhibited by heavy-metal ions and were stabilized, to some extent, by thiols. The reversible effects of Fe^{2+}, Co^{2+}, and Mn^{2+} ions and H_4edta on the activity of these enzymes were investigated. Tissue preparations varied widely in the levels of the enzymes most active at pH 6.5, although latent enzymic activity was released on incubation with activating metal ions.

The cytosolic form of the α-D-mannosidase from rat liver has been purified (1.2×10^4-fold) using D-mannopyranosylamine and 1,4-dithioerythritol as stabilizers.[159] The enzyme (mol. wt. $3.72—4.90 \times 10^5$, pH optimum 5.5—5.9, K_m for 4-nitrophenyl α-D-mannopyranoside 0.14 mmol l^{-1}) is composed of four subunits (mol. wt. 1.10×10^5), is competitively inhibited by D-mannopyranosylamine, and is stabilized by Co^{2+}, Mn^{2+}, and Fe^{2+} ions.

Two forms of α-D-mannosidase from yellow wax beans (*Phaseolus vulgaris*) have been purified by immunoadsorption.[160] A high degree of immunological identity has been demonstrated between α-D-mannosidase I and II from *P. vulgaris*. This made possible the purification of both forms of the enzyme by chromatography on an immunoadsorbent prepared from anti-(α-D-mannosidase I) antiserum.

The properties of a partially purified α-D-mannosidase from pineapple fruit have been reported.[161]

Affinity chromatography of glycoside hydrolases from soybeans on glycose-substituted agaroses has shown that specific binding of α-D-mannosidase cannot

[155] A. L. Beaudet and B. L. Nichols, jun., *Biochem. Biophys. Res. Comm.*, 1976, **68**, 292.
[156] G. Mersmann and E. Buddecke, *F.E.B.S. Letters*, 1977, **73**, 123.
[157] B. Hultberg, P. K. Masson, and S. Sjöblad, *Biochim. Biophys. Acta*, 1976, **445**, 398.
[158] S. M. Snaith, *Biochem. J.*, 1977, **163**, 557.
[159] V. A. Shoup and O. Touster, *J. Biol. Chem.*, 1976, **251**, 3845.
[160] E. Paus, *F.E.B.S. Letters*, 1976, **72**, 39.
[161] Y. Nakagawa and N. Takahashi, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 455.

be achieved, although purification by non-specific adsorption is possible.[59] The α-D-mannosidase activities from several wood-rotting fungi have been investigated.[162] The α-D-mannosidase activity secreted by an *Acinetobacter* sp. growing on a medium containing yeast cells has been studied in detail.[163]

A highly purified form of the β-D-mannosidase (mol. wt. 6.4×10^4, pH optimum 2.4—3.4) from the fruiting bodies of the mushroom *Polyporus sulfureus* has been recommended for use in structural studies on glycoproteins.[164]

Low levels of α-D-mannosidase activity occur in axenic culture media of the myxomycete *Physarum polycephalum*.[62]

Neuraminidases

The properties of a neuraminidase in the intestines of young mammals have been investigated.[165]

The fibroblasts from a patient with mucolipidosis I were found to be severely deficient in an 'acid' neuraminidase.[166] Since normal levels of other glycoside hydrolases were found, the basic metabolic lesion appears to involve a defect in the degradation of compounds containing 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonic acid.

Although the addition of sodium butyrate to HeLa cells in culture increased the synthesis of G_{M_3} ganglioside, the levels of sialidase and other glycoside hydrolases were not affected.[167] The sialidase hydrolysed G_{M_3} and $G_{D_{1a}}$ gangliosides, but not fetuin; the enzyme has a pH optimum of 5.0 and a K_m value of 75 μmol l^{-1} for the gangliosides. Although the cells produce a high level of sialidase activity (4—7 nmol mg^{-1} of protein per hour) and could bind exogenous [^{14}C]ganglioside G_{M_3}, no 'ecto'-sialidase activity could be detected in intact cells at physiological pH. This indicates that the ganglioside sialidase is not directly involved in the morphological and biochemical differentiation induced in HeLa by sodium butyrate.

A sensitive radiometric assay for the neuraminidase from calf brain uses a tritium-labelled $G_{D_{1a}}$ ganglioside as the substrate.[168] After incubation with the enzyme, the substrate and the product ([^{3}H]ganglioside G_{M_1}) are separated by t.l.c. and their radioactivities measured.

The relationships between bacterial neuraminidases and alterations in the immunological behaviour of neuraminidase-treated mammalian cells have been reviewed.[169]

Commercial neuraminidase preparations from *Clostridium perfringens* are contaminated with protease and *endo*-glycoside hydrolase activities, which hydrolyse casein, human serum albumin, and haemoglobin, *etc.*, and release oligosaccharides containing a 2-acetamido-2-deoxy-D-glucopyranosyl residue at the

[162] Z. Zouchová, J. Kocourek, and V. Musílek, *Folia Microbiol.*, 1977, **22**, 61.
[163] S. Katohda, Y. Sawaya, K. Asatsuma, F. Suzuki, and M. Hayashibe, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 331.
[164] C. C. Wan, J. E. Muldrey, S.-C. Li, and Y.-T. Li, *J. Biol. Chem.*, 1976, **251**, 4384.
[165] J. J. Dickson and M. Wesser, *Proc. Austral. Biochem. Soc.*, 1977, **10**, 35.
[166] M. Cantz, J. Gehler, and J. Spranger, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 732.
[167] J. F. Tallman, P. H. Fishman, and R. C. Henneberry, *Arch. Biochem. Biophys.*, 1977, **182**, 556.
[168] J. Schraven, C. Čáp, G. Nowoczek, and K. Sandhoff, *Analyt. Biochem.*, 1977, **78**, 333.
[169] P. K. Ray, *Adv. Appl. Microbiology*, 1977, **21**, 227.

reducing end from intact ovalbumin, respectively.[170] An *endo*-β-D-2-acetamido-2-deoxyglucanase was also detected.

The neuraminidase from *Vibrio cholerae* has been purified (10-fold) by affinity chromatography on agarose substituted with (4-nitrophenyl)oxamic acid.[171]

That polycation-stimulated mutants of fowl-plague virus contain an altered neuraminidase has been shown by changes in the sensitivity of the enzyme to inhibition, by its behaviour with specific antisera, and by ion-exchange chromatography of the disintegrated virus.[172] The chemistry and the enzymology of orthomyxoviruses have been reviewed.[173]

Investigations of the neuraminidase from Sendai virus have shown that it is a cell-surface glycoprotein (mol. wt. 1.4×10^5) composed of subunits of molecular weight 7.5×10^4 (rather than of mol. wt. 5.3×10^4 or 1.14×10^5, as suggested by others).[174]

α-L-Rhamnosidases

An α-L-rhamnosidase from the seeds of saracen corn (*Fagopyrum esculentum*) has been purified to electrophoretic homogeneity by precipitation, gel filtration, and chromatography on Ultrogel.[175] The enzyme (mol. wt. 7.0×10^4, p*I* 3.7) interacted with an immobilized form of concanavalin A, suggesting that it contains residues of α-D-mannopyranose or α-D-glucopyranose or both. L-Rhamnose, L-lyxose, 6-deoxy-D-glucose, and methyl α-D-mannopyranoside are competitive inhibitors of the enzyme, and the 6-deoxy group of L-rhamnose appears to be important for binding to the enzyme.

endo-β-D-Acetamidodeoxyglucanases and *endo*-β-D-Acetamidodeoxyglycanases

A new, improved assay for *endo*-β-D-2-acetamido-2-deoxyglucanase activity uses a dansylated derivative of the glycoprotein (4) as the substrate.[176] The assay measures the release of the heptasaccharide (5), which is separated from the

(D-[^{3}H]Man*p*)$_6$-(D-Glc*N*Ac*p*)$_2$-Asn (4)

(D-[^{3}H]Man*p*)$_6$-D-Glc*N*Ac*p* (5)

other product of hydrolysis by ion-exchange chromatography. The principal advantage of the assay is that radiolabelled products are not released from the substrate by *exo*-glycosidases (*e.g.* α-D-mannosidase).

The *endo*-β-D-2-acetamido-2-deoxyglucanase from rabbit serum has been purified by affinity chromatography on 2-acetamido-2-deoxy-β-D-glucopyranose immobilized on an agarose support.[55] The enzyme hydrolysed synthetic glycosides and interior-chain residues of chito-oligosaccharides.

Two *endo*-β-D-2-acetamido-2-deoxyglucanases that act on the carbohydrate

[170] S.-U. Chien, S. J. Yevich, S.-C. Li, and Y.-T. Li, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 683.
[171] R. Brossmer. D. Ziegler, and G. Keilich, *Z. physiol. Chem.*, 1977, **358**, 397.
[172] A. B. Germanov, L. Y. Zakstelskaya, E. V. Molibog, N. A. Evstigneeva, N. A. Parasiuk, and G. V. Kornilaeva, *Intervirology*, 1973/4, **2**, 273.
[173] E. S. Isaeva and M. I. Sokolov, *Vopr. Virusol.*, 1974, 515.
[174] L. M. Popa, R. Repanovici, I. Samuel, D. Smelt, and R. Portocala, *F.E.B.S. Letters*, 1975, **51**, 270.
[175] R. Bourbouze, F. Percheron, and J.-E. Courtois, *European J. Biochem.*, 1976, **63**, 331.
[176] A. L. Tarentino and F. Maley, *Analyt. Biochem.*, 1977, **77**, 185.

residues of glycoproteins have been obtained from culture fluids of *Clostridium perfringens*.[177] Although both enzymes are most active at neutral pH and are inhibited by 4-chloromercuribenzenesulphonate, they exhibit different substrate specificities; one of them is similar to the *endo*-β-D-2-acetamido-2-deoxyglucanase 'D' isolated from *Diplococcus pneumoniae*, whereas the other is similar to the *endo*-β-D-2-acetamido-2-deoxyglucanase 'H' isolated from *Streptomyces griseus*.

The *endo*-glycan hydrolase that contaminates commercial preparations of the neuraminidase from *Clostridium perfringens* has been identified as an *endo*-β-D-2-acetamido-2-deoxyglucanase.[170] The enzyme hydrolysed (D-Man*p*)$_5$-(D-Glc*N*Ac)$_2$-Asn (from ovalbumin) to O^4-(2-acetamido-2-deoxy-β-D-glucopyranosylamino)-hydrogen aspartate and a hexasaccharide containing 2-acetamido-2-deoxy-D-glucopyranose as the reducing-end residue.

Most of the D-mannose-containing glycopeptides from fibroblasts transformed with Simian virus 40 were hydrolysed by the *endo*-β-D-2-acetamido-2-deoxyglucanase 'D' from *Diplococcus pneumoniae*—in the presence of β-D-galactosidase, β-D-2-acetamido-2-deoxyglucosidase, and neuraminidase—and by the *endo*-β-D-2-acetamido-2-deoxyglucanase 'H' from *Streptomyces griseus* to oligosaccharides, probably having the structure (6).[178] The 'D' enzyme preferentially released smaller oligosaccharides than those released by the 'H' enzyme. 4-Nitrophenyl

$$(\alpha\text{-D-Man}p)_n \rightarrow \text{D-Glc}N\text{Ac}p$$

(6) $n = 3, 5,$ or 6, *etc.*

α-D-mannopyranoside competitively inhibited the *endo*-β-D-2-acetamido-2-deoxyglucanase 'D' from *Diplococcus pneumoniae*; D-mannose and methyl α-D-mannopyranoside were also inhibitors, whereas D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and 4-nitrophenyl α-D-glucopyranoside had little effect.[179] This indicates that α-D-mannopyranosyl residues in the substrates play an important part in binding to the enzyme. By contrast, the *endo*-β-D-2-acetamido-2-deoxyglucanase 'H' from *Streptomyces griseus* was not inhibited by D-mannose and simple α-D-mannopyranosides, although it was inhibited by yeast D-mannan.

exo-β-D-2-Acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxyglucanases

4-Methylumbelliferyl *N*-acetylmuramide has been synthesized and used as the substrate in an assay of *exo*-β-D-2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxyglucanases (*exo*-β-acetamidodeoxymuramidases).[180]

The *exo*-β-D-2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxyglucanase (*exo*-β-*N*-acetylmuramidase) produced by *Bacillus subtilis* growing under defined conditions is partly secreted and partly bound to the cell wall.[181] The synthesis of this enzyme was induced, in decreasing order of efficiency, by a lysozymal digest of *Micrococcus lysodeikticus* cell walls, 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucopyranose, and 2-acetamido-4-*O*-[2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranosyl]-2-deoxy-D-

[177] S. Ito, T. Muramatsu, and A. Kobata, *Arch. Biochem. Biophys.*, 1975, **171**, 78.
[178] T. Muramatsu, N. Koide, and M. Ogata-Arakawa, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 881.
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[180] L. A. del Río and R. C. W. Berkeley, *Analyt. Biochem.*, 1975, **66**, 405.
[181] L. A. del Río and R. C. W. Berkeley, *European J. Biochem.*, 1976, **65**, 3.

glucopyranose. Extensive chromatography yielded two active enzyme fractions. The activity and properties of this *exo*-enzyme (mol. wt. 9.0×10^4) towards synthetic and natural substrates were examined.

Agarases

Factors controlling the synthesis of an extracellular agarase by *Cytophaga flevensis* have been studied with suspensions of resting cells.[182] Synthesis of the enzyme depended on the presence of an inducer such as melibiose or neoagaro-oligosaccharides, among which neoagarotetraose was the most effective.

Degradation of agarose by a β-agarase from *Pseudomonas atlantica* gave neoagaro-oligosaccharides of DP 4, 6, 8, and 10.[183] A β-neoagarotetraose hydrolyase cleaved the central β-linkage in neoagarotetraose and the penultimate β-linkage at the non-reducing end of neoagaro-hexaose and -octaose to yield neoagarobiose. The β-neoagarotetraose hydrolase, which is located on or outside the cytoplasmic membrane, is activated by NaCl, KCl, $CaCl_2$, $MnCl_2$, and $MgSO_4$, and has a K_m value of 3.4×10^{-3} mol^{-1} for neoagarotetraose; it was free from β-agarase and α-neoagarobiose hydrolase activities and exhibited no transglycosylase activity.

Alginate Lyases

A lyophilized preparation of alginate lyase from a dialysed extract of sonicated cells of a *Pseudomonas* sp. has been separated into three fractions by gel filtration.[184] As well as degrading alginate, the fractions degraded a poly-L-guluronide and a polyuronide composed of D-mannuronic and L-guluronic acid residues, but not a poly-D-mannuronide, so that they appear to be *endo*-poly(L-guluronic acid) lyases. The properties of these enzymes have been investigated.[185]

The use of alginate lyases in structural studies of alginates has been described.[186]

α-Amylases

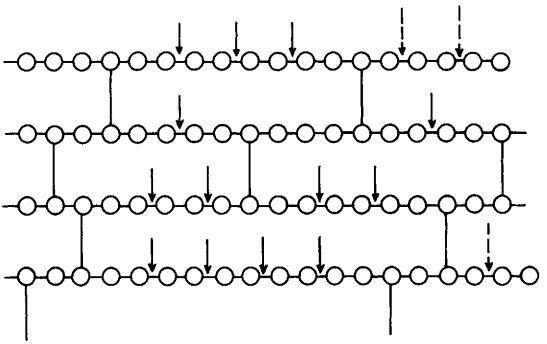
Aspects of the affinity chromatography of α-amylases on glycose-substituted agaroses have been investigated.[35] Affinity chromatography on cross-linked starch provides a simple and rapid purification of α-amylase (*e.g.* from bacteria and malt).[187] α-Amylase is retained on the column and is eluted with a solution of maltose; β-amylase is not retained, so that *endo*- and *exo*-amylase activities can be separated.

Natural and dyed amyloses (possible substrates for use in assays of α-amylases) have been characterized by ion-exclusion and gel-permeation chromatography and by enzymic degradation.[188] A new method for estimating the proportion of

[182] H. J. van der Meulen and W. Harder, *J. Microbiol. Serol.*, 1976, **41**, 277.
[183] D. Groleau and W. Yaphe, *Canad. J. Microbiol.*, 1977, **23**, 672.
[184] K. H. Min, S. F. Sasaki, Y. Kashiwabara, H. Suzuki, and K. Nisizawa, *J. Biochem. (Japan)*, 1977, **81**, 539.
[185] K. H. Min, S. F. Sasaki, Y. Kashiwabara, and K. Nisizawa, *J. Biochem. (Japan)*, 1977, **81**, 547.
[186] K. H. Min, S. F. Sasaki, Y. Kashiwabara, M. Umekawa, and K. Nisizawa, *J. Biochem. (Japan)*, 1977, **81**, 555.
[187] M. Weber, M. J. Foglietti, and F. Percheron, *Biochimie*, 1976, **58**, 1299.
[188] J. F. Kennedy, *Stärke*, 1976, **28**, 196.

α-(1 → 6)-linkages in starches is based on exhaustive hydrolysis with β-amylase.

The use of a cross-linked amylose as the substrate permits α- and β-amylase activities to be distinguished.[189] β-Amylase acts only on chain-ends that are too short to be attacked by α-amylase (Scheme 3).



Scheme 3

The Du Pont *aca* assay for α-amylase has been assessed and compared with assays using Roche Diagnostics 'Amylochrome' and Perkin-Elmer's Coleman 91.[190]

In a new technique for detecting isoenzymes of α-amylase after flat-bed gel electrophoresis, an amylase-sensitive test paper (dyed chromatography paper) is placed on top of the gel, whereupon the isoenzyme bands appear on the test paper as well-defined white lines on a pink background.[191] The gel can then be stained with other reagents.

The kinetics of the hydrolysis of starch by a mixture of α- and β-amylases in a membrane reactor have been analysed.[192]

A new assay for pancreatic and salivary amylases in sera requires neither electrophoresis nor chromatography.[193] It is based on inhibition of the salivary amylases with a protein from wheat, which allows the pancreatic amylases to be estimated separately. The assay was used to measure the levels of pancreatic and salivary amylases in the sera of healthy men and women and individuals with pancreatitis, mumps, and other diseases.

Antisera raised against purified human pancreatic α-amylase in rabbits inhibited human pancreatic, salivary, and serum amylases.[194] The interactions of the antisera with other amylases from mammalian sera were also investigated. Although antisera could not distinguish between the two isoenzymes of human pancreatic

[189] M. A. Mateescu, H. D. Schell, F. Mihailescu, and I. Cornoiu, *Biochimie*, 1976, **58**, 875.
[190] G. P. James, R. B. Passey, J. B. Fuller, and M. L. Giles, *Clinical Chem.*, 1977, **23**, 546.
[191] P. E. Burdett, A. E. Kipps, and P. H. Whitehead, *Analyt. Biochem.*, 1976, **72**, 315.
[192] T. V. Subramanian, *Biotechnol. and Bioeng.*, 1976, **18**, 1473.
[193] M. D. O'Donnell, D. Fitzgerald, and K. F. McGeeney, *Clinical Chem.*, 1977, **23**, 560.
[194] D. J. Langley and J. A. Carney, *Comp. Biochem. Physiol.*, 1976, **55B**, 563.

α-amylase, they inhibited serum, urinary, and salivary amylases.[195] The extent of inhibition depended on the size of the substrate, but was not enhanced by using a second antibody.

Ion-exchange chromatography separated four inhibitors of α-amylase from an alcoholic extract of wheat.[196] After extensive purification, one of them (mol. wt. 2.1×10^4) was 100 times more reactive towards human salivary α-amylase than towards human pancreatic α-amylase.

The use of chemically modified starches for the assay of serum α-amylase activity has been described.[197] α-Amylase acted on these substrates, in the presence of an excess of fungal glucoamylase, to give D-glucose in amounts directly proportional to the amount of α-amylase present. The amount of D-glucose released is measured using a specific enzymic assay. The results obtained with this assay are in good agreement with those obtained by a conventional saccharogenic assay, and the assay is more sensitive and less susceptible to interference than are most other assays for α-amylase.

The properties of eight amylase-linked immunoglobulins from human sera have been studied.[198] All were identified as macromolecular amylases by means of gel filtration on Sephadex G-200. These complexes dissociated at an alkaline pH and on affinity chromatography on Sepharose–concanavalin A; in the latter instance, dissociation of the complex was attributed to changes in the conformation of the amylase-linked immunoglobulin. The complexes of amylase and immunoglobulins are specific antigen–antibody complexes, which must be recognized as one of the circulating autoantibodies in plasma, and must be clearly distinguished from any others.

An abnormal amylase has been found in the serum of a patient with carcinoma of the lung.[199] It interacted strongly with concanavalin A and its electrophoretic mobility was reduced after treatment with neuraminidase. However, amylase extracted from the tumour and purified was indistinguishable from normal salivary amylase by electrophoresis, was not affected by neuraminidase, and did not exhibit an abnormal interaction with concanavalin A. The results suggest that the abnormal amylase contains residues of sialic acid and other sugars not normally found in human amylases.

The α-amylase from human urine has been crystallized.[200] The crystalline enzyme (mol. wt. 4.5×10^4) was homogeneous on examination by velocity sedimentation and gel filtration, *etc.*, although electrophoresis revealed the presence of, at least, five isoenzymes.

The levels of α-amylase activity in the sera of African elephants (*Loxodonta africana*) are ten times higher than those in human sera.[201] Electrophoresis revealed the presence of five isoenzymes—one in the γ-globulin region and four in the β-globulin region.

An improved method for the purification of porcine pancreatic α-amylase

[195] J. A. Carney, *Clinica Chim. Acta*, 1976, **67**, 153.
[196] M. D. O'Donnell and K. F. McGeeney, *Biochim. Biophys. Acta*, 1976, **422**, 159.
[197] J. J. Marshall, A. P. Iodice, and W. J. Whelan, *Clinica Chim. Acta*, 1977, **76**, 277.
[198] T. Kanno and K. Sudo, *Clinica Chim. Acta*, 1977, **76**, 67.
[199] K. Sudo and T. Kanno, *Clinica Chim. Acta*, 1976, **73**, 1.
[200] N. Minamiura, Y. Kimura, K. Tsujino, and T. Yamamoto, *J. Biochem. (Japan)*, 1975, **77**, 163.
[201] R. K. Skinner, I. R. F. Brown, and P. T. White, *Comp. Biochem. Physiol.*, 1976, **55B**, 363.

involves its interaction with polydextran gel, possibly by enzyme–substrate interactions.[202] The effects of 5,5′-dithiobis-(2-nitrobenzoic acid) on the enzyme were investigated.

Comparisons of the products resulting from the action of porcine pancreatic α-amylase on Amylose–Cibacron Blue 3G-A (a dyed amylose) and amylose indicated that enzymic hydrolysis of the dyed amylose is modified.[203] These observations were discussed with reference to the use of Amylose–Cibacron Blue 3G-A for the assay of α-amylase in clinical work.

The action of trypsin on porcine pancreatic α-amylase has been studied.[204] Characterization of the released fragments showed that trypsinolysis of this α-amylase is incomplete. Chemical studies (*e.g.* 2,4-dinitrophenylation and dansylation, *etc.*) have indicated that porcine pancreatic α-amylase contains an *N*-acetylated amino-acid at the NH_2-terminus and leucine at the CO_2H-terminus.[205] These findings support the view that the enzyme consists of a single chain, rather than of a subunit or a multi-chain structure.

The release of amylase in rat-parotid acinar cells stimulated by adrenaline, isoproterenol, phenylephrine, carbamoylcholine, dibutyryl cAMP, and 3-isobutyl-1-methylxanthine, *etc.*, has been studied.[206]

The α-amylase extracted from homogenized chicken pancreas is indistinguishable by electrophoresis from that secreted from pancreatic ducts.[207] The secreted amylase gave, at least, three amylolytic proteins on electrophoresis.

The mobility of malted-barley α-amylase on electrophoresis in polyacrylamide gels containing starch or amylose or the β-limit dextrin from amylopectin was retarded completely at low concentrations of the enzyme, but it was not affected at high concentrations.[208]

The amylolytic activity of an α-D-glucosidase (mol. wt. 8.0—8.8 × 10^4) from buckwheat seeds was inhibited by tris and erythritol and was stimulated by Na^+ and K^+ ions.[137]

α-Amylase has been isolated from Canadian red spring wheat by ion-exchange chromatography and complexation with glycogen.[209] Three multiple forms of the enzyme were separated by ion-exchange chromatography; they have similar molecular weights, activation energies, K_m values, and pH optima, but different p*I* values. The α-amylase from wheat has been purified by competitive affinity chromatography on glycogen covalently bound to macroporous agarose and elution with a dilute solution of glycogen.[210] A zymogram of the purified enzyme was obtained by placing the gel used for electrophoresis on top of a flat polyacrylamide gel containing starch, which was subsequently stained with a solution of iodine and potassium iodide: colourless areas showed where starch had been hydrolysed.

C.d. spectroscopy has been used to characterize a purified inhibitor of α-amylase

[202] M. Granger, B. Abadie, Y. Mazzei, and G. Marchis-Mouren, *F.E.B.S. Letters*, 1975, **50**, 276.
[203] J. F. Kennedy, *Stärke*, 1977, **29**, 114.
[204] M. Granger, B. Abadie, and G. Marchis-Mouren, *F.E.B.S. Letters*, 1975, **56**, 189.
[205] A. T. Romano and D. H. Strumeyer, *Biochim. Biophys. Acta*, 1976, **446**, 19.
[206] P. Kanagasuntheram and P. J. Randle, *Biochem. J.*, 1976, **160**, 547.
[207] I. M. Lehrner and G. M. Malacinski, *Comp. Biochem. Physiol.*, 1976, **53B**, 61.
[208] A. W. MacGregor, *Analyt. Biochem.*, 1977, **79**, 605.
[209] B. Marchylo, J. E. Kruger, and G. N. Irvine, *Cereal Chem.*, 1976, **53**, 157.
[210] R. Tkachuk, *F.E.B.S. Letters*, 1975, **52**, 66.

isolated from the kernels of hexaploid wheat.[211] The inhibition of other amylases was also examined.

The α-amylase from a wheat–rye hybrid [triticale (× *Triticosecale*)] has been purified by affinity chromatography on cyclohepta-amylose immobilized on a derivative of macroporous agarose.[212] Contaminating proteins were eluted with a sodium acetate buffer, whereafter α-amylase was eluted with a buffer containing cyclohepta-amylose. This chromatographic procedure gave a yield of 90% and ⩽180-fold purification.

The α-amylase (mol. wt. 5.0×10^4, p*I* 9.2) from a thermophilic bacterium (found in a hot spring in Japan) is more thermostable than α-amylases from mesophilic sources.[213] It contains two cysteine residues, but no disulphide linkages, and 20% of α-helix. The effects of pH, metal ions, and denaturing agents on the thermal stability of the enzyme were examined.[214] It is most stable at its isoelectric point and is stabilized by calcium ions: one of the bound Ca^{2+} ions could not be removed by electrodialysis unless the enzyme was first denatured.

Studies of the hydrolyses of cyclohexa-, cyclohepta-, and cyclo-octa-amyloses by Taka-amylase A (one of the α-amylases from *Aspergillus oryzae*) indicated that each cycloamylose binds to the same active site of the enzyme.[215] Since there is little difference in the respective ΔG, ΔH, and ΔS values for enzymic hydrolysis of these cycloamyloses, their binding modes appear to be similar. The extent of multiple attack on the cycloamyloses was not affected by temperature. A 4-phenylazobenzoyl derivative of Taka-amylase A has been used to investigate the active site of the enzyme.[216] *A. oryzae* α-amylase has a synergistic effect on the action of the glucoamylase from *A. awamori* var. *kawachi*.[217]

Two forms of α-amylase are present on the membrane of *Bacillus amyloliquefaciens*: one is loosely bound and the other is tightly bound, requiring the use of a detergent for its solubilization.[218] The loosely-bound form has an electrophoretic mobility similar to that of the tightly-bound form and, unlike the tightly-bound form, a sedimentation behaviour similar to that of the soluble α-amylase in the culture medium. A fraction containing six singly-branched heptaoses has been isolated following digestion of a β-limit dextrin with the liquefying α-amylase from *B. amyloliquefaciens*.[219] Each heptaose is composed of a 6^2-α-maltosylmaltotriose unit, to which either an α-maltosyl residue or two α-D-glucopyranosyl residues are attached at various points by (1 → 4)-linkages. The specificity of the enzyme was discussed in the light of this evidence.

Carbon dioxide stimulated the production of α-amylase by *B. subtilis* in batch cultures, the highest activity being obtained using air containing 6% (v/v) of carbon dioxide, although both the growth and the division of vegetative cells

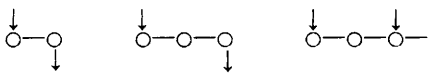
[211] T. Petrucci, A. Rab, M. Tomasi, and V. Silano, *Biochim. Biophys. Acta*, 1976, **420**, 288.
[212] M. P. Silvanovich and R. D. Hill, *Analyt. Biochem.*, 1976, **73**, 430.
[213] A. Hasegawa, M. Miwa, T. Oshima, and K. Imahori, *J. Biochem. (Japan)*, 1976, **79**, 35.
[214] A. Hasegawa and K. Imahori, *J. Biochem. (Japan)*, 1976, **79**, 469.
[215] N. Suetsugu, I. Fukuzawa, S. Koyoma, K. Takeo, and T. Kuge, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 2109.
[216] K. Omichi, S. Kasai, and Y. Matsushima, *J. Biochem. (Japan)*, 1975, **78**, 493.
[217] S. Ueda, R. Ohba, and S. Kano, *Stärke*, 1974, **26**, 374.
[218] L. Fernandez-Revira Rio and A. Arroyo-Begovich, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 161.
[219] K. Umeki and T. Yamamoto, *J. Biochem. (Japan)*, 1975, **78**, 889.

were inhibited when the concentration of carbon dioxide exceeded 3% (v/v).[220] Air containing 8% (v/v) of carbon dioxide increased (three-fold) the production of α-amylase by *B. subtilis* in chemostat cultures without affecting the growth of the cells.

The hydrolyses of phenyl α-maltoside and diverse substituted-phenyl α-maltosides by *B. subtilis* α-amylase have been examined under conditions that produce only maltose and the corresponding phenols, so that the anomeric configuration of the released disaccharide could be determined.[221] At the pH optimum (5.4) of the enzyme, maltose was released as the β-anomer (*cf.* a previous report), although some of the α-anomer was released at high pH values. The action of *B. subtilis* α-amylase on α- and β-maltose has been studied: plots of the initial rate against the substrate concentration gave sigmoidal curves in both cases.[222]

In connection with studies of the ability of α-amylases to catalyse the non-hydrolytic degradation of starch and glycogen, *B. subtilis* α-amylase has been shown to yield 4-nitrophenyl malto-oligosaccharides from starch and 4-nitrophenyl D-glucopyranosides.[223] Calculations based on the product yields indicated that glycosyl residues are transferred from starch to 4-nitrophenyl β-D-glucopyranoside with a frequency of 0.234 relative to all transfers to water. Less striking, but similar, findings were obtained with α-amylases from porcine pancreas and *Aspergillus oryzae*. The results clearly demonstrate that α-amylases are able to use HO-4 of certain D-glucopyranosides as an acceptor site.

Two highly-branched dextrin fractions, each containing 9—10 D-glucopyranosyl residues, have been isolated following digestion of the β-limit dextrin from rice starch with *B. subtilis* α-amylase.[224] Fragmentation analysis showed that each fraction contains four triply-branched dextrins that have 6^3-α-D-glucopyranosylmaltotriose or 6^2-α-D-glucopyranosylmaltose or both as linking units and a branching configuration represented by (7) or (8) or (9).



(7) (8) (9)

○ = α-D-glucopyranosyl residue, ↓ = (1 → 6)-α-D-glucosidic linkage
— = (1 → 4)-α-D-glucosidic linkage

C.d. spectroscopy has been used to study the conformational transitions of *B. subtilis* α-amylase in the pH range 2.75—12 in the absence or the presence of sodium dodecyl sulphate.[225] The effects of the detergent on the conformation of the enzyme were discussed.

Hybridization of a hyperderepressed strain of *Neurospora crassa* with a normal strain increased the amylase activity.[68]

Two amylases isolated from cultures of a *Streptomyces* sp. and a filamentous

[220] A. P. Ghandi and L. Kjaergaard, *Biotechnol. and Bioeng.*, 1975, **17**, 1109.
[221] T. Shibaoka, K. Ishikura, K. Hiromi, and T. Watanabe, *J. Biochem. (Japan)*, 1975, **77**, 1215.
[222] T. Shibaoka, K. Inatani, K. Hiromi, and T. Watanabe, *J. Biochem. (Japan)*, 1975, **77**, 965.
[223] M. Takeshita and E. J. Hehre, *Arch. Biochem. Biophys.*, 1975, **169**, 627.
[224] K. Umeki and T. Yamamoto, *J. Biochem. (Japan)*, 1975, **78**, 897.
[225] Y. Y. Su and B. Jirgensons, *Arch. Biochem. Biophys.*, 1977, **181**, 137.

fungus (27591 RP) caused both saccharification and dextrinization of starch.[226] The *Streptomyces* enzyme is most active at 40 °C and pH 5.9, whereas the other is most active at 54 °C and pH 4.0. The amylolytic activity of the *Streptomyces* enzyme towards wheat-meal dough is very much greater than that of the fungal enzyme. Conditions for maximal production of amylase by the thermophilic actinomycete *Thermomonospora viridis* have been reported.[227] Purification of the enzyme was also described. Traces of extracellular α-amylase are produced by *Trichoderma viride*.[228]

α-Amylase reacted with dextran cyclic imidocarbonate to give an active immobilized, water-soluble derivative.[229]

β-Amylases

The kinetics of the hydrolysis of starch by a mixture of β- and α-amylases in a membrane reactor have been examined.[192] Cross-linked amylose has been used to determine the β- and α-amylase activities of an amylolytic preparation (see Scheme 3).[189]

The β-amylase (mol. wt. 6.0×10^4) from defatted soybean meal has been purified by fractional precipitation, ion-exchange chromatography, and gel filtration.[230] Two homogeneous β-amylase components crystallized from ammonium sulphate solution; each component forms large single crystals that have a hexagonal lattice and unit-cell dimensions $a = 86.1$ and $c = 144.4$ Å. Soybean β-amylase (mol. wt. 5.7×10^4) is composed of 494 amino-acid residues.[231] No differences were found in the molecular weights and compositions of two components of β-amylase separated by ion-exchange chromatography. Modification of the sulphydryl groups produced a change in the molecular conformation. Some preliminary crystallographic data for crystals formed at pH 4 were obtained; the inactivation of β-amylase by various heavy-metal compounds, which were used for isomorphous replacement in the crystallographic investigation, was also studied.

β-Amylase has been reversibly immobilized, with retention of activity, by hydrophobic attachment to hexyl-substituted, epichlorohydrin-cross-linked agarose[232–234] and to octyl-substituted, epichlorohydrin-cross-linked agarose containing mercapto-groups in the side-chains.[232]

Amylo-1,6-D-glucosidases

A glycogen-debranching enzyme (amylo-1,6-D-glucosidase) from dogfish muscle has been purified by a procedure that takes advantage of its adsorption onto

[226] A. Belloc, J. C. Palla, and J. Verrier, *Ann. Technol. Agric.*, 1974, **23**, 141.
[227] M. E. Upton and W. M. Fogarty, *Appl. Environ. Microbiol.*, 1977, **33**, 59.
[228] J. A. Schellart, F. M. W. Visser, T. Zandstra, and W. J. Middelhoven, *J. Microbiol. Serol.*, 1976, **42**, 229.
[229] J. J. Marshall, *Carbohydrate Res.*, 1976, **49**, 389.
[230] Y. Morita, S. Aibara, H. Yamashita, F. Yagi, T. Suganuma, and K. Hiromi, *J. Biochem. (Japan)*, 1975, **77**, 343.
[231] Y. Morita, F. Yagi, S. Aibara, and H. Yamashita, *J. Biochem. (Japan)*, 1976, **79**, 591.
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[233] K. D. Caldwell, R. Axén, M. Bergwall, and J. Porath, *Biotechnol. and Bioeng.*, 1976, **18**, 1573.
[234] K. D. Caldwell, R. Axén, M. Bergwall, I. Olsson, and J. Porath, *Biotechnol. and Bioeng.*, 1976, **18**, 1605.

concanavalin A immobilized on macroporous agarose when glycogen is present.[235] Similar molecular weights ($1.62 \pm 0.05 \times 10^5$) were obtained for this enzyme by sedimentation equilibriation and gel electrophoresis, but the low (6.5*S*) sedimentation constant suggested that the molecule is asymmetric. The enzyme appears to consist of a single polypeptide chain with no multiple repeating sequences.

Arabinanases

The occurrence, purification, properties, and modes of action of L-arabinanases have been reviewed.[4]

exo-Cellobiohydrolases

Four distinct *exo*-cellobiohydrolases (1,4-β-D-glucan cellobiohydrolases) have been isolated from cultures of *Trichoderma viride*.[236] They have similar u.v. spectra, amino-acid and amino-sugar compositions, thermal stabilities, molecular weights, specific activities, and CO_2H-terminal residues, but have different contents and compositions of hexoses.

Cellulases

The properties and modes of action of cellulases[237] and technological aspects of the enzymic depolymerization of cellulosic materials[238] have been discussed.

New sensitive assays for cellulase activity use as substrates modified carboxymethylcelluloses containing either 2,4,6-trinitrophenyl groups (for spectrophotometric assay) or fluorescamine groups (for fluorometric assay).[239] A simple and rapid staining procedure for qualitative and quantitative determinations of plant (*Citrus sinensis*) and fungal (*T. viride*) cellulases is based on the incorporation of carboxymethylcellulose into polyacrylamide gels.[240] After electrophoresis of the crude extract, the gel is incubated in a phosphate buffer, acidified, and treated with iodide–iodine: no colour develops in areas containing cellulase activity.

In the presence of high concentrations of auxin, etiolated pea (*Pisum sativum*) epicotyls synthesized a buffer-soluble cellulase (pH optimum 5.5) and a salt-soluble cellulase (pH optimum 7.0).[241] The buffer-soluble enzyme gave two bands, and the salt-soluble enzyme several bands, on polyacrylamide gel electrophoresis. It was concluded that ethylene- and auxin-treated pea epicotyls synthesize different forms of cellulase. The kinetics and substrate specificities of cellulases from auxin-treated pea epicotyls have been investigated.[242]

Aspergillus sydowi released extracellular cellulolytic enzymes during growth in liquid culture media containing a soluble or an insoluble cellulosic source of carbon.[243] Gel filtration and ion-exchange chromatography gave three cellulases

[235] J. U. Becker, T. J. Long, and E. H. Fischer, *Biochemistry*, 1977, **16**, 291.
[236] E. K. Gum and R. D. Brown, *Biochim. Biophys. Acta*, 1977, **192**, 225.
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[239] J. S. Huang and J. Tang, *Analyt. Biochem.*, 1976, **73**, 369.
[240] R. Goren and M. Huberman, *Analyt. Biochem.*, 1976, **75**, 1.
[241] T. E. Ferrari and P. G. Arnison, *Plant Physiol.*, 1974, **54**, 487.
[242] G. B. Fincher, Y. S. Wong, and G. A. Maclachlan, *Proc. Austral. Biochem. Soc.*, 1977, **10**, 3.
[243] P. O. Olutiola and O. O. Cole, *Plant Physiol.*, 1977, **39**, 243.

of high molecular weight, one of which resembles C_1 cellulase. Synergistic effects were observed among the enzymes.

The properties of a cellulolytic activity from soil, which hydrolyses cellulose powder, newspaper, and paper towels, *etc.*, have been compared with those of *Cellulomonas flavigena*.[244] Possible uses of the activity in disposing of refuse were discussed.

Cellvibrio gilvus synthesizes a cellulase when growing in media containing either cellulose or carboxymethylcellulose, but no cellulase is synthesized in the presence of either D-glucose or cellobiose.[245] Repeated additions of small amounts of D-glucose to the culture media prevented the synthesis of the cellulase in the presence of cellulose. The induction and repression of the cellulase were discussed.

The effects of temperature on the synthesis of a cellulase (*endo*-β-1,4-D-glucanase) by cultures of the compost fungus *Chaetomium thermophile* have been investigated.[246] Although this fungus grows on a number of carbohydrates, synthesis of the cellulase activity was induced only in the presence of cellulose or D-xylan. The enzyme exhibited optimal activity at 58 °C and is most stable at pH 6.0.

The synthesis of extracellular cellulase activity by three strains of *Clostridium thermocellum* growing on cellulose has been shown to be directly related to the degradation of cellulose and to the growth of the bacteria.[247] Cellulase was not detected when the cells grow on cellobiose. The activities of the *exo*-D-glucanase (pH optimum 5.2, temperature optimum 65 °C) and *endo*-D-glucanase (pH optimum 5.4, temperature optimum 64 °C) were compared. D-Glucose and cellobiose were released when the crude cellulase acted on cellulose, cellotetraose, and cellotriose *in vitro*.

An (*endo*-)cellulase from culture filtrates of *Irpex lacteus* (*Polyporus tulipiferae*) showed a less-random hydrolysis of cellulose after extensive purification.[248] This homogeneous component (mol. wt. 5.6×10^4) contains 2.2% of carbohydrate consisting principally of residues of D-glucose and D-mannose. Another homogeneous (*endo*-)cellulase (carboxymethylcellulase) obtained from culture filtrates of *I. lacteus* also exhibited xylanase activity.[249] Both activities are always present in the same ratio, regardless of the purification procedure used, and appear to be present in the same species. Investigations of the substrate specificities of three (*endo*-)cellulase components isolated from culture filtrates of *I. lacteus* suggested that cotton and other celluloses are degraded mainly by synergistic action of the components.[250]

The fungus *Papulaspora thermophila* has been shown to degrade filter paper and soluble carboxymethylcellulose.[251] Synthesis of the cellulolytic enzymes could be induced by cellulosic material added to the growth medium. A cytosolic β-D-glucosidase from *Pseudomonas fluorescens* possesses some *exo*-cellulase activity.[144]

[244] A. Kaufmann, J. Egan, P. Doleac, C. Gainer, D. Wittich, and A. Glann, *J. Gen. Microbiol.*, 1976, **94**, 405.
[245] C. Breuil and D. J. Kushner, *Canad. J. Microbiol.*, 1976, **22**, 1776.
[246] J. Eriksen and J. Goksöyr, *Arch. Mikrobiol.*, 1976, **110**, 233.
[247] T. K. Ng, P. J. Weimer, and J. G. Zeikus, *Arch. Mikrobiol.*, 1977, **114**, 1.
[248] T. Kanda, K. Wakabayashi, and K. Nisizawa, *J. Biochem. (Japan)*, 1976, **79**, 977.
[249] T. Kanda, K. Wakabayashi, and K. Nisizawa, *J. Biochem. (Japan)*, 1976, **79**, 989.
[250] T. Kanda, K. Wakabayashi, and K. Nisizawa, *J. Biochem. (Japan)*, 1976, **79**, 997.
[251] E. S. Chapman, E. Evans, M. C. Jacobelli, and A. A. Logan, *Mycologia*, 1975, **67**, 608.

Rhizoctonia lamellifera growing in a liquid culture containing soluble or insoluble celluloses released cellulolytic enzymes into the medium.[252] Gel filtration and ion-exchange chromatography separated the enzymes into three components of high molecular weight and four components of low molecular weight, one of which resembles C_1 cellulase. When the components are combined, they release more reducing saccharides from cellulosic substrates than when they are used singly.

Two extracellular cellulases that act on carboxymethylcellulose have been isolated from the supernatant fluids of cultures of *Sporocytophaga myxococcoides* by gel-filtration and ion-exchange chromatography.[253] More cellulase II (mol. wt. 5.2×10^4 by gel electrophoresis, p*I* 4.75, pH optimum 5.5—7.5) was present than cellulase I (mol. wt. 4.6×10^4, p*I* 7.5, pH optimum 6.5—7.5). Both cellulases have very low contents of carbohydrate, possibly as impurities, and similar amino-acid compositions, and are *endo*-enzymes. A cell-bound cellulase was also partly purified.

Optimal conditions for the synthesis of cellulase by *Trichoderma viride* growing on cellulose have been reported.[254] The level of cellulase synthesized by *T. viride* could be doubled by controlling the pH of a medium containing 2% of cellulose.[255] The continuous production of cellulase by *T. viride* in a single-stage, stirred tank-reactor has been investigated.[256] Compared to the time taken for a single culture of *T. viride* to produce its maximum yield of cellulase activity, that taken by a mixed culture of *T. viride* and either *Saccharomyces cerevisiae* or *Candida utilis* (growing on alkali-treated barley straw) is reduced by several days.[257] Sophorose, a cellulase inducer, was rapidly catabolized to carbon dioxide and water by *T. viride*; little sophorose was needed to induce the synthesis of cellulase, although repeated additions of small amounts of sophorose induced the synthesis of more cellulase activity than did a single equivalent dose.[258] The catabolic pathway seems to have a higher capacity and a lower affinity for sophorose than the inductive pathway.

A plate-clearing assay for screening high-producing cellulase mutants of *T. viride* uses either Rose Bengal or oxgall (to limit the size of the colony) and tributyl-2,4-dichlorobenzylphosphonium chloride (Phosphon D) (to aid in the detection of cellulase) in combination with acid-swollen cellulose (as the substrate) on agar plates.[259] The assay was used to isolate constitutive cellulase mutants of *T. viride* and to determine the concentrations of D-glucose and glycerol that inhibit the synthesis of cellulase by the wild-type strains.

Ion-exchange chromatography of an extract of Cellulase–Onozuka (a commercial *T. viride* preparation) gave three cellulase components, one (mol. wt. 5.3×10^4) of which exhibited both β-D-glucosidase and *exo*-cellulase activities.[145] The

[252] P. O. Olutiola, *J. Gen. Microbiol.*, 1976, **97**, 251.
[253] K. Osmundsvag and J. Goksöyr, *European J. Biochem.*, 1975, **57**, 405.
[254] N. Peitersen, *Biotechnol. and Bioeng.*, 1977, **19**, 337.
[255] D. Sternberg, *Biotechnol. and Bioeng.*, 1976, **18**, 1751.
[256] G. Mitra and C. R. Wilke, *Biotechnol. and Bioeng.*, 1975, **17**, 1.
[257] N. Peitersen, *Biotechnol. and Bioeng.*, 1975, **17**, 1291.
[258] J. R. Loewenberg and C. M. Chapman, *Arch. Mikrobiol.*, 1977, **113**, 61.
[259] B. S. Montencourt and D. E. Eveleigh, *Appl. Environ. Microbiol.*, 1977, **33**, 178.

hydrolysis of cellulose and cello-oligosaccharides by this enzyme was examined. The enzyme also degraded D-xylan by an *exo*-mechanism.

A β-1,4-D-glucan cellobiohydrolase (mol. wt. 4.18—4.20 × 10^4) from *T. viride* contains a high proportion of acidic amino-acids and a low proportion of basic amino-acids, eight disulphide bridges, no free sulphydryl groups, and 9.2% of carbohydrate consisting of D-mannose (16), D-galactose (2), and D-glucose (6 residues per molecule).[260] The enzyme (pH optimum 4.8) degraded microcrystalline cellulose (~ 80%) within 72 h, provided that the reaction products (mainly cellobiose) were continuously removed by ultrafiltration. A model has been devised to represent the hydrolysis of cellulose by the enzyme system of *T. viride*, based on the assumption that non-competitive inhibition by cellobiose dominates the reaction kinetics.[261] The ability of a purified cellulase from *T. viride* to produce cellobiose from fibrous α-cellulose has been examined.[262] Kinetic data obtained for inhibition by cellobiose are compatible with competitive product inhibition, rather than with anti- or non-competitive product inhibition.

An (*endo*-)cellulase component—which depolymerizes cellulose randomly—from a commercial preparation of *T. viride* cellulase has been subjected to limited proteolysis with a protease from the same fungus.[263] The degraded products were still able to hydrolyse cellulosic substrates, and limited proteolysis of cellulase components may be responsible, in part, for the multiple forms found *in vivo*.

A batch reactor for the degradation of amorphous celluloses by *T. viride* cellulase gave high conversions; at some stage the enzyme is immobilized by adsorption onto the substrate.[264]

Primary sludges can be rendered filterable by treatment with *T. viride* cellulase.[265] The use of sludges as growth media for *T. viride* producing cellulase was also investigated.

Chitinases (*endo*-β-D-Acetamidodeoxyglucanases)

The chitinolytic activity in extracts of the gastric mucosa from a prosimian mammal (*Perodicticus potto*) has been purified by chromatography on colloidal chitin and by gel chromatography.[266] The purified enzyme appears to be a specific chitinase. Procedures have been described for the isolation of chitinases from the pancreas and gastric mucosa of frogs (*Rana temporaria temporaria*).[267] Precautions were taken to avoid denaturing the enzyme, whose behaviour on polyacrylamide gel electrophoresis was examined.

The synthesis of chitinase by an *Arthrobacter* sp. that degrades *Fusarium roseum* cells[268] and the properties of two chitinases from *Streptomyces orientalis*[269] have been examined.

[260] L. E. R. Berghem, L. G. Pettersson, and U.-B. A. Fredriksson, *European J. Biochem.*, 1975, **53**, 55.
[261] J. A. Howell and J. D. Stuck, *Biotechnol. and Bioeng.*, 1975, **17**, 873.
[262] R. J. Maguire, *Canad. J. Biochem.*, 1977, **55**, 644.
[263] M. Nakayama, Y. Tomita, H. Suzuki, and K. Nisizawa, *J. Biochem. (Japan)*, 1976, **79**, 955.
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[267] G. Dandrifosse, *Biochimie*, 1975, **57**, 829.
[268] R. F. Morrissey, E. P. Dugan, and J. S. Koths, *Soil Biol. Biochem.*, 1976, **8**, 23.
[269] Y. Tominaga and Y. Tsujisaka, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 2325.

Chondroitin Sulphate Lyases AC

The chondroitin sulphate lyase AC (mol. wt. 7.5—8.0 × 10^4) from *Arthrobacter aurescens* is a glycoprotein containing residues of D-mannose, D-glucose, 2-amino-2-deoxy-D-glucose, and D-glucuronic acid (molar proportions 3:5:4:2).[270] The amounts of α-helix and β-structure present in the protein chain were estimated to be 16 and 25%, respectively, by o.r.d. and c.d. spectroscopy, *etc.*

Dextranases

New sensitive assays for dextranase activity are based on the use of Sephadex substituted with either 2,4,6-trinitrophenyl groups (for spectrophotometric assay) or fluorescamine groups (for fluorometric assay).[239] 3,5-Dinitrosalicylic acid has been used in automated assays to determine the reducing sugars released on hydrolysis of the appropriate substrates with soluble or insoluble dextranases and other polysaccharide hydrolases.[9]

A dextran–dextranase system has been used to examine the synergistic effect of *exo-* and *endo*-enzymes acting jointly on polymeric substrates.[271] An *endo*-dextranase (from *Penicillium funiculosum*) and an *exo*-dextranase (from *Streptococcus mitis*) produced D-glucose more rapidly from dextrans than the summed effects of the individual enzymes.

The prevention of dental caries in rats by a dextranase from a strain of *Spiearia violacea* has been studied.[272]

Active immobilized derivatives of dextranase have been obtained by chelation with hydrous zirconium(IV) oxide[147] and porous titanium(IV) oxide.[9]

D-Galactanases

The occurrence, purification, properties, and modes of action of D-galactanases have been reviewed.[4]

Enzymes that hydrolyse α-D-galactopyranosyl residues in saccharides have been found in *Trichomonas foetus*.[116] Three of the enzymes are specific for (1 → 3)-, (1 → 4)-, and (1 → 6)-linked α-D-galactopyranosyl residues.

endo-β-1,3-D-Glucanases

A new, improved assay of *endo*-β-1,3-D-glucanases, which combines the simplicity of a reductiometric method with the specificity of a viscosimetric method, measures the amount of blue dye released from a Cibachron Blue derivative of pachyman.[273]

Purification of an *endo*-β-1,3-D-glucanase from a green-malt extract gave a fraction containing two protein components, one of which is enzymically active.[274] This enzyme (p*I* 9.8) was stimulated by sodium chloride and exhibited optimal activity towards laminarin and carboxymethylpachyman at pH 5.0 and 5.8,

[270] K. Hiyama and S. Okada, *J. Biochem. (Japan)*, 1975, **78**, 1183.
[271] M. A. Wheatley and M. Moo-Young, *Biotechnol. and Bioeng.*, 1977, **19**, 219.
[272] S. Hamada, T. Ooshima, N. Masuda, J. Mizuno, and S. Sobue, *Jap. J. Microbiol.*, 1976, **20**, 321.
[273] W. A. Philpott and J. M. Chapman, *Analyt. Biochem.*, 1977, **79**, 257.
[274] G. M. Ballance and W. O. S. Meredith, *J. Inst. Brewing*, 1976, **82**, 64.

respectively. The synthesis of polysaccharide hydrolases, including *endo*-β-1,3-D-glucanase, by germinating rye has been studied.[275]

An enzyme (zymolyase) that degrades viable yeast cells has been isolated from culture fluids of *Arthrobacter luteus* and purified (75-fold) by procedures that included fractionation with ammonium sulphate and chromatography on Biogel CM-100.[276] The purified enzyme (mol. wt. 2.1×10^4), which appeared to be homogeneous on examination by electrophoresis and ultracentrifugation, degraded viable yeast cells in the absence of other enzymes or additives. Studies of the hydrolyses of (1 → 3)-β-D-glucans (*e.g.* pachyman, curdlan, *etc.*) revealed that zymolyase is an *endo*-β-1,3-glucanase that releases laminaripentaose as the smallest fragment. With (1 → 3)-β-D-glucans of high molecular weight that show strong molecular aggregation (*e.g.* yeast D-glucan), it behaves as an *exo*-enzyme, releasing laminaripentaose. Zymolyase exhibited greater activity towards insoluble D-glucans than towards soluble D-glucans.

An *Acinetobacter* sp. growing on cells of baker's yeast as the only source of carbon secreted an *endo*-β-1,3-D-glucanase activity.[163]

endo-β-1,4-D-Glucanases

The synthesis of polysaccharide hydrolases, including *endo*-β-1,4-D-glucanases, by germinating rye has been examined.[275]

Among other activities, glucanase GV (a commercial enzyme preparation of fungal origin) contains an *endo*-β-1,4-D-glucanase, which could be separated from an endogenous *exo*-β-1,3-D-glucanase by gel filtration.[277]

endo-β-1,6-D-Glucanases

An *Acinetobacter* sp. growing on cells of baker's yeast secreted, among others, an *endo*-β-1,6-D-glucanase activity.[163]

exo-β-1,3-D-Glucanases

Among other activities, glucanase GV contains an *exo*-β-1,3-D-glucanase which has a relatively high specificity for (1 → 3)-β-D-glucosidic linkages, but which does not attack lichenin.[277]

A purified *exo*-β-1,3-D-glucanase obtained from culture fluids of the yeast *Candida utilis* contains a high proportion of acidic amino-acids and 20% of carbohydrate, is more reactive towards laminarin than towards 4-nitrophenyl β-D-glucopyranoside and the (1 → 3)-β-D-glucans in yeast cell walls, and is strongly inhibited by lactones and some heavy-metal ions.[278]

An *exo*-β-1,3-D-glucanase (mol. wt. 4.3×10^4) has been isolated from culture fluids of a *Fungi imperfecti* by fractional precipitation, ion-exchange chromatography, gel filtration, and affinity chromatography on powdered pachyman.[279] This enzyme removes single D-glucopyranosyl residues from the non-reducing end of (1 → 3)-β-D-glucans, with inversion of the anomeric configuration, and is

[275] G. M. Ballance and D. J. Manners, *Biochem. Soc. Trans.*, 1975, **3**, 989.
[276] K. Kitamura, T. Kaneko, and Y. Yamamoto, *J. Gen. Appl. Microbiol.*, 1974, **20**, 323.
[277] D. J. Manners, J. R. Stark, G. Wilson, and J. Y. Brodie, *Carbohydrate Res.*, 1976, **49**, 383.
[278] T. G. Villa, V. Notario, T. Benítez, and J. R. Villanueva, *Canad. J. Biochem.*, 1976, **54**, 927.
[279] S. Nagasaki, K. Saito, and S. Yamamoto, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 493.

active against laminarin and hexa-, penta-, and tetra-oses derived from laminarin (K_m values 0.066×10^3, 0.83×10^{-3}, 2.30×10^{-3}, and 18.60×10^{-3} mol l^{-1}, respectively).

D-Glucanases (Miscellaneous)

Following the removal of α-amylase from normal human urine by adsorption onto starch, three fractions of a D-glucanase, which could be distinguished from glucoamylase and α-D-glucosidase, were separated by ion-exchange chromatography and gel filtration.[280] All the fractions hydrolysed starch and glycogen (releasing only D-glucose) and maltose.

Studies of the effects of nojirimycin (an inhibitor of *exo*-β-D-glucanases) on excised *Avena sativa* coleoptiles and pea-stem (*Pisum sativum*) segments have indicated that *exo*-β-D-glucanases are involved in cell-wall extension induced by indole-3-acetic acid and in modification of the non-cellulosic D-glucan in the cell wall.[281]

The hydrolysis of pachyman [a (1 → 3)-β-D-glucan] by culture filtrates of more than 700 strains of micro-organisms has been examined.[282] Several commercial and gastropode enzyme preparations were also tested for β-1,3-D-glucanase activity. A strain of *Streptomyces*, which is related to *S. arabicus*, synthesizes an appreciable amount of β-1,3-D-glucanase activity.

Commercially available enzymes used in the preparation of fungal cell walls have been examined for α- and β-D-glucanases, including β-1,3-D-glucanase.[15]

The production of β-D-glucanase activity during the cell-cycle of *Bacillus subtilis* has been investigated.[283]

When growing in a mineral medium with either yeast cell walls or yeast D-glucan as the only source of carbon, *Bacillus circulans* synthesized a β-1,3-D-glucanase and wall-lytic and non-lytic β-1,6-D-glucanases.[284] A homogeneous lytic β-1,6-D-glucanase was obtained from the culture media by adsorption onto insoluble yeast D-glucan. This enzyme (pH optimum 6.0, mol. wt. 5.4×10^4) hydrolysed pustulan (K_m 0.29 mol l^{-1}) to gentio-oligosaccharides and D-glucose, and gave small amounts of gentiobiose with laminarin and pachyman, but no β-(1 → 3)-linked D-gluco-oligosaccharides were found. The lytic β-1,6-D-glucanase has a specific activity lower than that of the non-lytic β-1,6-D-glucanase towards souble pustulan.

Rapid purification of the β-1,3-D-glucanase in culture fluids of the yeast *Candida utilis* can be achieved by affinity chromatography on a purified (1 → 3)-β-D-glucan.[285]

An enzyme (pH optimum 5.6) that debranches branched D-glucans has been isolated from *E. coli* by ion-exchange chromatography.[286] It hydrolysed the

[280] N. Minamiura, H. Chiura, K. Tsujino, and T. Yamamoto, *J. Biochem. (Japan)*, 1975, **77**, 1015.
[281] D. J. Nevins, *Plant Cell Physiol.*, 1975, **16**, 347.
[282] Y. Shibata and T. Fukimbara, *J. Ferm. Technol.*, 1974, **52**, 210.
[283] R. Borriss, *Z. allgem. Mikrobiol.*, 1976, **16**, 475.
[284] F. M. Rombouts and H. J. Phaff, *European J. Biochem.*, 1976, **63**, 109.
[285] T. G. Villa, V. Notario, and J. R. Villanueva, *Appl. Environ. Microbiol.*, 1976, **32**, 185.
[286] R. Jeanningros, N. Creuzet-Sigal, C. Frixon, and J. Cattaneo, *Biochim. Biophys. Acta*, 1976, **438**, 186.

(1 → 6)-α-D-glucosidic linkages in amylopectin and β-limit dextrins derived from glycogen, but exhibited little or no activity towards glycogen and pullulan. This enzyme does not appear to be an isoamylase.

The properties of multiple forms of a lytic D-glucanase from a strain of *Flavobacterium dormitator* have been studied.[287] The principal component acted only on such (1 → 3)-β-D-glucans as pachyman, yeast D-glucan, and laminarin, releasing mainly a pentasaccharide from laminarin. Bacterial cells became osmotically sensitive following treatment with this enzyme.

Papulaspora thermophila produced extracellular amylolytic enzymes, even in the absence of starch.[251] The yields of these enzymes under various conditions of growth, *etc.*, were examined.

Glucoamylases

Aspects of the affinity chromatography of glucoamylase on glycose-substituted agaroses have been studied.[35] The kinetics of a multiple enzyme system (consisting of glucoamylase, D-glucose oxidase, and D-gluconolactonase) used to convert maltose into D-gluconic acid have been investigated.[288]

Porcine-spleen 'acid' α-D-glucosidase has been shown to possess glucoamylase activity.[134]

The glucoamylase system of black koji mould (*Aspergillus awamori* var. *kawachi*) has been fractionated into four glucoamylases by adsorption onto corn starch and chromatography on DEAE-cellulose.[217] One of the fractions (glucoamylase I) had strong debranching activity and was very active towards raw starch. The hydrolysis of raw waxy corn starch by glucoamylase I was accelerated in the presence of either α-amylase or isoamylase, whereas that of raw non-waxy corn starch was accelerated more in the presence of α-amylase than in the presence of isoamylase. Another fraction (glucoamylase II) had weak debranching activity; the limited hydrolysis of raw waxy corn starch by this enzyme was enhanced in the presence of isoamylase, whereas it was not affected by α-amylase.

Photo-oxidation of the glucoamylase I from *Aspergillus niger* in the presence of either Methylene Blue or Rose Bengal resulted in a pH-dependent loss of enzymic activity, owing to the destruction of tryptophanyl residues.[289] Maltose (a substrate for the enzyme) protected the enzymic activity and tryptophanyl residues from photo-degradation. The tryptophanyl residues of glucoamylase I, which had been inactivated by reaction of its carboxylic acid residues with glycine methyl ester, were also substantially protected by maltose. It was concluded that the active centre of glucoamylase I is a cleft lined with tryptophanyl residues that participate in the binding of substrates, and that one or more carboxylic acid residues are involved in bond cleavage.

The properties of a glucoamylase activity isolated from cultures of *Corticium rolfsii* growing in the presence of sucrose have been investigated.[290] The enzyme is stable to acid.

The results of mixed (D-glucono-1,5-lactone) and competitive (phenyl α-D-

[287] H. Mori, S. Yamamoto, and S. Nagasaki, *Agric. and Biol. Chem. (Japan)*, 1977, **41**, 611.
[288] Y. K. Cho and J. E. Bailey, *Biotechnol. and Bioeng.*, 1977, **19**, 185.
[289] M. E. Jolley and C. J. Gray, *Carbohydrate Res.*, 1976, **49**, 361.
[290] A. Kaji, M. Sato, M. Kobayashi, and T. Murao, *J. Agric. Chem. Soc. Japan*, 1976, **50**, 509.

glucopyranoside) inhibitions of the hydrolysis of maltodextrin by *Rhizopus niveus* glucoamylase are consistent with a mechanism in which the inhibitor binds to subsite 1 or 2, respectively.[291] U.v. difference spectra have been obtained for *R. niveus* glucoamylase in the presence of the inhibitors D-glucose, D-glucono-1,5-lactone, methyl β-D-glucopyranoside, cellobiose, and cyclohexa- and cyclohepta-amyloses.[292] The values obtained for the dissociation constants of complexes between the enzyme and maltose or D-glucose or D-glucono-1,5-lactone (1.2, 51, and 1.5 mmol l^{-1}, respectively) are in good agreement with the values of K_m or K_i derived from steady-state kinetics. The spectroscopic data suggest that maltose and D-glucono-1,5-lactone are bound at subsite 1, where a tryptophanyl residue is located and where the non-reducing end of the substrate is normally bound in a productive complex. D-Glucono-1,5-lactone (a transition-state analogue) has been found to quench some of the protein fluorescence of the glucoamylase from *R. niveus*.[293] Stopped-flow methods indicated that this quenching occurs in two steps, *viz.* a rapid bimolecular association followed by a slow unimolecular isomerization (to conformational isomers of the enzyme–D-glucono-1,5-lactone complex)—the latter process is accompanied by a change in the fluorescence intensity.

The degradation of starch has been studied with a strain of the fungus *Trichoderma viride* that utilizes starch or dextrins but not maltose.[228] The amylolytic enzyme system is extracellular and consists principally of glucoamylase; its synthesis is completely inhibited by D-glucose and glutamic acid.[294] During growth of the fungus on starch, the dextrinizing and saccharifying activities are synthesized at different rates, indicating that, at least, two distinct enzymes are present. Control of the synthesis of the dextrinizing activity was discussed.

The glucoamylase from *T. viride* has a molecular weight of 7.5×10^4 (by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate).[295] The K_m and V_{max} values were determined for its action on glycogen, amylose, amylopectin, soluble starch, and maltose. The use of pure α- and β-D-glucopyranoses, under conditions which limit their mutarotation, showed that the transglycosylation reactions catalysed by glucoamylase require the donor to have a specific anomeric configuration.[296] Thus, a highly purified glucoamylase from *T. viride* catalysed the synthesis of maltose and isomaltose from β-D-glucopyranose only.

Glucoamylase has been immobilized, with retention of activity, by reaction with cellulose beads treated with tolylene 2,4-di-isocyanate, [297] or with an activated derivative of collagen,[298, 299] or with diazotized 1,4-diaminobenzene in various forms,[300] by glutaraldehyde-coupling to aminoalkylsilyl glass,[301] by glutaralde-

[291] M. Ohnishi, T. Yamashita, and K. Hiromi, *J. Biochem. (Japan)*, 1976, **79**, 1007.
[292] M. Ohnishi, H. Kegai, and K. Hiromi, *J. Biochem. (Japan)*, 1975, **77**, 695.
[293] M. Ohnishi, T. Yamashita, and K. Hiromi, *J. Biochem. (Japan)*, 1977, **81**, 99.
[294] J. A. Schellart, E. J. F. van Arem, M. A. J. S. van Boekl, and W. J. Middelhoven, *J. Microbiol. Serol.*, 1976, **42**, 239.
[295] G. Okada, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 143.
[296] G. Okada, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 147.
[297] L. F. Chen and G. T. Tsao, *Biotechnol. and Bioeng.*, 1976, **18**, 1507.
[298] J.-M. Brillouet, P. R. Coulet, and D. C. Gautheron, *Biotechnol. and Bioeng.*, 1976, **18**, 1821.
[299] J.-M. Brillouet, P. R. Coulet, and D. C. Gautheron, *Biotechnol. and Bioeng.*, 1977, **19**, 125.
[300] M. Kierstan and C. Bucke, *Biotechnol. and Bioeng.*, 1977, **19**, 387.
[301] D. R. Marsh and G. T. Tsao, *Biotechnol. and Bioeng.*, 1976, **19**, 349.

hyde-coupling to, and glutaraldehyde cross-linking on, hornblende and activated hornblendes,[302] by attachment to a poly(acrylonitrile) resin by an amidination reaction,[303] by adsorption onto acid-activated molecular sieves or alumina[304] or hexyl-substituted, epichorohydrin-cross-linked agarose,[305] and by entrapment in gels formed from dimethylacrylamide or 2-hydroxyethyl acrylate.[75] Glucoamylase has been reversibly immobilized by hydrophobic attachment to hexyl-substituted, epichlorohydrin-cross-linked agarose or octyl-substituted, epichlorohydrin-cross-linked agarose containing mercapto-groups on the side-chains.[232]

endo-β-1,4-D-Glucuronanases

An enzyme from human placenta that degrades heparan sulphate has been partially purified.[306] This enzyme, which appears to be an *endo*-D-glucuronanase, released oligosaccharides from heparan sulphate and is most active at pH values < 6.5.

Glycanases (Miscellaneous)

The lysis of intact yeast cells and isolated cell walls by the inducible enzyme system of an *Arthrobacter* species has been investigated.[307]

An enzyme that degrades the cell walls of red yeast has been isolated from the soil fungus *Penicillium lilacinum* by ion-exchange chromatography and gel filtration.[308] The enzyme is most active at pH 4.0—4.5 and 50°C, is stable between pH 3.5 and 7.0, and has no action on *Saccharomyces* yeasts.

Hemicellulases

The synthesis of extracellular hemicellulases by the fungus *Cephalosporium sacchari* has been studied.[309] One of two hemicellulases (mol. wts. 1.07×10^4 and 9.55×10^3, p*I* values 9.40 and 6.0, respectively) obtained by precipitation with ammonium sulphate and isoelectric focusing hydrolysed hemicellulose B to oligosaccharides without releasing monosaccharides.

Heparan Sulphate Hydrolases

An enzyme from human placenta that specifically degrades heparan sulphate appears to be an *endo*-D-glucuronanase.[306]

Hyaluronate Lyases

The synthesis of hyaluronate lyase by a group A *Streptococcus* type 4 was enhanced by an infusion of trypsinized veal in the presence of oligosaccharides derived from hyaluronic acid.[310] The purified enzyme (mol. wt. $5—7 \times 10^4$, pH optimum 6.0) gave a single band on polyacrylamide gel electrophoresis in the presence of

[302] D. B. Johnson and M. Costelloe, *Biotechnol. and Bioeng.*, 1976, **18**, 421.
[303] T. Handa, S. Goto, T. Shinoda, T. Yoda, and S. Odawara, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 124.
[304] B. Solomon and Y. Levin, *Biotechnol. and Bioeng.*, 1975, **17**, 1323.
[305] K. D. Caldwell, R. Axén, M. Bergwall, and J. Porath, *Biotechnol. and Bioeng.*, 1976, **18**, 1589.
[306] U. Klein and K. von Figura, *Biochem. Biophys. Res. Comm.*, 1976, **73**, 569.
[307] M. Vršanská, Z. Krátký, and P. Biely, *Z. allgem. Mikrobiol.*, 1977, **17**, 391.
[308] M. Arai, R. Yamamoto, and S. Murao, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 27.
[309] G. N. Richards and T. Shambe, *Carbohydrate Res.*, 1976, **49**, 371.
[310] J. Hill, *Infection and Immunity*, 1976, **14**, 726.

sodium dodecyl sulphate. Inactivation of the enzyme could be prevented by reducing agents.

Hyaluronidases

A spectrophotometric assay of hyaluronidase activity is based on binding a carbocyanine dye (λ_{max} 640 nm) to hyaluronic acid, which results in a shift of λ_{max} in proportion to the amount of hyaluronic acid remaining.[311]

The hyaluronidase (pH optimum 3.9, p*I* 5.2, mol. wt. 7×10^4) obtained from human placenta, by gel filtration and ion-exchange chromatography, is a typical lysosomal enzyme that also depolymerizes chondroitin and chondroitin 4- and 6-sulphates.[312]

The level of hyaluronidase activity has been monitored in the sera of women during pregnancy.[313]

Inulinases

Inulinase has been converted into an active immobilized form by entrapment in gels of calcium alginate.[300]

Isoamylases

When growing under appropriate conditions, *Bacillus amyloliquefaciens* synthesizes a polysaccharide hydrolase (isoamylase) that selectively cleaves D-glucosidic linkages in branched α-D-glucans.[314] This enzyme appears to assist α-amylase in depolymerizing branched α-D-glucans.

An isoamylase from a *Pseudomonas* sp. has a synergistic effect on the glucoamylase of *Aspergillus awamori* var. *kawachi*.[217]

Laminarinases

Comparison of the properties of the β-1,3-D-glucanases (laminarinases) from *Aspergillus nidulans*, a *Basidiomycetes* sp., and *Trichoderma viride* showed that the enzyme from *A. nidulans* differs in its action on laminarin.[315]

Two β-1,3-D-glucanases that degrade yeast cell walls have been isolated from culture media of *Bacillus circulans* by adsorption onto a yeast D-glucan.[316] One of the enzymes of mol. wt. 4×10^4 hydrolysed laminarin (K_m 0.105 mol l^{-1}, pH optimum 5.5; *cf.* the pH optimum of 6.5 for the other enzyme) to laminaribiose and D-glucose, and had a synergistic effect on a lytic β-1,6-D-glucanase from the same bacterium.

Limit Dextrinases

The levels of limit dextrinase in germinating rye have been monitored.[275]

A carbohydrase-free limit dextrinase from malted sorghum flour readily

[311] L. C. Benchetrit, S. L. Pahoja, E. D. Gray, and R. D. Edstrom, *Analyt. Biochem.*, 1977, **79**, 431.
[312] M. Yamaoa, E. Hasegawa, and M. Kanamori, *J. Biochem. (Japan)*, 1977, **81**, 485.
[313] M. L. Salkie and C. L. Hannah, *Enzyme*, 1977, **22**, 52.
[314] H. Urlaub and G. Wöber, *F.E.B.S. Letters*, 1975, **57**, 1.
[315] E. H. Hadibi, R. Donati, and M. Bastide, *Compt. rend.*, 1976, **283**, *D*, 1397.
[316] F. M. Rombouts and H. J. Phaff, *European J. Biochem.*, 1976, **63**, 121.

hydrolysed α-limit dextrins containing either maltosyl or maltotriosyl side-chains, pullulan, and amylopectin β-limit dextrin.[317] Glycogen β-limit dextrin and amylopectin were hydrolysed less rapidly, and glycogen hardly at all, by the enzyme.

Lysozymes

The affinity chromatography of lysozymes on glycose-substituted agaroses has been investigated.[35]

A general kinetic analysis developed for unimolecular, three-species models has been applied both to the reversible folding of lysozyme and to the folding of lysozyme in the presence of guanidinium hydrochloride.[318] The results and implications of a theoretical study of the hinge-bending mode in lysozyme have been discussed.[319] Free-energy calculations have been used in an attempt to predict the three-dimensional structures of enzyme–substrate and enzyme–inhibitor complexes of lysozyme.[320] A global search method, involving the use of a disaccharide fragment, was first used to determine all the favourable binding regions at the active site, and it was shown that (non-fragmented) oligomers of 2-acetamido-2-deoxy-D-glucopyranose bind in a highly specific way. *X*-Ray structures of compounds bound at the active site were considered. The structures of lysozyme, lysozyme–inhibitor complexes, and the inhibitors in solution and in the crystals have been compared.[321]

Quantitative kinetic studies of lysozyme-catalysed hydrolyses can be achieved only in limited circumstances; for example, the use of chromophoric substrates is beset by difficulties and no substrate of low molecular weight has gained acceptance in standard assays.[322] An efficient and rapid assay of the lysozyme-catalysed hydrolysis and transglycosylation of [^{14}C]chito-oligosaccharides uses h.p.l.c. to separate the products and liquid scintillation counting to determine their concentrations.

The stability of the carbocation intermediate formed on cleavage of a glycosidic linkage by lysozyme has been examined by a hybrid classical–quantum mechanical approach (Figure 1).[17] It was found that electrostatic stabilization is important in increasing the rate of the reaction leading to the carbocation intermediate, whereas steric factors, such as the strain introduced into the substrate on binding, do not seem to contribute significantly. In general, it seems much easier to control the rate of the enzymic reaction by electrostatic interactions, which vary gradually with distance, than by steric interactions, which vary rapidly with distance and which can be relieved by small movements of the atoms.

Assays of lysozyme by a diffusion technique, which employs an agarose gel impregnated with substrate organisms (lysoplates), differed greatly from those obtained by spectrophotometric and immunological techniques.[323] Different agaroses had unpredictable effects on the determination of lysozyme in normal

[317] D. G. Hardie, D. J. Manners, and D. Yellowlees, *Carbohydrate Res.*, 1976, **50**, 75.
[318] P. J. Hagerman, *Biopolymers*, 1977, **16**, 731.
[319] J. A. McCammon, B. R. Gelin, M. Karplus, and P. G. Wolynes, *Nature*, 1976, **262**, 325.
[320] M. R. Pincus, S. S. Zimmerman, and H. A. Scheraga, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 4261.
[321] A. A. Timchenko, O. B. Ptitsyn, I. N. Serdiuk, B. A. Fedorov, and N. A. Kravchenko, *Doklady Akad. Nauk S.S.S.R.*, 1976, **230**, 458.
[322] P. van Eikeren and H. McLaughlin, *Analyt. Biochem.*, 1977, **77**, 513.
[323] R. A. Greenwald and W. W. Moy, *Clinica Chim. Acta*, 1976, **73**, 299.

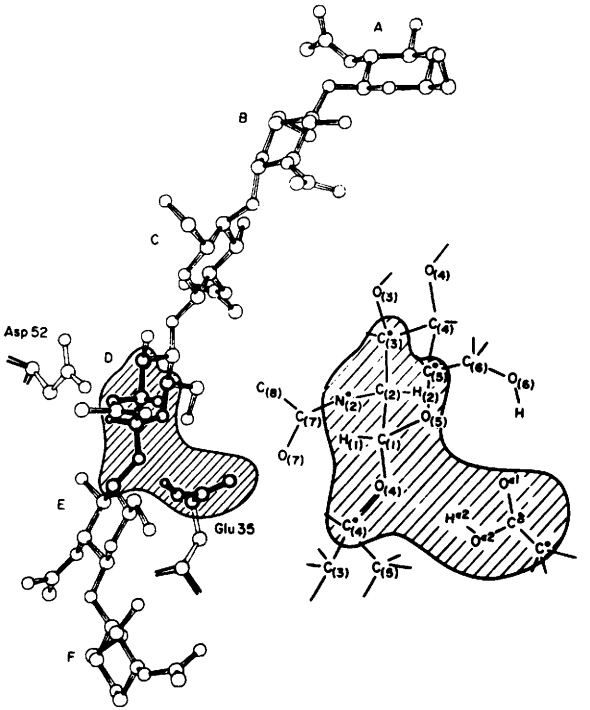


Figure 1 *Partition of the lysozyme–substrate system into quantum mechanical and classical regions. The shaded area defines the quantum mechanical region, which is presented in greater detail on the right-hand side of the Figure. Only part of the enzyme is drawn, although the whole enzyme is included in the calculation.*

sera, and the results obtained with one gel did not agree with either those found with other gels or independent assays. The lysoplate technique should be employed only with uniform layers of agarose and when extensive calibration is possible.

C.d. spectroscopy has been used to study the interaction of tri-*N*-acetylchitotriose with human lysozymes at various pH values.[324] The binding constants for the trisaccharide and different microscopic protonated forms of lysozyme were estimated using the binding constants determined at various pH values and the microscopic ionization constants of the catalytic carboxylic acid groups (Glu-35 and Asp-52). Unlike hen and turkey lysozymes, all four species of human lysozyme showed similar binding with tri-*N*-acetylchitotriose.

Human saline and milk lysozymes and hen egg-white lysozyme have been purified by affinity chromatography on a matrix of 4-aminophenyl 2-acetamidó-2-deoxy-β-D-glucopyranoside attached, by linkage-extension, to agarose cyclic imidocarbonate.[325] The purification achieved by this single-step procedure depends on the nature of the enzyme.

[324] S. Kuramitsu, K. Ikedá, K. Hamaguchi, S. Miwa, and K. Nakashima, *J. Biochem. (Japan)*, 1975, **78**, 327.

[325] E. Junowicz and S. E. Charm, *F.E.B.S. Letters*, 1975, **57**, 219.

The association constants for the binding of various saccharides to human and hen egg-white lysozymes have been used to deduce the role of ground-state strain in reactions catalysed by lysozyme.[326] The binding constants for saccharides, which must have a residue of *N*-acetylmuramic acid or 2-acetamido-2-deoxy-D-glucopyranose or 2-acetamido-2-deoxy-D-xylopyranose bound at subsite D, indicated that there is no strain involved in binding 2-acetamido-2-deoxy-D-glucopyranose and that the lactyl group, rather than the hydroxymethyl group, of *N*-acetylmuramic acid is responsible for the strain introduced on binding at subsite D.

The steady-state and transient fluorescence properties of turkey egg-white and human lysozymes and bovine α-lactalbumin have been compared with those of hen egg-white lysozyme and its derivatives.[327] The fluorescence of the tryptophanyl residues appears to be sufficiently sensitive to the environment for a small change in the sequence of amino-acids to alter its properties. The results indicated that human lysozyme assumes a number of conformations in solution and that interconversion of the conformers is slow compared to the fluorescence lifetime.

The primary structure of rat lysozyme has been determined and compared with those of human and other mammalian lysozymes.[328] Lysozyme activity has been detected in the gastric mucosa of a prosimian mammal (*Perodicticus potto*).[266] The dependence on pH of the binding of 2-acetamido-2-deoxy-D-glucopyranose and methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides to turkey and hen egg-white lysozymes has been examined with the aid of c.d. measurements.[329] The similarity of the pH-dependence curves suggested that aspartic acid-48, as well as aspartic acid-66 and -52 and glutamic acid-35, is perturbed on binding a monomer.

The volume of solution needed to elute hen egg-white lysozyme from an agarose column far exceeds the total volume of the column; similar results were obtained using columns of polyacrylamide–agarose gels.[330] This anomalous behaviour is due to interaction of the enzyme with agarose, which in some ways resembles the physiological interaction of the enzyme with substrates. The binding of 2-acetamido-2-deoxy-D-glucopyranose and chito-oligosaccharides to hen egg-white lysozyme at 40 °C has been investigated.[331] The fluorescence that results from the interaction of 1-anilinonaphthalene-8-sulphonate with hen egg-white lysozyme has been compared with that resulting from the corresponding interaction with bovine α-lactalbumin.[332]

Hen egg-white lysozyme and three derivatives of its reduced form have been examined by c.d. spectroscopy and the proportions of the protein chain present as α-helix, β-structure, and disordered structure were estimated by a computer-adapted curve-fitting program.[333] The proportion of α-helix ranged from 2 to 33% in the derivatives of the reduced form (*cf.* 26—31% for native lysozyme), whereas

[326] M. Schindler, Y. Assaf, N. Sharon, and D. M. Chipman, *Biochemistry*, 1977, **16**, 423.
[327] C. Formoso and L. S. Forster, *Biochim. Biophys. Acta*, 1976, **427**, 377.
[328] T. J. White, G. A. Mross, E. F. Osserman, and A. C. Wilson, *Biochemistry*, 1977, **16**, 1430.
[329] Y. Nakae, E. Ryo, S. Kuramitsu, K. Ikeda, and K. Hamaguchi, *J. Biochem. (Japan)*, 1975, **78**, 589.
[330] J. M. Fernandez-Sousa and R. Rodriguez, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 1426.
[331] P. Jollés, J. Saint-Blancard, M. Allary, J. P. Périn, and P. Cozzone, *F.E.B.S. Letters*, 1975, **55**, 165.
[332] V. Versee and A. O. Barel, *Bull. Soc.chim. belges*, 1976, **85**, 585.
[333] F. H. White, *Biochemistry*, 1976, **15**, 2906.

that of the β-structure varied from 23 to 62% (*cf.* 11—16% for native lysozyme). Structures that might form at least part of a precursor conformation in the formation of the native structure were discussed.

Differential scanning calorimetry has been used to investigate changes in the conformation of hen egg-white lysozyme brought about by the combined actions of temperature and denaturants.[334] The effects of a wide range of alcoholic denaturants indicated that hen egg-white lysozyme, whether in solution or in crystalline form, displays structural features that are generally strikingly similar.

The binding of 2-acetamido-2-deoxy-α- and -β-D-glucopyranoses, methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides, di-*N*-acetylchitobiose, and tri-*N*-acetylchitotriose to hen egg-white lysozyme at various pH values and the effects of these saccharides on the charge-transfer binding of *N'*-methylnicotinamide have been examined.[335] Whether or not tryptophan-62 ionizes when lysozyme binds saccharides depends on the structure of the saccharide and on the binding configuration. That a relationship exists between the state of ionization of tryptophan-62 and that of glutamic acid-35, which is distant from it, was also confirmed for complexes between lysozyme and various saccharides and for the free enzyme.

A third antigenic site has been identified in hen egg-white lysozyme using a synthetic 'surface-simulation' approach, which reveals contributions from residues that are close to the site in the three-dimensional structure although distant from it in the amino-acid sequence.[336] It appears that the antigenic site need not always include residues that are in direct peptide linkage.

Lysozyme failed to penetrate the outer membrane of stationary phase cells of *E. coli* when it was added to suspensions of plasmolysed cells, although it penetrated the outer membrane of cells subjected to mild osmotic shock.[337] Added Mg^{2+} ions stabilized the membrane, so that no enzyme penetrated during osmotic shock.

The dependence on pH of an extrinsic band in the c.d. spectrum of a lysozyme with tryptophan-62 selectively (2-nitrophenylsulphenyl)ated has been studied.[338] Analysis of the peptides in tryptic hydrolysates of two forms of lysozyme oxidized with singlet oxygen established that tryptophan-62 is destroyed.[339] This was confirmed by the effect exerted by chitin, which protected tryptophan-62 from oxidation. The possible oxidation of other tryptophanyl residues by singlet oxygen was also discussed.

Hen egg-white lysozyme has been partially *N*-acetylated in order to facilitate a study of the interactions between the carboxylic acid groups and amino-groups.[340] Structural considerations and the results of pH titration indicated that the carboxylic acid groups of aspartic acid-48, -66, and -87 are not titratable in the acetylated lysozyme, which contains fewer titratable groups in the pH range 7—10 than does the native enzyme.

[334] J. Vincentelli, Y. Looze, and J. Léomis, *Biochim. Biophys. Acta*, 1976, **427**, 38.
[335] K. Ikeda and K. Hamaguchi, *J. Biochem. (Japan)*, 1975, **77**, 1.
[336] C.-L. Lee and M. Z. Atassi, *Biochem. J.*, 1976, **159**, 89.
[337] B. Witholt, H. van Heerikhuizen, and L. de Leij, *Biochim. Biophys. Acta*, 1976, **344**, 534.
[338] Y. Nakae, K. Ikeda, and K. Hamaguchi, *J. Biochem. (Japan)*, 1975, **77**, 993.
[339] N. I. Churakova, N. A. Kravchenko, É. P. Serebryakov, and E. D. Kavernzneva, *Biochemistry (U.S.S.R.)*, 1976, **41**, 810.
[340] T. Okuda and S. Sugai, *J. Biochem. (Japan)*, 1975, **78**, 1241.

A carboxyethyl derivative of lysozyme and synthetic disulphide peptides have been used to study the enzymic and immunochemical properties of lysozyme around the disulphide bridge (linking residues 6—127) at the reactive site.[341] One of the six tryptophanyl residues, possibly either tryptophan-62 or -63, of hen egg-white lysozyme has been oxidized selectively with ozone in aqueous solution.[342] *N'*-Formylkynurenine was formed, with concomitant loss of the enzymic activity, although there was apparently little change in the gross conformation of the molecule.

The enzymic properties of lysozymes from the invertebrates *Asterias rubens* and *Nephthys hombergii* have been compared with those of hen egg-white lysozyme.[343] Whereas *N. hombergii* lysozyme exhibits enzymic properties similar to those of the avian enzyme, *A. rubens* lysozyme appears to be a new type of lysozyme.

A crystalline lysozyme (mol. wt. 2.0—2.3 × 10^4), which acts on the cells of *Streptococcus mutans*, has been obtained from culture filtrates of *Streptomyces griseus* by ion-exchange chromatography and gel filtration.[344] The enzyme was homogeneous on examination by polyacrylamide gel electrophoresis.

Lysozyme has been conjugated with fibrinogen glycopeptides that had been activated by reaction with *N*-ethyl-5-phenylisoxazolium-3'-sulphonate.[345] Lysozyme has been immobilized, with retention of activity, on a collagen membrane using an impregnation process.[121]

D-Mannanases (Miscellaneous)

The occurrence, purification, properties, and modes of action of D-mannanases have been reviewed.[4]

A homogeneous β-D-mannanase (mol. wt. 4.0 × 10^4, pH optimum 5.2, temperature optimum 50 °C) from germinating alfalfa seeds hydrolysed galacto- and gluco-mannans and D-manno-oligosaccharides by preferential cleavage of the third linkage from the non-reducing end of the chain, although D-galactopyranosyl residues reduced the accessibility of the D-mannan chain of galactomannans.[346]

An *endo*-β-D-mannanase activity in the endosperm of germinating fenugreek (*Trigonella foenum-graecum*) seeds is required to hydrolyse the storage galactomannan.[347] The level of *endo*-β-D-mannanase activity is controlled *in vivo* by the aleurone layer.

Germinating lettuce (*Lactuca sativa*) seeds synthesize large amounts of an *endo*-β-D-mannanase that hydrolyses D-mannose-containing polysaccharides in the endosperm cell walls.[348] This enzyme appears to mobilize the cell-wall polysaccharides as a source of nutrient for the growing embryo.

The D-mannanase activities of some wood-rotting fungi have been investigated.[162]

[341] M. Z. Atassi, J. Koketsu, and A. F. S. A. Habeeb, *Biochim. Biophys. Acta*, 1976, **420**, 358.
[342] M. Kuroda, F. Sakiyama, and K. Narita, *J. Biochem. (Japan)*, 1975, **78**, 641.
[343] J. P. Périn and P. Jollès, *Biochimie*, 1976, **58**, 657.
[344] K. Yokogawa, S. Kawata, and Y. Yoshimura, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 661.
[345] E. Moczar and C. Sepulchre, *Biochimie*, 1975, **57**, 1241.
[346] H. Villarroya and F. Petek, *Biochim. Biophys. Acta*, 1976, **438**, 200.
[347] J. S. G. Reid, C. Davies, and H. Meier, *Planta*, 1977, **133**, 219.
[348] P. Halmer, J. D. Bewley, and T. A. Thorpe, *Planta*, 1976, **130**, 189.

The α-D-mannanase activity secreted by an *Acinetobacter* sp. growing on baker's yeast has been separated into a number of components by ion-exchange chromatography; one of them (an *exo*-α-1,2-D-mannanase) hydrolysed *Saccharomyces cerevisiae* D-mannan and oligosaccharides derived from yeast D-mannan.[163]

A *Streptomyces* sp. from soil produces a β-D-mannanase that depolymerized an insoluble β-D-mannan.[349]

Pectate Hydrolases (Miscellaneous) and Lyases

The assay, purification, occurrence, action patterns, and specificities of pectate hydrolases and lyases have been reviewed.[5]

Poly-D-galacturonases

The poly-D-galacturonases have been reviewed.[5]

The binding of (*endo*-)poly-D-galacturonase to pectic acid cross-linked with epichlorohydrin has been studied in connection with the biodegradability of the modified material.[350] The poly-D-galacturonase from snails (*Helix pomatia*) has been purified by affinity chromatography on a cross-linked poly-D-galacturonic acid.[351] Poly-D-galacturonase activity has been detected in sugar-cane stalks.[64]

The synthesis of poly-D-galacturonase activities during the fermentation of citric acid by *Aspergillus niger* has been investigated.[352] The poly-D-galacturonase activity of Pectinol (a commercial preparation from *A. niger*) has been separated into two forms of comparable activity by ion-exchange chromatography; the forms have different pH optima (4.1 and 3.8), molecular weights (3.5×10^4 and and 8.5×10^4), and electrophoretic mobilities, *etc.*, but neither form is affected by iodoacetate and they are inhibited at similar rates by diethyl pyrocarbonate.[353] The presence of two carbethoxyhistidyl residues in the inactivated enzymes indicated that histidyl residues are essential for enzymic activity.

The synthesis of extracellular poly-D-galacturonase activity by two strains of *Colletotrichum lagenarium* has been compared.[354] Poly-D-galacturonase activity present on the surface of these cells could be removed by washing the mycelia.

Pseudomonas cepacia growing on onions (*Allium cepa*) synthesized an (*endo*-)-poly-D-galacturonase (pH optimum 4.4—4.6).[355] The ability of the purified enzyme (labelled with fluorescein isothiocyanate) to penetrate the host cells was investigated.

Poly-D-galacturonate Lyases

The poly-D-galacturonate lyase and pectinesterase activities of *Clostridium multifermentans* appear to be associated in a single complex.[356] Kinetic data suggested that the enzymes act on their respective substrates by a single-chain

[349] C. Coulombel, M. Charpentier, F. Percheron, and M. J. Foglietti, *Compt. rend. Soc. Biol. Fil.*, 1976, **170**, 965.
[350] R. Kohn, L. Rexová-Benkova, O. Luknár, and L. Kuniak, *Coll. Czech. Chem. Comm.*, 1976, **41**, 1879.
[351] M. J. Foglietti, H. Girerd, and F. Percheron, *Biochimie*, 1975, **57**, 667.
[352] F. A. Hamissa, S. S. Mabrouk, and A. F. Abdel-Fattah, *J. Gen. Appl. Microbiol.*, 1977, **23**, 23.
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[355] J. M. Ulrich, *Physiol. Plant Pathol.*, 1975, **5**, 37.
[356] M. I. Sheiman, J. D. Macmillan, L. Miller, and T. Chase, jun., *European J. Biochem.*, 1975, **64**, 565.

mechanism and in a co-ordinate manner—the polysaccharide chain passing from the esterase site to the lyase site without dissociation and re-binding.

A poly-D-galacturonate lyase released from *Erwinia rubrifaciens* cells by osmotic shock has been purified by ion-exchange chromatography and isoelectric focusing.[357] The purified enzyme (mol. wt. 4.1×10^4, $S_{20,w}$ 3.09*S*, p*I* 6.25, pH optimum 9.5, temperature optimum 37 °C) requires Ca^{2+} ions, but not Mg^{2+} ions, for activity and prefers unmethylated poly(D-galacturonic acid) as a substrate. Ways of releasing more of the enzyme from the cells were reported.

Pseudomonas cepacia growing in continuous culture at pH 7 synthesized a poly-D-galacturonate lyase.[355]

exo-Poly-D-galacturonate Lyases

exo- Poly-D-galacturonate lyase activity has been detected in cultures of *Sphaeropsis malorum* growing on apple tissues.[358]

endo-Poly-L-guluronate Lyases

Components of an alginate lyase from a *Pseudomonas* sp. appear to possess specific *endo*-poly-L-guluronate lyase activity;[184] the properties of these enzymes and the effects of salts, *etc.*, on their activities were investigated.[185] The use of *endo*-poly-L-guluronate lyase in structural studies of alginates has been discussed.[186]

Pullulanases

An extracellular form and a cell-bound form of pullulanase (mol. wts. 1.26×10^5 and 1.41×10^5, respectively) from *Klebsiella aerogenes* have been purified by ion-exchange chromatography, gel filtration, and affinity chromatography.[359] The cell-bound form was also released by proteolysis. Comparisons of their physicochemical properties and amino-acid compositions suggested that the enzymes are derived from the same gene product, which can be cleaved selectively by endogenous or added proteases.

αα-Trehalases

Hybridization of a hyperderepressed strain and a normal strain of *Neurospora crassa* produced an increase in the level of αα-trehalase activity.[68]

αα-Trehalase from *Saccharomyces cerevisiae* has been purified by precipitation, ion-exchange chromatography, and affinity chromatography on immobilized concanavalin A; it contains no other carbohydrase activity and did not act on D-glucose 1- and 6-phosphates.[360]

αα-Trehalase has been immobilized, with retention of activity, by reaction with agarose cyclic imidocarbonate.[118]

endo-1,4-β-D-Xylanases

The levels of polysaccharide hydrolases, including *endo*-1,4-β-D-xylanase, in germinating rye have been monitored.[275]

[357] J. M. Gardner and C. I. Kado, *J. Bacteriol.*, 1976, **127**, 451.
[358] G. Bompeix and B. Poiret, *Compt. rend.*, 1974, **279**, *D*, 275.
[359] C. J. Brandt, B. J. Catley, and W. M. Awad, *J. Bacteriol.*, 1976, **125**, 501.
[360] P. J. Kelly and B. J. Catley, *Analyt. Biochem.*, 1976, **72**, 353.

Xylanases (Miscellaneous)

The properties of xylanases[237] and the modes of action of β-1,3- and β-1,4-D-xylanases have been discussed.[4]

An enzyme from culture filtrates of *Irpex lacteus* exhibited both xylanase and cellulase activities, which are present in the same species.[249]

Carbohydrate Isomerases

Aldose–Ketose Isomerases.—The reactions catalysed by aldose–ketose isomerases, including proton-migration, stereochemical transformations, amination, and deamination, *etc.*, have been reviewed.[361]

D-Glucose Isomerases.—D-Glucose isomerase has been purified by affinity chromatography on an immobilized xylitol derivative.[362]

Theoretical modes for the conversion of D-glucose into D-fructose by whole cells containing D-glucose isomerase and for inactivation of the enzyme have been proposed and verified experimentally.[363] The model could be used to predict the rate of production of D-fructose in a batch reactor.

D-Glucose isomerase has been immobilized, with retention of activity, by reaction with a polyacrylonitrile containing methylimidate hydrochloride groups,[364] by glutaraldehyde-coupling to an aminoalkylsilyl derivative of porous glass,[365] by adsorption onto, and glutaraldehyde-coupling to, chitin,[366] by attachment to DEAE-cellulose,[367] and by reaction with a derivative of DEAE-cellulose.[297] The D-glucose isomerase in cells of *Streptomyces phaeochromogenus* can be immobilized by heat.[368]

Carbohydrate Oxidases

D-Galactose Oxidases.—Several commercial samples of D-galactose oxidase contained proteolytic activities (*e.g.* towards fibrinogen, transferrin, albumin, and casein), which could be removed by affinity chromatography on agarose.[369] The relative affinities of the enzyme for agarose and Sephadex were used to elucidate the mechanism by which the enzyme is adsorbed.

The role of tryptophanyl residues in the catalytic activity of a fungal D-galactose oxidase has been investigated.[370] Studies of the fluorescence of the native enzyme and of the NBS-oxidized enzyme indicated that one or more tryptophanyl residues are present at or near the Cu^{II} atom and the substrate-binding site of the enzyme.[371] The dependence on pH of the oxidation of methyl β-D-galactopyranoside by a fungal D-galactose oxidase has been determined; the velocity (k_{cat}) exhibited a bell-shaped dependence on the ionization of, at least, two groups

[361] I. A. Rose, *Adv. Enzymol.*, 1975, **43**, 491.
[362] Y. H. Lee, P. C. Wankat, and A. H. Emery, *Biotechnol. and Bioeng.*, 1976, **18**, 1639.
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[364] T. Handa, S. Goto, T. Shindo, and K. Imai, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 169.
[365] Y. Y. Lee, A. R. Fratzke, K. Wun, and G. T. Tsao, *Biotechnol. and Bioeng.*, 1976, **18**, 389.
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[371] R. E. Weiner, M. J. Ettinger, and D. J. Kosman, *Biochemistry*, 1977, **16**, 1602.

(pK_b, 6.3 and pK_a, 7.1) in the enzyme–substrate complex.[372] Investigation of the interaction of an alkylated (iodoacetamide) derivative of the enzyme with this substrate suggested that the histidyl residue at the active site has both a catalytic function and a subtle conformational role.

The D-galactose oxidase from *Dactylium dendroides* has been used to prepare UDP-D-[^{14}C]galacturonic acid and UDP-D-[6-^{3}H]galactose of high specific activity.[373]

D-Galactose oxidase has been immobilized, with retention of activity, by attachment to a benzoyl azido derivative of porous glass.[374,375]

D-Glucose Oxidases.—D-Glucose oxidase, in combination with glucoamylase and D-gluconolactonase, has been used to convert maltose into D-gluconic acid.[288] A study has been made of the efficiency of a D-glucose oxidase–catalase system, both with the free and immobilized enzymes.[376]

A purified D-glucose oxidase (mol. wt. 1.6×10^5) from *Aspergillus niger* contains two atoms of iron, flavin adenine dinucleotide, neutral sugars (16.4%), and 2-amino-2-deoxyhexoses (2.4%).[377] The holoenzyme is composed of two identical subunits (mol. wt. 7.9×10^4) that have the same molecular weight as the apoenzyme. Flavin hypoxanthine dinucleotide is as effective a coenzyme as flavin adenine dinucleotide.

Oxidation of *A. niger* D-glucose oxidase with periodate indicated that the carbohydrate component (12% by weight) stabilizes the protein, although it has neither catalytic nor immunological activity and is not involved in maintaining the conformation of the molecule.[378]

Some of the chemical and immunological properties of the D-glucose oxidases from *A. niger* and *Penicillium glaucum* have been compared.[379] Although c.d. spectroscopy indicated that the protein chains have essentially identical conformations, small variations in their structures were revealed by differences in the thermal stabilities, catalytic properties, and immunological activities of the enzymes.

D-Glucose oxidase has been immobilized, with retention of activity, by chelation with hydrous metal oxides of iron(III), tin(II), vanadium(III), and zirconium(IV),[147] by reaction with diazotized 1,4-diaminobenzene[148] and with agarose cyclic imidocarbonate,[380] by covalent coupling to derivatives of non-porous glass,[381] by glutaraldehyde-coupling to a silanized, nickel-impregnated silica–alumina support,[376,382] by glutaraldehyde-coupling with β-D-galactosidase to derivatized porous glass,[125] by glutaraldehyde-cross-linking and -coupling to inorganic supports[383] and manganese(III) oxide,[384] and by entrapment in gels of calcium alginate.[300]

[372] L. D. Kwiatkowski, L. Siconolfi, R. E. Weiner, R. S. Giordano, R. D. Bereman, M. J. Ettinger, and D. J. Kosman, *Arch. Biochem. Biophys.*, 1977, **182**, 712.
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[374] S. K. Dahodwala, M. K. Weibel, and A. E. Humphrey, *Biotechnol. and Bioeng.*, 1976, **18**, 1679.
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[378] S. Nakamura, S. Hayashi, and K. Koga, *Biochim. Biophys. Acta*, 1976, **445**, 294.
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[382] J. C. Bouin, M. T. Atallah, and H. O. Hultin, *Biotechnol. and Bioeng.*, 1976, **18**, 179.
[383] M. T. Atallah, B. P. Wasserman, and H. O. Hultin, *Biotechnol. and Bioeng.*, 1976, **18**, 1833.
[384] Z. Duvnjak and M. D. Lilly, *Biotechnol. and Bioeng.*, 1976, **18**, 737.

L-Gulonolactone Oxidases.—A rapid and quantitative assay for L-gulonolactone oxidase in mammalian livers has been described; maximal recovery of the enzyme was achieved by homogenization of the livers in a solution of sodium deoxycholate.[385] The effects of sampling and of storing on the enzymic activity were assessed.

Carbohydrate Transferases

Cyclodextrin D-Glucanotransferases.—Investigations of the transglycosylation reactions catalysed by cyclodextrin D-glucanotransferase showed that L-sorbose, D-xylose, and D-galactose are efficient acceptors.[386] The enzyme transferred D-glucanosyl residues to O-3 of L-sorbose and to O-4 of D-xylose, yielding a series of malto-oligosaccharides containing either 3-*O*-α-D-glucopyranosyl-L-sorbose or 4-*O*-α-D-glucopyranosyl-D-xylose units at the reducing end.

An 'acid' cyclodextrin D-glucanotransferase from an alkalophilic *Bacillus* sp. exhibits a specificity similar to those of the transferases from *B. circulans* and *B. macerans*, although different proportions of cyclohexa-, cyclohepta-, and cyclo-octa-amyloses are formed.[387] The cyclodextrin D-glucanotransferase from an alkalophilic *Bacillus* sp. has been used, either alone or in combination with pullulanase, to produce cyclohepta-amylose.[388] A succinylated derivative of this cyclodextrin D-glucanotransferase has been immobilized, with retention of activity, by adsorption onto a vinylpyridine polymer.

D-Galactosyltransferases.—The D-galactosyltransferase associated with the lactose synthase system in cows' milk exists as a monomer (mol. wt. 4.6×10^4), although its aggregation is promoted by 2-acetamido-2-deoxy-D-glucose.[389] Complexation of the enzyme with α-lactalbumin was investigated.

Glycopeptide Linkage Hydrolases

4-L-Aspartyl-β-D-glucosylamine Amidohydrolases.—4-L-Aspartyl-β-D-glucosylamine amidohydrolases in the livers of humans and rats are associated with post-nuclear and lysosomal fractions.[390] Neither enzyme could be separated into isoenzymes.

Proteinases

Bromelains.—Bromelains (stem and juice) have been purified by a process that involved blocking the sulphydryl groups with phenylmercury(II) acetate.[391]

Carboxypeptidases.—A study of the biosynthesis of yeast carboxypeptidase Y (a mannoprotein) has revealed that a membrane fraction contains a precursor of the enzyme, which, although it has a higher molecular weight than the enzyme, is not a complex of the enzyme and its specific inhibitor.[392]

[385] K. M. Ayaz, R. Jenness, and E. C. Birney, *Analyt. Biochem.*, 1976, **72**, 161.
[386] S. Kitahata and S. Okada, *J. Biochem. (Japan)*, 1976, **79**, 641.
[387] N. Nakamura and K. Horikoshi, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1647.
[388] N. Nakamura and K. Horikoshi, *Biotechnol. and Bioeng.*, 1977, **19**, 87.
[389] R. J. Ivatt and M. A. Rosemeyer, *European J. Biochem.*, 1976, **64**, 233.
[390] H. Somer, J. Palo, H. Savolainen, and A. Konttinen, *Clinica Chim. Acta*, 1975, **60**, 219.
[391] S. Ota, H. Umi, E. Muta, and Y. Okamoto, *J. Biochem. (Japan)*, 1975, **78**, 627.
[392] A. Hasilik and W. Tanner, *Biochem. Biophys. Res. Comm.*, 1976, **72**, 1430.

Pepsins and Pepsinogens.—The carbohydrate compositions and some sequences of amino-acids have been determined for glycopeptides derived from pepsinogens isolated from Japanese monkeys.[393] Glycopeptides released by successive treatments of pepsinogen I with thermolysin and aminopeptidase contained 2-amino-2-deoxy-D-glucose (4), D-galactose (6), D-mannose (6—8), and L-fucose (8—10 residues per molecule). The carbohydrate component is attached to the asparagyl residue in the sequence Ile → Gly → Ile → Gly → Thr → Pro → Glu → Ala → Asn.

Phosphatases

Alkaline Phosphatases.—Since an alkaline phosphatase from *Dictyostelium discoideum* interacted with concanavalin A, it appears to be a glycoprotein.[394]

Sulphatases

Arylsulphatases.—An arylsulphatase from the marine gastropod *Charonia lampas* hydrolysed sulphatide (cerebroside 3-sulphate), sulphogalactolipids, and seminolipid.[395] The enzymic activity was stimulated by sodium taurodeoxycholate and manganese(II) chloride, and was inhibited by 4-nitrophenyl sulphate, 4-nitrophenyl catechol sulphate, or L-ascorbic acid 2-sulphate, but not by D-glucose 6-sulphate. The action pattern of the enzyme was compared with those of other sulphatase activities in *C. lampas*.

The purification and properties of arylsulphatases from *Klebsiella aerogenes* have been reported.[396]

Glycolipid Sulphate Sulphatases.—Proteins of low molecular weight from secondary lysosomes activated a number of sphingolipid hydrolases, including cerebroside sulphatase (E.C. 3.1.6.8).[36]

Glycosulphatases.—A glycosulphatase from the liver of the marine gastropod *Charonia lampas* slowly hydrolysed sulphogalactolipids, sulphatide (cerebroside 3-sulphate), and seminolipid.[395] Two glycosulphatases (mol. wts. 1.12×10^5 and 7.9×10^4, pH optima 5.5, K_m of each for D-glucose 6-sulphate 25 mmol l^{-1}) from this source appear to be glycoproteins containing different carbohydrate components.[397]

Miscellaneous Enzymes

Acetylcholinesterases.—Acetylcholinesterase from the head tissues of house flies can be purified by affinity chromatography on immobilized concanavalin A.[398]

Adenosine 3′,5′-Phosphate Phosphodiesterases.—Since it interacted with immobilized concanavalin A, an adenosine 3′,5′-phosphate phosphodiesterase from *Dictyostelium discoideum* appears to be a glycoprotein.[394]

Dextransucrases.—A dextransucrase from the cell-free supernatant fluid of cultures of *Leuconostoc mesenteroides* has been separated into two active forms,

[393] T. Kageyama and K. Takahashi, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 789.
[394] E. V. Crean and E. F. Rossomando, *Biochem. Biophys. Res. Comm.*, 1977, **75**, 488.
[395] H. Hatanaka, F. Egami, I. Ishizuka, and Y. Nagai, *Biochim. Biophys. Acta*, 1976, **438**, 176.
[396] H. Okamura, T. Yamaoa, Y. Muraoka, and T. Harada, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 2071.
[397] H. Hatanaka, Y. Ogawa, and F. Egami, *J. Biochem. (Japan)*, 1976, **79**, 27.
[398] R. W. Steele and B. N. Smallman, *Biochim. Biophys. Acta*, 1976, **445**, 147.

one (pH optimum 6.0 and temperature optimum 40 °C) of which is an aggregate of the other (pH optimum 5.5, temperature optimum 35 °C, mol. wt. 4.8×10^4).[399] The dextransucrase activity was stimulated by Mg^{2+}, Fe^{2+}, and Co^{2+} ions and was inhibited by H_4edta.

α-1,4-D-Glucan Phosphorylase.—A homogeneous α-1,4-D-glucan phosphorylase (mol. wt. 1.8—1.9 × 10^5, p*I* 5.3, pH optimum 5.9) has been obtained from *Klebsiella pneumoniae*.[400] This enzyme dissociates into two polypeptide chains (mol. wts. 8.8—9.0 × 10^4) in the presence of sodium dodecyl sulphate or guanidinium hydrochloride, contains pyridoxal phosphate, and is stable in the pH-range 5.9—10.5. The amino-acid composition of the enzyme was also determined.

α-Lactalbumins.—A review of the chemistry and biochemistry of the lactose synthase system includes a discussion of the covalent and three-dimensional structures, and the chemical, immunological, and physical properties, of α-lactalbumin.[401]

A sensitive and specific radioimmunoassay has shown that some men and women have detectable levels of α-lactalbumin in their blood and that α-lactalbumin is also present in some cultures of malignant tissues from women with breast cancer.[402] The synthesis of α-lactalbumin was stimulated by ovine prolactin in some tumours. Other workers have concluded that the ability of breast carcinoma cells to synthesize α-lactalbumin is intrinsic rather than induced.[403]

α-Lactalbumin from cows' milk has been purified by fractional precipitation with ammonium sulphate and gel filtration.[404] Moving-boundary electrophoresis of α-lactalbumin at acid pH values has been used to study how the electrophoretic behaviour reflects the conformation of the molecule.[405] The appearance of a single boundary only in the transition region indicated that the conformational transition is rapid, with a half-life of < 7 min.

Comparisons of the steady-state and transient fluorescence of bovine α-lactalbumin with those of several lysozymes suggested that α-lactalbumin adopts a number of conformations in solution and that the rates of interconversion among the conformers are slower than the fluorescence lifetime.[327]

The reversible unfolding of bovine α-lactalbumin in the presence of low concentrations of guanidinium hydrochloride has been examined by spectroscopic and pH-jump methods.[406] Unfolding was shown to occur between the native and denatured states, since the apparent rate constants for unfolding and refolding depend only on pH.

^{1}H N.m.r. measurements have yielded information on the environments of the three histidyl residues in bovine α-lactalbumin.[407] The chemical shifts indicated that histidine-68 is an external residue, histidine-32 is partially buried in the vicinity of aromatic residues, and histidine-107 is partially buried and, probably,

[399] M. Kobayashi and K. Matsuda, *J. Biochem. (Japan)*, 1976, **79**, 1301.
[400] D. Linder, G. Kurz, H. Bender, and K. Wallenfels, *European J. Biochem.*, 1976, **70**, 291.
[401] R. L. Hill and K. Brew, *Adv. Enzymol.*, 1975, **43**, 411.
[402] D. L. Kleinberg, *Science*, 1975, **190**, 276.
[403] H. N. Rose and C. M. McGrath, *Science*, 1975, **190**, 673.
[404] R. I. Kaplanas and A. I. Antanavichyus, *Biochemistry (U.S.S.R.)*, 1975, **40**, 493.
[405] K. Kuwajima, K. Nitta, and S. Sugai, *J. Biochem. (Japan)*, 1975, **78**, 205.
[406] N. Kita, K. Kuwajima, K. Nitta, and S. Sugai, *Biochim. Biophys. Acta*, 1976, **427**, 350.
[407] J. H. Bradbury and R. S. Norton, *European J. Biochem.*, 1975, **53**, 387.

hydrogen bonded. The interactions of α-lactalbumin with the D-galactosyltransferase from cows' milk[389] and with 1-anilinonaphthalene-8-sulphonate[332] have been reported.

A study of the mRNAs that participate in the synthesis of milk proteins in guinea pigs identified pre-α-lactalbumin as a translation product in a heterologous cell-free system.[408]

The interactions of immobilized α-lactalbumin with D-galactosyltransferase and with anti-(α-lactalbumin) antibodies have been studied by fluorometry.[409]

Lactose Synthases.—The chemistry, biochemistry, and immunology of the lactose synthase system have been reviewed.[401]

A complex (mol. wt. 6.0×10^4) containing one molecule each of D-galactosyltransferase and α-lactalbumin has been isolated from cows' milk by gel filtration in the presence of an excess of α-lactalbumin and either D-glucose or 2-acetamido-2-deoxy-D-glucose.[389] The complex is stable in the presence of D-galactosyltransferase and α-lactalbumin.

Levansucrases.—The levansucrase from *Bacillus subtilis* has an average molecular weight of 5.4×10^4.[410] Estimates of the size and shape of the molecule were comparable with those obtained by electron microscopy.

Kinetic equations derived for the 'ping-pong' mechanism proposed for reactions catalysed by the levansucrase from *B. subtilis* allowed the exchange properties and hydrolytic activities of the enzyme to be predicted.[411] The mechanisms and efficiencies of enzyme- and acid-catalysed hydrolyses of sucrose were discussed.

5'-Nucleotidases.—Since the 5'-nucleotidase from *Dictyostelium discoideum* interacted with immobilized concanavalin A, it appears to be a glycoprotein.[394]

Pectinesterases.—The occurrence, formation, assay, purification, and specificities of pectinesterases have been reviewed.[5] Pectinesterase activity was detected both in healthy and diseased onions (*Allium cepa*), but not in cultures of the invading bacterium (*Pseudomonas cepacia*).[355] Both the pectinesterase and poly-D-galacturonate lyase activities of *Clostridium multifermentans* are associated with a single complex.[356]

Pectinesterase has been immobilized, with retention of activity, on a porous glass to which pendant benzoyl azide groups are attached.[412]

Peroxidases.—Horseradish peroxidase has been purified by affinity chromatography on immobilized concanavalin A; the nature of the binding suggests that the enzyme is a glycoprotein.[413]

Proline, 2-Oxoglutarate Dioxygenases.—Part of the enzymic activity was lost when the proline, 2-oxoglutarate dioxygenase from chicken embryos interacted with [^{3}H-acetyl]concanavalin A, suggesting that the enzyme contains a carbohydrate component.[414]

[408] R. K. Craig, P. A. Brown, D. G. Harrison, D. McIlreavy, and P. N. Campbell, *Biochem. J.*, 1976, **160**, 57.
[409] J. P. Prieels and A. O. Barel, *Biochim. Biophys. Acta*, 1975, **393**, 496.
[410] G. Gonzy-Tréboul, R. Chambert, and R. Dedonder, *Biochimie*, 1975, **57**, 17.
[411] R. Chambert and G. Gonzy-Tréboul, *European J. Biochem.*, 1976, **62**, 55.
[412] M. K. Weibel, R. Barrios, R. Delotto, and A. E. Humphrey, *Biotechnol. and Bioeng.*, 1975, **17**, 85.
[413] H. G. Brattain, M. E. Marks, and T. G. Pretlow, *Analyt. Biochem.*, 1976, **72**, 346.
[414] N. A. Guzman, R. A. Berg, and D. J. Prockop, *Biochem. Biophys. Res. Comm.*, 1976, **73**, 279.

Index of Enzymes Referred to in Chapter 6

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
β-D-2-Acetamido-2-deoxy-galactosidase	2-Acetamido-2-deoxy-β-D-galacto-side acetamidodeoxygalacto-hydrolase	3.2.1.53	374
endo-β-D-2-Acetamido-2-deoxyglucanase	*see* Chitinase	3.2.1.14	406
β-D-2-Acetamido-2-deoxy-glucosidase	2-Acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.30	374
β-D-2-Acetamido-2-deoxy-hexosidase	2-Acetamido-2-deoxy-β-D-hexoside acetamidodeoxyhexohydrolase	3.2.1.52	374
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Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
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Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
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* Sialidase is a term generally used to include neuraminidase and unspecified acylneuraminyl hydrolases. Since there is little evidence for the existence of acylneuraminyl hydrolases that do not cleave 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonyl residues, use of the term neuraminidase is preferred.

7
Glycolipids

BY R. J. STURGEON

Introduction

The chemistry and biochemistry of the glycolipids of mammalian cell membranes[1] and the biosynthesis and functions of gangliosides[2] have been reviewed. A book on the biological functions of sialic acid has discussed the chemistry and analysis of sialic acids, the biosynthesis of glycolipids, the degradation of gangliosides, and disorders of the catabolism of gangliosides.[3] It also deals with the metabolism of gangliosides in cultured and virus-transformed cell lines. The biochemistry of sphingolipid-storage diseases has been reviewed.[4]

A general procedure that enables a nitroxide spin-label to be attached as phosphate ester to the oligosaccharide residue of gangliosides has been reported.[5] Under the conditions used, some of the primary hydroxy-groups of the oligosaccharide residue are esterified using 2,2,6,6-tetramethylpiperidine-1-oxide-4-phosphate. Gangliosides so labelled and incorporated into lipid-bilayer vesicles gave sharp, three-line e.s.r. spectra that are characteristic of highly mobile structures. Another method for labelling gangliosides is based on selective oxidation of the *N*-acetylneuraminic acid residue(s) with periodate and reduction of the oxidized residue(s) with sodium borotritide.[6]

Anti-glycolipid (D-galactosylcerebroside) antibodies were absorbed onto D-galactosylcerebroside-containing liposomes in a simple technique for purifying specific anti-glycolipid (D-galactosylcerebroside) antibodies from antisera.[7] The absorbed antibodies were recovered, after washing the liposomes, by elution with M-sodium iodide solution or by extraction with a saline–chloroform mixture. It was shown that hydrophobic regions of D-galactosylcerebroside and of the surrounding phospholipids influence the binding of antibodies. Some of the conditions that might influence the specificity of the purified antibodies towards certain D-galactose-containing glycolipids were identified.[8] The neuraminidase from *Clostridium perfringens* hydrolysed the neuraminic acid residue at O-3 of the D-galactosyl residue in the gangliosides G_{M_1} {β-D-Gal*p*-(1 → 3)-β-D-Gal*N*Ac*p*-(1 → 4)-[α-Neu*N*Ac*p*-(2 → 3)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide} and

[1] S. Hakomori, in 'Carbohydrates', ed. G. O. Aspinall, MTP International Review of Science, Organic Chemistry, Series Two, Butterworths, London, 1976, vol. 7, p. 223.
[2] P. H. Fishman and R. O. Brady, *Science*, 1976, **194**, 906.
[3] 'Biological Roles of Sialic Acid', ed. A. Rosenberg and C. L. Schengrund, Plenum Press, New York, 1976.
[4] K. Sandhoff, *Angew. Chem. Internat. Edn.*, 1977, **16**, 273.
[5] F. J. Sharom and C. W. M. Grant, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 1039.
[6] R. W. Veh, A. P. Corfield, M. Sander, and R. Schauer, *Biochim. Biophys. Acta*, 1977, **486**, 145.
[7] C. R. Alving and R. L. Richards, *Immunochemistry*, 1977, **14**, 373.
[8] C. R. Alving and R. L. Richards, *Immunochemistry*, 1977, **14**, 383.

G_{M_2}-{β-D-Gal*N*Ac*p*-(1 → 4)-[α-Neu*N*Ac*p*-(2 → 3)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide}, without prior hydrolysis of the *O*-β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-D-galactopyranosyl and 2-acetamido-2-deoxy-D-galactopyranosyl residues, respectively, indicating that residues adjacent to the neuraminic acid residue do not prevent the neuraminidase from attacking the gangliosides.[9] Quantitative isolation of glycosphingolipids from small volumes of plasma has been achieved by, among other steps, chromatography of the acetylated glycosphingolipids on magnesia–silica gel; the glycosphingolipids could then be assayed by the orcinol procedure.[10]

Animal Glycolipids and Gangliosides

In a new procedure for the isolation of brain gangliosides, the extracted lipids were chromatographed on silica gel, without the prior use of partition procedures.[11] Although some differences were observed, cultured cerebellar cells from Tay–Sachs foetuses accumulated ganglioside G_{M_2} in a way similar to that found in foetal Tay–Sachs disease, so that the cultured cells can be studied as an *in vitro* model of the disease.[12] The pre- and post-natal development of gangliosides in various parts of human brain have been investigated.[13] Human-liver hexosaminidase A, but not the corresponding hexosaminidase B, hydrolysed the 2-acetamido-2-deoxy-D-galactopyranosyl residue from the principal Tay–Sachs ganglioside, G_{M_2}, and also from the corresponding ceramide-free derivative β-D-Gal*N*Ac*p*-(1 → 4)-[α-Neu*N*Ac*p*-(2 → 3)]-β-D-Gal*p*-(1 → 4)-D-glucitol.[14] Further evidence that ganglioside G_{M_3} [α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide] is necessary for the morphological differentiation of HeLa cells by butyrate has been presented.[15] The radiolabelled gangliosides G_{M_3} (with an α-Neu*N*[^{14}C]Ac*p* label) and $G_{D_{1a}}$-{α-Neu*N*[^{14}C]Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*N*Ac*p*-(1 → 4)-[α-Neu*N*Ac*p*-(2 → 3)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide} have been synthesized from CMP-Neu*N*[^{14}C]Ac by using the appropriate precursor glycolipid and specific sialyltransferases from rat brain. The radiolabelled gangliosides were isolated and used as substrates to assay neuraminidase activity in HeLa cells.[16]

The structures (1)—(5) of five gangliosides from human kidneys have been reported.[17] The similarities in structure between the gangliosides (3) and (4) and blood-group substances of a glycosphingolipid nature were noted.

Two of the principal neutral glycolipids from human gastric secretions have been partially characterized as an *O*-acyl-*O*-alkylglyceryl D-glucohexaoside and the corresponding D-gluco-octaoside.[18] Although sulphated derivatives of glyceryl D-glucolipids have not been found previously in mammalian secretions, one of the

[9] H. Rauvala, *F.E.B.S. Letters*, 1976, **65**, 229.
[10] W. Atzpodien and G. J. Kremer, *J. Clin. Chem. Clin. Biochem.*, 1977, **15**, 293.
[11] N. Kawamura and T. Taketomi, *J. Biochem. (Japan)*, 1977, **81**, 1217.
[12] L. M. Hoffman, D. Amsterdam, S. E. Brooks, and L. Schenck, *J. Neurochem.*, 1977, **29**, 551.
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[14] K. Sandhoff, E. Conzelmann, and H. Nehrkorn, *Z. physiol. Chem.*, 1977, **358**, 779.
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[18] A. Slomiany and B. L. Slomiany, *Biochem. Biophys. Res. Comm.*, 1977, **76**, 115.

α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide

(1)

α-Neu*N*Ac*p*-(2 → 8)-α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide

(2)

α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide

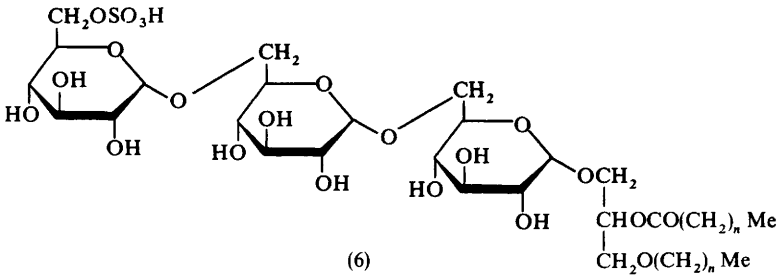
(3)

α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
3
↑
1
α-L-Fuc*p*

(4)

α-Neu*N*Ac*p*
2
↓
3
α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*N*Ac*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide

(5)



(6)

principal components of human gastric secretions has been tentatively identified as (6) on the basis of the results of partial hydrolysis with acid, oxidations with periodate ion and chromium trioxide, and methylation analysis.[19,20] The diglyceride portion of this sulphated glycolipid consists of 65% of 2-*O*-acyl-1-*O*-alkylglycerol and 35% of 1-*O*-acyl-2-*O*-alkylglycerol.

The levels of plasma D-glucosylceramide in eight patients with Gaucher's disease are higher than normal, whereas the levels of D-glucosylceramide in their erythrocytes are within the normal range.[21] Most of the excess of gangliosides in the patients' plasma is associated with the low-density lipoprotein fraction. The results do not support the view that D-glucosylceramide is exchanged between the plasma lipoprotein and the erythrocyte membrane in human blood.

Gangliosides inhibited the binding of [^{125}I]-labelled human chorionic gonadotrophin (hCG) to the membranes of rat testes, suggesting that hCG, like thyrotrophin (TSH), interacts with a receptor that has a ganglioside or ganglioside-like structure and, on interaction, undergoes a change of conformation.[22] Presumably hCG recognizes a specific oligosaccharide sequence on the ganglioside, since the most effective inhibitors of hCG-binding are gangliosides G_{T_1} and $G_{D_{1a}}$, each of which has a terminal sialic acid residue on the oligosaccharide chain, whereas the most effective inhibitors of TSH-binding to thyroid plasma membranes have a disialyl residue linked to a D-galactopyranosyl residue in the interior of the oligosaccharide chain. The receptor for thyrotrophin on thyroid plasma membranes has been isolated and fractionated into a glycoprotein component and a ganglioside component.[23]

Fibroblasts from a patient with mucolipidosis I showed a five-fold increase in the level of sialic acid and a lower-than-normal level of lysosomal neuraminidase, which results in impaired catabolism of glycopeptides and glycolipids containing sialic acid.[24] A D-mannosyltransferase has been found in suspensions of Balb/c fibroblasts incubated with GDP-D-[^{14}C]mannose; it appears to be present at the cell surface, where it is involved in the synthesis of glycolipids and glycoproteins.[25]

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[24] M. Cantz, J. Gehler, and J. Spranger, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 732.
[25] L. M. Patt and W. J. Grimes, *Biochim. Biophys. Acta*, 1976, **444**, 97.

The cell-surface glycosphingolipids and other lipids in the fibroblasts from a family with familial hypercholesterolemia have been examined after they had been oxidized with D-galactose oxidase and then reduced with sodium borotritide.[26] Comparisons of the radiolabel incorporated by homozygous, heterozygous, and normal fibroblast cells suggested that the defective metabolism of lipids in this disease is more extensive than previously realized. Gangliosides G_{M_2} and asialo-G_{M_2} have been shown to accumulate in the cerebrospinal fluid of a patient with Sandhoff's disease.[22]

The structure of a glycolipid from human testes has been established as a 2-*O*-acyl-1-*O*-alkyl-3-*O*-(β-D-galactopyranosyl 3-sulphate)-*sn*-glycerol.[28] The molecular ion could be detected in the chemical-ionization mass spectra of the TMS derivatives of D-glucosyl-sphingosines, -cerebrosides, and -ceramides, thus making this technique more useful than electron-impact m.s. for determining the molecular weights of cerebrosides, *etc.*[29] This technique, in combination with g.l.c., was used to examine the D-glucosylcerebrosides from the spleen of a patient with Gaucher's disease.

Human-erythrocyte membranes have been shown to contain glycosphingolipids of unusual complexity, which may contain as many as 60 monosaccharides in the carbohydrate portion of the molecule.[30] These polyglycosylceramides (formerly megaloglycolipids) have the general formula (7) and are probably the principal recognition sites for A, B, H, and I antigens on human erythrocytes. The immunological properties of these complex glycosphingolipids, which were isolated from human blood-group A, A_2, and O erythrocytes, were also reported.

$$(\text{L-Fuc}p)_{3 \text{ or } 4} \rightarrow (\text{D-Gal}p)_n \rightarrow (\text{D-Glc}N\text{Ac}p)_{n-2} \rightarrow \text{D-Glc}p \rightarrow \text{sphingosine}$$

(7)

In the absence of other proteins, choleragen binds specifically to liposomes containing ganglioside G_{M_1}.[31] That choleragen induced changes in the permeability of the liposomes was demonstrated by the release of D-glucose from inside the liposomes. The release of D-glucose depended only on interaction of the ganglioside-binding site on the B protomer of choleragen with ganglioside G_{M_1} in the liposomes.[32] The principal ganglioside synthesized by choleragen-responsive human fibroblasts has been identified as G_{M_3}, with only trace amounts of other gangliosides, including G_{M_1} (reputedly the choleragen receptor), being produced.[33] Although the cells require three glycosyltransferases for ganglioside synthesis, 2-acetamido-2-deoxy-D-galactosyltransferase activity, which is needed for the conversion of ganglioside G_{M_3} into more complex gangliosides, was not detected.

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[32] J. Moss, R. L. Richards, C. R. Alving, and P. H. Fishman, *J. Biol. Chem.*, 1977, **252**, 797.
[33] P. H. Fishman, J. Moss, and V. C. Manganiello, *Biochemistry*, 1977, **16**, 1871.

The cell-surface glycolipids of normal human fibroblasts and of transformed murine fibroblasts have been labelled by treating the intact cells with either D-galactose oxidase or sodium periodate and reduction of the oxidized sugar residues with sodium borotritiide.[34] Maximal binding of choleragen completely prevented the oxidation of G_{M_1} ganglioside with D-galactose oxidase in intact fibroblasts, but it only partly protected the *N*-acetylneuraminic acid residue in G_{M_1} ganglioside from oxidation with the periodate ion. Choleragen and its A protomer catalysed the hydrolysis of nicotinamide adenine dinucleotide (NAD^+) to ADP-D-ribose and nicotinamide.[35] The NADase activity was inhibited by several gangliosides, including G_{M_1}, G_{M_2}, G_{M_3}, and $G_{D_{1a}}$, and analogues of NAD^+. Studies of the lipolytic action of choleragen on fat cells have not supported earlier views that ganglioside G_{M_1} is the membrane receptor for the toxin, or that G_{M_1} ganglioside enhances the toxin's activity at either saturating or low concentrations of the toxin.[36] Equilibrium displacement dialysis has been used to investigate the binding between choleragen and oligosaccharides related to those occurring in gangliosides.[37] At low concentrations of choleragen, the monosialyltetra-itol (8) derived from G_{M_1} ganglioside was bound more strongly than the parent ganglioside. The terminal D-galactopyranosyl residue and an intact carboxylic acid group in the *N*-acetylneuraminic acid residue are necessary for binding choleragen.

β-D-Gal*p*-(1 → 3)-β-D-Gal*N*Ac*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-D-glucitol
3
↑
2
α-Neu*N*Ac*p*

(8)

The binding between gangliosides and polypeptide fragments from tetanus toxin has been examined.[38—40] An interaction between tetanus toxin and a ganglioside containing two *N*-acetylneuraminic acid residues linked in sequence to one another was demonstrated using a method involving the radiolabelled ganglioside and tetanus toxin adsorbed onto Sephadex.[41] Removal of the ceramide portion from the ganglioside resulted in complete loss of the binding activity, whereas removal of the terminal *N*-acetylneuraminic acid residue from the intact ganglioside had no effect.

Ultracentrifugation has shown that changes in the content of either sialic acid or C_{20}-sphingosine affect the size of the micelles formed by bovine-brain gangliosides–increases in the content of sialic acid decreasing the size of the micelle.[42] The gangliosides from bovine brain possess physical properties that differ from

[34] J. Moss, V. C. Manganiello, and P. H. Fishman, *Biochemistry*, 1977, **16**, 1876.
[35] J. Moss, J. C. Osborne, P. H. Fishman, H. B. Brewer, M. Vaughan, and R. O. Brady, *Proc. Nat. Acad. Sci. U.S.A.*, 1977, **74**, 74.
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[38] S. van Heyningen, *F.E.B.S. Letters*, 1976, **68**, 5.
[39] B. Bizzini, K. Stoeckel, and M. Schwab, *J. Neurochem.*, 1977, **28**, 529.
[40] T. B. Helting and O. Zwisler, *J. Biol. Chem.*, 1977, **252**, 187.
[41] T. B. Helting, O. Zwisler, and H. Wiegandt, *J. Biol. Chem.*, 1977, **252**, 194.
[42] H. C. Yohe, D. E. Roark, and A. Rosenberg, *J. Biol. Chem.*, 1976, **251**, 7083.

those of membrane phospholipids.[43] Freeze-etching techniques and studies of the inhibition of haemagglutination have demonstrated that brain gangliosides in lipid vesicles are effective receptors for influenza virus.[44] Multilamellar liposomes containing mixed brain gangliosides specifically bind bovine thyrotrophin.[45] The binding is characterized by high affinity and is decreased with increasing ionic strength and temperature, properties similar to those displayed in the binding of thyrotrophin to thyroid plasma membranes. Gangliosides from the membranes of chromaffin granules isolated from bovine adrenal medulla have been identified as haematosides (ganglioside G_{M_3}), which migrated on thin-layer chromatograms as three principal species.[46] Endogenous lipids and proteins in subcellular fractions of bovine retina have been labelled with the aid of CMP-*N*-[^{3}H]acetylneuraminic acid and GDP-D-[^{14}C]mannose. *N*-Acetylneuraminic acid was located on lipid material indistinguishable from gangliosides, while D-mannose was incorporated into polyprenyl D-mannopyranosyl phosphate, which is possibly further utilized in the synthesis of glycoproteins.[47] The principal ganglioside in bovine retina has been identified as a disialyldihexosylceramide by m.s. of the fully methylated, the methylated and reduced, and the methylated, reduced, and trimethylsilylated derivatives.[48] Proof was obtained that the *N*-acetylneuraminic acid residues are linked to one another to form a terminal disaccharide unit.

The initial rate of synthesis of dolichyl D-mannopyranosyl phosphate in white matter from the brains of actively myelinating pigs is higher than that in similar preparations from adult pigs.[49] Exogenous dolichyl phosphate enhanced the incorporation of D-[^{14}C]mannose into an oligosaccharyl-lipid and glycoproteins, supporting the view that alterations in the cellular level of dolichyl phosphate may regulate the lipid-mediated synthesis of glycoproteins. Microsomal enzymes from pig liver catalysed the transfer of D-glucose from dolichyl D-glucopyranosyl phosphate to an endogenous acceptor, which was identified as a P^1-dolichyl P^2-oligosaccharyl pyrophosphate containing residues of D-glucose, D-mannose, and some hexosamine.[50] The principal glycolipids in pig adipose tissues have been isolated and characterized as tetraglycosylceramide (globoside, α-D-Gal*N*Ac*p*-(1 → 3)-α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide) and the disialylganglioside $G_{D_{1a}}$.[51] Small amounts of haematosides, L-fucosylmonosialylgangliosides, and a disialylganglioside containing both *N*-acetyl- and *N*-glycolylneuraminic acids are also found in these tissues.

Five branched fucolipids extracted from pig gastric mucosa with organic solvents have been purified to homogeneity by chromatography on DEAE-

[43] W. Curatolo, D. M. Small, and G. G. Shipley, *Biochim. Biophys. Acta*, 1977, **468**, 11.
[44] F. J. Sharom, D. G. Barratt, A. E. Thede, and C. W. M. Grant, *Biochim. Biophys. Acta*, 1976, **455**, 485.
[45] S. M. Aloj, L. D. Kohn, G. Lee, and M. F. Meldolesi, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 1053.
[46] H. Dreyfus, D. Aunis, S. Harth, and P. Mandel, *Biochim. Biophys. Acta*, 1977, **489**, 89.
[47] J. A. Curtino, H. J. Maccioni, and R. Caputto, *J. Neurochem.*, 1977, **28**, 803.
[48] M. Holm, I. Pascher, and B. E. Samuelsson, *Biomed. Mass Spectrometry*, 1977, **4**, 77.
[49] J. B. Harford, C. J. Waechter, and F. L. Earl, *Biochem. Biophys Res. Comm.*, 1977, **76**, 1036.
[50] E. Tabora and N. H. Behrens, *Mol. Cell. Biochem.*, 1977, **16**, 193.
[51] M. Ohashi and T. Yamakawa, *J. Biochem. (Japan)*, 1977, **81**, 1675.

Sephadex and magnesia–silica gel.[52, 53] Partial hydrolysis with acid, oxidations with the periodate ion and chromium trioxide, methylation analysis, and serological studies established their structures as (9)—(13), which are similar to those of oligosaccharides in blood-group-active glycoproteins and glycolipids from pig gastric mucosa.

α-D-Gal*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
2 6
↑ ↑
1 1
α-L-Fuc*p* R

(9) R = β-D-Gal*p*
(10) R = β-D-Glc*N*Ac*p*-(1 → 4)-β-D-Gal*p*
(11) R = α-D-Gal*N*Ac*p*-(1 → 3)-β-D-Gal*p*

α-D-Gal*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
2 6
↑ ↑
1 1
α-L-Fuc*p* β-D-Gal*p*
3
↑
1
α-D-Gal*N*Ac*p*

(12)

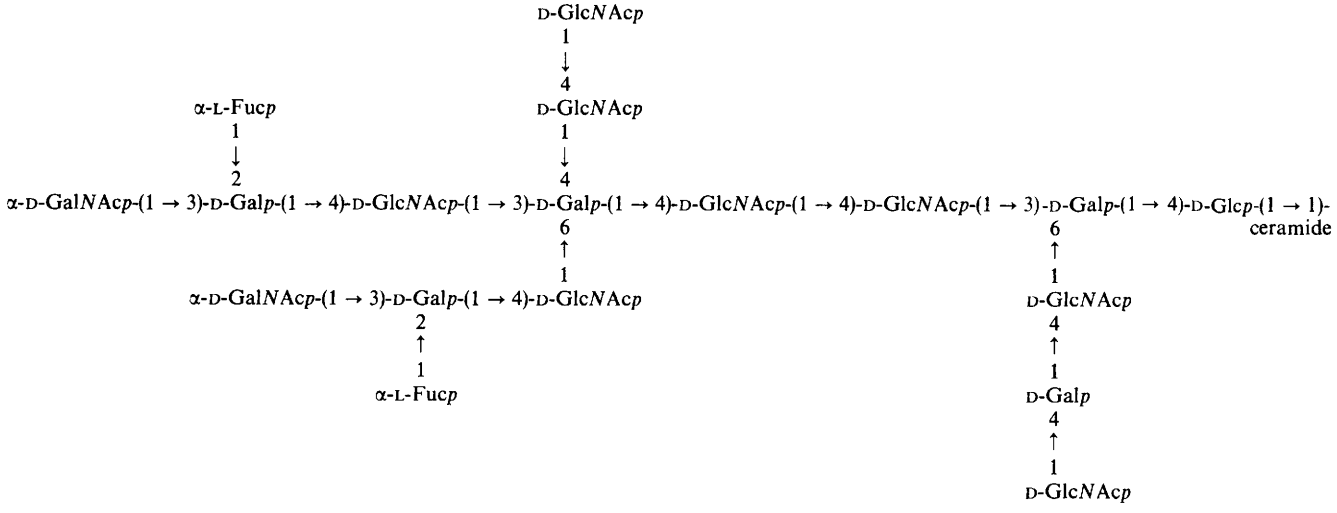
β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 4)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
3 6
↑ ↑
1 1
α-L-Fuc*p* β-D-Gal*p*
4
↑
1
β-D-Glc*N*Ac*p*

(13)

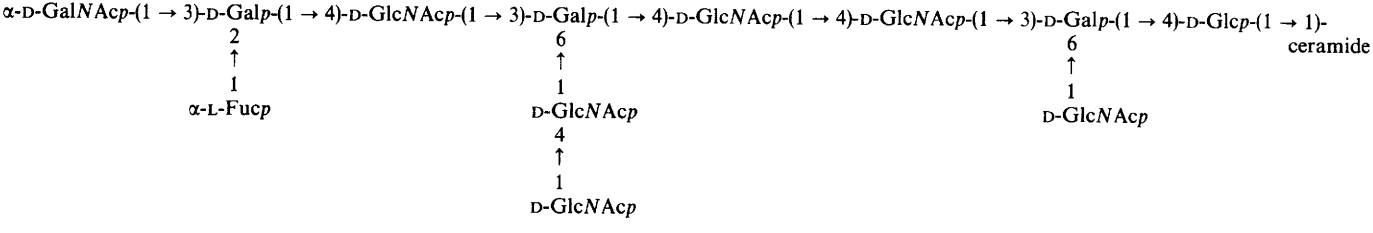
Two glycolipids (14) and (15), which contain 18 and 12 monosaccharide residues, respectively, and which exhibit blood-group A activity, have been isolated from a water-soluble glycolipid fraction from pig gastric mucosa.[54] They have several structural features in common, although differing in the number and nature of the side-chains attached to interior D-galactopyranosyl residues.

Water-soluble glycosphingolipids containing residues of D-galactose, D-glucose, and 2-acetamido-2-deoxy-D-galactose from canine gastric mucosa have been identified as a pentaglycosylceramide having the same carbohydrate structure as the Forssman hapten [α-D-Gal*N*Ac*p*-(1 → 3)-β-D-Gal*N*Ac*p*-(1 → 3)-α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide] and the branched heptaglycosyl-

[52] B. L. Slomiany and A. Slomiany, *Biochim. Biophys. Acta*, 1977, **486**, 531.
[53] B. L. Slomiany and A. Slomiany, *Chem. Phy. Lipids*, 1977, **20**, 57.
[54] B. L. Slomiany and A. Slomiany, *F.E.B.S. Letters*, 1977, **73**, 175.


D-GlcNAcp
1
↓
4
α-L-Fucp
1
↓
2
D-GlcNAcp
1
↓
4
α-D-GalNAcp-(1 → 3)-D-Galp-(1 → 4)-D-GlcNAcp-(1 → 3)-D-Galp-(1 → 4)-D-GlcNAcp-(1 → 4)-D-GlcNAcp-(1 → 3)-D-Galp-(1 → 4)-D-Glcp-(1 → 1)-
ceramide
6
↑
1
α-D-GalNAcp-(1 → 3)-D-Galp-(1 → 4)-D-GlcNAcp
2
↑
1
α-L-Fucp
6
↑
1
D-GlcNAcp
4
↑
1
D-Galp
4
↑
1
D-GlcNAcp

(14)


α-D-GalNAcp-(1 → 3)-D-Galp-(1 → 4)-D-GlcNAcp-(1 → 3)-D-Galp-(1 → 4)-D-GlcNAcp-(1 → 4)-D-GlcNAcp-(1 → 3)-D-Galp-(1 → 4)-D-Glcp-(1 → 1)-
ceramide
2
↑
1
α-L-Fucp
6
↑
1
D-GlcNAcp
4
↑
1
D-GlcNAcp
6
↑
1
D-GlcNAcp

(15)

ceramide (16).[55,56] An octaglycosylceramide containing residues of L-fucose, D-galactose, D-glucose, and 2-acetamido-2-deoxy-D-galactose was also isolated.

α-D-Gal*N*Ac*p*-(1 → 3)-β-D-Gal*N*Ac*p*-(1 → 3)-α-D-Gal*p*-(1 → 4)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
4
↑
1
β-D-Gal*N*Ac*p*
3
↑
1
β-D-Gal*p*

(16)

G_{D_3}[α-Neu*N*Ac*p*-(2 → 8)-α-Neu*N*Ac*p*-(2 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide], the principal ganglioside in mammalian retina, appears to be degraded *in vivo* in two stages.[57] During the first stage (2—4 weeks) following intraocular injection of 2-[^{3}H]acetamido-2-deoxy-D-mannose (a precursor), 50% of ganglioside G_{D_3} was degraded, whereas the other retinal gangliosides did not undergo catabolism, indicating that ganglioside G_{D_3} has a different cellular or subcellular location from the others.

Neuraminic acid residues in glycolipids and glycoproteins present in the synaptic membrane of rats are released *in situ* by the action of a neuraminidase, whose action may be required to modify the physical properties of the membrane.[58] Two clonal lines of astroblasts—one derived from tumorous rat astroblasts and the other from baby hamster astroglia—have been shown to contain a simple pattern of gangliosides, with G_{M_3} as the principal component.[59] The effect of anti-ganglioside serum on suspensions of cerebellar cells from immature rats indicated that gangliosides are located on the outer surface of the plasma membrane in most cells.[60] This finding suggests that immunohistochemical methods could be usefully applied in locating these haptens on the cell membrane. The marked reduction in the ability of thyrotrophin to bind to tumorous thyroid plasma membranes in rats has been related to the absence from the membranes of such gangliosides as $G_{D_{1b}}$ {β-D-Gal*p*-(1 → 3)-β-D-Gal*N*Ac*p*-(1 → 4)-[α-Neu*N*Ac*p*-(2 → 8)-α-Neu*N*Ac*p*-(2 → 3)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide}, $G_{T_{1a}}$ {α-Neu*N*Ac*p*-(2→ 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*N*Ac*p*-(1 → 4)-[α-Neu*N*Ac*p*-(2 → 8)-α-Neu*N*Ac*p*-(2 → 3)]-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide}, and G_{M_1}, which in the normal membrane are able to bind thyrotrophin.[61]

The fragmentation patterns of the TMS derivatives of five reference 2-acetamido-2-deoxy-3- or -4- or -6-*O*-hexopyranosyl-D-hexitols have been examined following g.l.c.–m.s.; the information was then used to determine the position of

[55] A. Slomiany and B. L. Slomiany, *European J. Biochem.*, 1977, **76**, 491.
[56] A. Slomiany, B. L. Slomiany, and C. Annese, *F.E.B.S. Letters*, 1977, **81**, 157.
[57] M. Holm, *F.E.B.S. Letters*, 1977, **77**, 225.
[58] H. C. Yohe and A. Rosenberg, *J. Biol. Chem.*, 1977, **252**, 2412.
[59] J. Robert, G. Rebel, and P. Mandel, *J. Lipid Res.*, 1977, **18**, 517.
[60] N. A. Gregson, M. Kennedy, and S. Leibowitz, *Nature*, 1977, **266**, 461.
[61] M. F. Meldolesi, P. H. Fishman, S. M. Aloj, L. D. Kohn, and R. O. Brady, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, 73, 4060.

the linkage in related disaccharide alditols derived from rat-brain glycoproteins and gangliosides.[62]

The glycoproteins and glycolipids in hyperplastic nodules induced in rat liver by *N*-(2-fluorenyl)acetamide have been shown to contain high proportions of sialic acid.[63] Rat-liver preparations rapidly take up liposomes containing ganglioside G_{M_1} and the related asialoganglioside.[64] Competitive experiments with asialofetuin suggested that this uptake is mediated by specific recognition of the terminal β-D-galactopyranosyl residue of the glycolipid in the liposomes by a receptor on the plasma membrane of rat-liver parenchymal cells. Analyses of the fatty acids and long-chain bases in glycolipids from rat intestines suggested that haematoside (G_{M_3}) and trihexosylceramide, which are abundant in villus and crypt cells, respectively, may be derived from different lactosylceramide precursors.[65] A study of glycolipids during the growth of granulomas in rats revealed the presence of a tetrahexosylceramide and haematoside, *etc.*, although another glycolipid containing residues of D-glucose, D-galactose, 2-acetamido-2-deoxy-D-galactose, and L-fucose (molar proportions 1:3:1:1) is present only at an early stage.[66] The synthesis of fucolipids in normal rat cells and in rat cells transformed with murine sarcoma virus has been examined as a function of the cell-population density.[67]

The synthesis of D-galactopyranosyl retinyl phosphate by a particulate enzyme fraction from murine mastocytomas was stimulated by detergents and by retinol.[68] The synthase has an absolute requirement for Mn^{2+} or Mg^{2+} ions. Long-chain gangliosides in the neuronal cells of embryonic mice have been shown to bind tetanus toxin.[69] Two myelin-specific glycosphingolipids – a D-galactosylceramide and its 3-*O*-sulphate – have been found in cloned cell lines derived from murine neurotumours.[70] The incorporation of labelled precursors into sialoglycosphingolipids has been used to study the synthesis of gangliosides in murine neuroblastoma cells.[71] The results agree with those of previous studies on the glycosyltransferases in these cells and support the view that gangliosides are synthesized by the pathway $G_{M_3} \rightarrow G_{M_2} \rightarrow G_{M_1}$.[72] Mice and humans with mammary carcinomas have elevated levels of circulating glycolipids in their sera.[73] Although the increased levels of circulating glycolipids may result from secondary responses to the tumours, they could provide a basis for the early detection of this type of cancer. Chemically transformed cells of murine fibroblasts lacking ganglioside G_{M_1} were found to be unresponsive to choleragen, although they responded to choleragen after incorporating exogenous ganglioside G_{M_1}.[74] The

[62] J. Finne, I. Mononen, and J. Kärkkäinen, *Biomed. Mass Spectrometry*, 1977, **4**, 281.
[63] W. D. Merritt, T. W. Keenan, and D. J. Morré, *Cancer Biochem. Biophys.*, 1976, **1**, 179.
[64] A. Surolia and B. K. Bachhawat, *Biochim. Biophys. Acta*, 1977, **497**, 760.
[65] J. F. Bouhours and R. M. Glickman, *Biochim. Biophys. Acta*, 1977, **487**, 51.
[66] E. Hanada, K. Konno, S. Handa, and T. Yamakawa, *J. Biochem. (Japan)*, 1977, **81**, 1079.
[67] M. Gacto and S. Steiner, *Biochim. Biophys. Acta*, 1976, **444**, 11.
[68] P. A. Peterson, L. Rask, T. Helting, L. Ostberg, and Y. Fernstedt, *J. Biol. Chem.*, 1976, **251**, 4986.
[69] W. Dimpfel, R. T. C. Huang, and E. Habermann, *J. Neurochem.*, 1977, **29**, 329.
[70] G. Dawson, N. Sundarraj, and S. E. Pfeiffer, *J. Biol. Chem.*, 1977, **252**, 2777.
[71] S. F. Kemp and A. C. Stoolmiller, *J. Biol. Chem.*, 1976, **251**, 7626.
[72] S. F. Kemp and A. C. Stoolmiller, *J. Neurochem.*, 1976, **26**, 723.
[73] T. M. Kloppel, T. W. Keenan, M. J. Freeman, and D. J. Morré, *Proc. Nat. Acad. Sci. U.S.A.*, 1977, **74**, 3011.
[74] J. Moss, P. H. Fishman, V. C. Manganiello, M. Vaughan, and R. O. Brady, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 1034.

cells are unable to synthesize ganglioside G_{M_1} owing to the absence of the enzymes CMP-sialic acid:lactosylceramide sialyltransferase and UDP-D-galactose:G_{M_2}-D-galactosyltransferase.[75] The cells are also able to bind other gangliosides present in the culture medium, although none of them conferred a responsiveness to choleragen. Changes in the level of ganglioside G_{M_2} and of 2-acetamido-2-deoxy-D-galactosyltransferase activity in a temperature-sensitive mutant of murine 3T3 cells transformed with SV 40 virus have been attributed to factors controlling cell growth.[76]

A particulate preparation from the pupae of the insect *Ceratitis capitata* contained enzymes that transfer D-glucose, D-mannose, and 2-acetamido-2-deoxy-D-glucose from nucleotide-linked precursors to an endogenous lipid acceptor of the polyprenyl phosphate type.[77] The transfer reactions were stimulated by a partially purified lipid extract from the insect and also by liver dolichyl phosphate.

Each of three globosides from spermatozoa of the fresh-water bivalve *Hyriopsis schlegelii* contains D-mannose instead of D-galactose in the carbohydrate portion of the molecule.[78] The structure (17) was deduced for one of them on the basis of methylation analysis, enzymic hydrolysis, and oxidation with chromium trioxide.

β-D-Glc*N*Ac*p*-(1 → 2)-β-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
(17)

Microbial Glycolipids

The disaccharide covalently attached to lipid by enzyme preparations from *Acetobacter xylinium* has been identified as maltose and not cellobiose, as earlier suggested.[79] The maltosyl-lipid might act as a primer for the synthesis of the α-D-glucan. 6,6′-Di-*O*-corynomycolyltrehalose and 6-*O*-corynomycolyltrehalose have been isolated from glycolipids obtained from *Brevibacterium vitarumen*, thus demonstrating a similarity between this strain and typical aerobic Corynebacteria.[80]

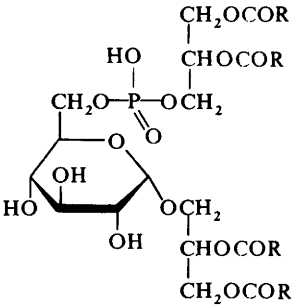
The formation of lipid-linked sugars *in vitro* by *Bacillus stearothermophilus*, *Salmonella typhimurium*, and *Shigella flexneri* requires an unsaturated α-isoprenyl residue in a polyprenyl phosphate, in contrast to that in rat-liver microsomes which requires a saturated α-isoprenyl residue.[81] D-Glucopyranosyl polyprenyl phosphate was formed on incubation of UDP-D-[^{14}C]glucose with particulate preparations from *B. stearothermophilus*, and it may serve as a D-glucosyl donor in the synthesis of an extracellular polysaccharide.[82] The properties of the membrane-associated sialyltransferase complexes of *Escherichia coli* have been examined.[83] These complexes catalyse the incorporation of *N*-acetylneuraminic

[75] P. H. Fishman, J. Moss, and M. Vaughan, *J. Biol. Chem.*, 1976, **251**, 4490.
[76] K. Itaya and S. Hakomori, *F.E.B.S. Letters*, 1976, **66**, 65.
[77] L. A. Quesada Allue, L. R. Maréchal, and E. Belocopitow, *F.E.B.S. Letters*, 1976, **67**, 243.
[78] T. Hori, M. Sugita, J. Kanbayashi, and O. Itasaka, *J. Biochem. (Japan)*, 1977, **81**, 107.
[79] H. Sandermann, *F.E.B.S. Letters*, 1977, **81**, 294.
[80] M.-A. Lanéelle and J. Asselineau, *Biochim. Biophys. Acta*, 1977, **486**, 205.
[81] T. Mańkowski, W. Sasak, E. Janczura, and T. Chojnacki, *Arch. Biochem. Biophys.*, 1977, **181**, 393.
[82] W. Sasak and T. Chojnacki, *Arch. Biochem. Biophys.*, 1977, **181**, 402.
[83] I. K. Vijay and F. A. Troy, *J. Biol. Chem.*, 1975, **250**, 164.

acid from CMP-*N*-acetylneuraminic acid into polymeric products and a lipid fraction.[84] Reconstitution of the enzymic activity in lipid-depleted membrane complexes depends on undecaprenyl phosphate—this C_{55}-isoprenoid alcohol accounts for 95% of the polyisoprenols, the other 5% being the C_{60}-homologue. The complexes dissociate in organic solvents.

Studies with mutant strains of *E. coli* have demonstrated that there is a connection between the turnover of membrane phospholipids and the synthesis of the so-called 'membrane-derived oligosaccharides', which are D-gluco-oligosaccharides variously substituted with *sn*-glycerol phosphate, ethanolamine phosphate, and succinate on the outer envelope of the cell.[85] The glycerol phosphate residues of these oligosaccharides appear to be donated by phosphatidylglycerol or the lipid cardiolipin or both.

The synthesis of di-*O*-acyl-D-glucuronosylglycerol from UDP-D-glucuronic acid and di-*O*-acylglycerol has been accomplished using particulate preparations from *Pseudomonas diminuta*; UDP-D-glucuronic acid could not be replaced by D-glucuronic acid, D-glucuronic acid 1-phosphate, or other nucleotide-linked sugars in this system.[86] The *in vitro* synthesis of this glycolipid requires exogenous di-*O*-acylglycerols containing unsaturated and shorter chain fatty acids. A phospholipid isolated from *P. diminuta* has been identified as the tetra-acylated derivative



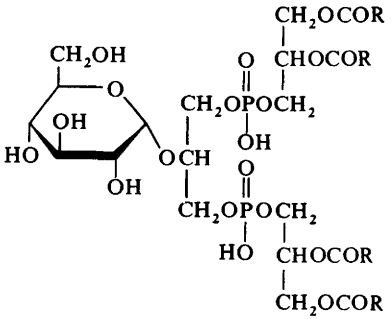
R is a fatty-acid residue

(18)

(18).[87] The biosynthesis of this phospholipid involves the transfer of the phosphatidyl residue from phosphatidylglycerol to O-6′ of a diacyl-D-glucosylglycerol. Evidence for this comes from studies of the kinetics, stoicheiometry, substrate specificity, and products, as well as from labelling studies. A particulate preparation from *P. halosaccharolytica* was able to transfer the D-glucosyl residue from UDP-D-glucose to a phospholipid, probably a phosphatidylglycerol.[88]

Among the polar lipids isolated from group B *Streptococci* was a novel glycophospholipid containing residues of D-glucose, glycerol, fatty acids, and phosphate (molar proportions 1:3:4:2).[89] The products obtained from acid and alkaline

[84] F. A. Troy, I. K. Vijay, and N. Tesche, *J. Biol. Chem.*, 1975, **250**, 156.
[85] H. Schulman and E. P. Kennedy, *J. Biol. Chem.*, 1977, **252**, 4250.
[86] J. M. Shaw and R. A. Pieringer, *J. Biol. Chem.*, 1977, **252**, 4391.
[87] J. M. Shaw and R. A. Pieringer, *J. Biol. Chem.*; 1977, **252**, 4395.
[88] Y. Ohno, I. Yano, and M. Masui, *J. Biochem.* (*Japan*), 1976, **80**, 1429.
[89] W. Fischer, *Biochim. Biophys. Acta*, 1977, **487**, 74.



R is a fatty-acid residue

(19)

hydrolyses, Smith degradation, and periodate oxidation and hydrazinolysis, *etc.*, identified it as (19).

Polymers composed of glycerol, fatty acids, neutral sugars, and *N*-acylated amino-sugars have been isolated from mycoplasmas.[90] The neutral sugars, the proportions of which varied in polymers from four *Acholeplasma* sp., two *Anaeroplasma* sp., and *Mycoplasma neurolyticum*, were identified as D-glucose, D-galactose, and D-mannose, while the amino-sugars include 2-amino-2,6-dideoxygalactose (fucosamine), 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and an unidentified amino-sugar.

The principal sphingolipids from *Aspergillus oryzae* have been identified, mainly by g.l.c.–m.s., as *N*-(2-hydroxystearoyl)-1-*O*-D-glucopyranosyl-17(?)-methylsphingadienine and the corresponding *N*-(2-hydroxyoctadecenoic acid) derivative.[91]

Glycosyl-transfer reactions in the nuclear membranes of *Saccharomyces cerevisiae* are mediated by a lipid intermediate.[92] Studies with UDP-2-acetamido-2-deoxy-D-glucose and GDP-D-mannose revealed that relatively high levels of P^1-2-acetamido-2-deoxy-D-glucopyranosyl P^2-dolichyl pyrophosphate and a D-mannosylated protein are formed, consistent with the role of P^1-2-acetamido-2-deoxy-D-glucopyranosyl P^2-dolichyl pyrophosphate as an intermediate in the glycosylation of nuclear glycoproteins.

Plant Glycolipids

Novel glycolipids isolated from ripening corn seeds contain residues of D-galactose or D-glucose, ethanediol, and fatty acids.[93] Analysis of the deacylation products of one of the isolated glycolipids showed that they are derived from a 1-*O*-acyl-2-*O*-β-D-galactopyranosylethanediol, which normally contains palmitic, stearic, or oleic acid as the fatty-acid component. The synthesis of digalactosyldiglycerol by

[90] P. F. Smith, T. A. Langworthy, and W. R. Mayberry, *J. Bacteriol.*, 1976, **125**, 916.
[91] Y. Fujino and M. Ohnishi, *Biochim. Biophys. Acta*, 1977, **486**, 161.
[92] G. Palamarczyk and E. Janczura, *F.E.B.S. Letters*, 1977, **77**, 169.
[93] V. A. Vaver, K. G. Todria, N. V. Prokazova, B. V. Rozynov, and L. D. Bergelson, *Biochim. Biophys. Acta*, 1977, **486**, 60.

cell-free preparations from pea leaves involves the transfer of D-galactose from UDP-D-galactose to D-galactosyldiglycerol.[94] The level of (D-glucosyl 6-sulphate)-diglyceride was reduced substantially in cells of *Chlorella pyrenoidosa* growing on cysteine as the only source of sulphur.[95]

[94] M. Siebertz and E. Heinz, *Z. physiol. Chem.*, 1977, **358**, 27.
[95] M. Sinensky, *J. Bacteriol.*, 1977, **129**, 516.

8

Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, and Glycolipids

BY J. F. KENNEDY

Synthesis of Polysaccharides, Oligosaccharides, Glycoproteins, Enzymes, and Glycolipids

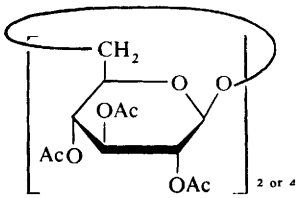
Polysaccharides.—Melt-polymerization of 1,2,3,4-tetra-*O*-acetyl-β-D-galactopyranose with 5% of zinc chloride yielded, after deacetylation, 6-*O*-β-D-galactopyranosyl-D-galactopyranose and a β-D-galactan which methylation analysis showed to be predominantly (1 → 6)-linked.[1]

The PF_5-catalysed copolymerization of 1,6-anhydro-2,3,4-tri-*O*-(4-methylbenzyl)-β-D-glucopyranose and 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-mannopyranose gave stereoregular polymers, as predicted by classical theory.[2] Conformational analysis was invoked to explain differences in the reactivities of the monomers and the derived cations towards polymerization and copolymerization.

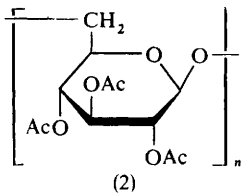
D-Glucose in anhydrous hydrogen fluoride at 20°C formed oligosaccharides and low-molecular-weight polysaccharides composed of D-glucose and 3,6-anhydro-D-glucose in ratios of 10:1 to 5:1, depending on the reaction conditions.[3]

Derivatives of 2,3,3′,4,4′-penta-*O*-acetylsucrose containing as many as three 4-vinylbenzoyl groups gave pseudopolysaccharides on copolymerization with styrene or methyl methacrylate.[4] The glass-transition temperatures of the copolymers were determined before and after removal of the acetyl groups.

Polycondensation of 2,3,4-tri-*O*-acetyl-6-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranosyl bromide in acetonitrile containing mercuric salts yielded the cyclic and linear oligomers (1) and (2), respectively.[5] Methylation analy-



(1)



(2)

[1] E. E. Lee, G. Keaveney, A. Hartigan, and P. S. O'Colla, *Carbohydrate Res.*, 1976, **49**, 475.
[2] K. Kobayashi and C. Schuerch, *J. Polymer Sci., Polymer Chem.*, 1977, **15**, 913.
[3] U. Kraska and F. Micheel, *Carbohydrate Res.*, 1976, **49**, 195.
[4] R. D. Guthrie, A. D. Jenkins, and T. J. Lucas, *European Polymer J.*, 1977, **13**, 409.
[5] D. Gagnaire and M. Vignon, *Carbohydrate Res.*, 1976, **51**, 140.

sis and ^{1}H and ^{13}C n.m.r. measurements showed that (2) contains β-D-(1 → 6)-linked residues.

Oligosaccharides.—The synthesis of oligosaccharides (*e.g.* 3-*O*-α-D-altropyranosyl-D-glucose) by way of cycloaddition reactions[6] was mentioned in last year's Report (p. 92), while the synthesis of such trisaccharides as *O*-α-D-mannopyranosyl-(1 → 4)-*O*-α-L-rhamnopyranosyl-(1 → 3)-D-galactopyranose[7] is covered in Part I of this Report.

Glycoproteins.—αα-Trehalose has been covalently linked to bovine serum albumin through HO-6 or -6′ or both by way of 4-aminobenzoic acid.[8]

Synthetic glycoproteins have been prepared by reductive amination of proteins (*e.g.* bovine serum albumin) and reducing disaccharides (*e.g.* maltose, lactose, and melibiose) in the presence of sodium cyanoborohydride.[9] The reaction proceeded readily in aqueous solution over a broad pH-range and gave high degrees of substitution which could be determined by amino-acid analyses, since the secondary amine linkage formed with the ε-amino-group of lysyl residues is stable to acid conditions that hydrolyse the peptide linkages.

Disaccharides representing the antigenic determinants of the O-antigenic polysaccharide chains of *Salmonella* sero-groups B and D have been coupled by way of their 4-isothiocyanatophenyl glycosides to bovine serum albumin to give pseudoglycoproteins, which have been used as immunogens for the preparation of specific anti-*Salmonella* O antisera.[10]

Aldonic acid derivatives of oligosaccharides have been linked to amino-groups in proteins following activation of the carboxylic acid group with a water-soluble carbodi-imide.[11] The immunological and other properties of pseudoglycoproteins obtained from bovine serum albumin and melibionic, gentiobionic, lactobionic, maltobionic, or maltotrionic acids were assessed using antibodies raised against the conjugates and the phytohaemagglutinins, respectively. Affinity matrices suitable for use in the purification of lectins have been prepared by direct reductive amination of a disaccharide (*e.g.* lactose, maltose, melibiose, and di-*N*-acetylchitobiose) and an aminoethyl polyacrylamide gel with sodium cyanoborohydride in aqueous solution at pH 9, followed by *N*-acetylation of any remaining amino-groups.[12] Columns of such materials are very stable, have good flow-rates, and high binding-capacities.

Copolymerization of allyl α-D-galactopyranoside and acrylamide produced an insoluble pseudoglycoprotein that was used in the purification of peanut lectin.[13]

Enzymes.—Peptides related to some of the sequences of hen egg-white lysozyme have been synthesized to aid in identifying the antigenic sites of the enzyme.[14] A semi-synthetic approach to fragments of hen egg-white lysozyme has been de-

[6] S. David, A. Lubineau, and J.-M. Vatèle, *J.C.S. Perkin I*, 1976, 1831.
[7] N. K. Kochetkov, E. M. Klimov, and V. I. Torgov. *Bull. Acad. Sci. U.S.S.R.*, 1976, **25**, 154.
[8] W. J. Gensler and I. Alam, *J. Org. Chem.*, 1977, **42**, 130.
[9] B. A. Schwartz and G. R. Gray, *Arch. Biochem. Biophys.*, 1977, **181**, 542.
[10] G. Ekborg, K. Eklind, P. J. Garegg, B. Gotthammar, H. E. Carlsson, A. A. Lindberg, and B. Svenungsson, *Immunochemistry*, 1977, **14**, 153.
[11] J. Lönngren, I. J. Goldstein, and J. E. Niederhuber, *Arch. Biochem. Biophys.*, 1976, **175**, 661.
[12] R. J. Baues and G. R. Gray, *J. Biol. Chem.*, 1977, **252**, 57.
[13] K. Sutoh, L. Rosenfeld, and Y. C. Lee, *Analyt. Biochem.*, 1977, **79**, 329.
[14] C.-L. Lee and M. Z. Atassi, *Biochem. J.*, 1976, **159**, 89.

scribed and used to obtain five fragments containing between 31 and 51 amino-acid residues.[15] Disulphide peptides related to those in lysozyme have been synthesized, purified, and characterized and used in studies of the enzymic and immunochemical properties of lysozyme.[16]

Glycolipids.—3-*O*-[6-*O*-(6-*O*-α-D-Galactopyranosyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-1,2-di-*O*-stearoyl-L-glycerol (3), a 'trigalactosyl diglyceride', has been synthesized by the sequence of reactions shown in Scheme 1.[17]

Modification of Polysaccharides and Oligosaccharides, and Uses of Modified Polysaccharides and Oligosaccharides

Introduction.—A book entitled 'Polysaccharide Shapes' has discussed how the units of polysaccharides link together to confer a unique set of properties on the chain.[18] Papers presented at a symposium on the thermal uses and properties of carbohydrates and lignins have covered some aspects of the pyrolysis of natural polysaccharides.[19]

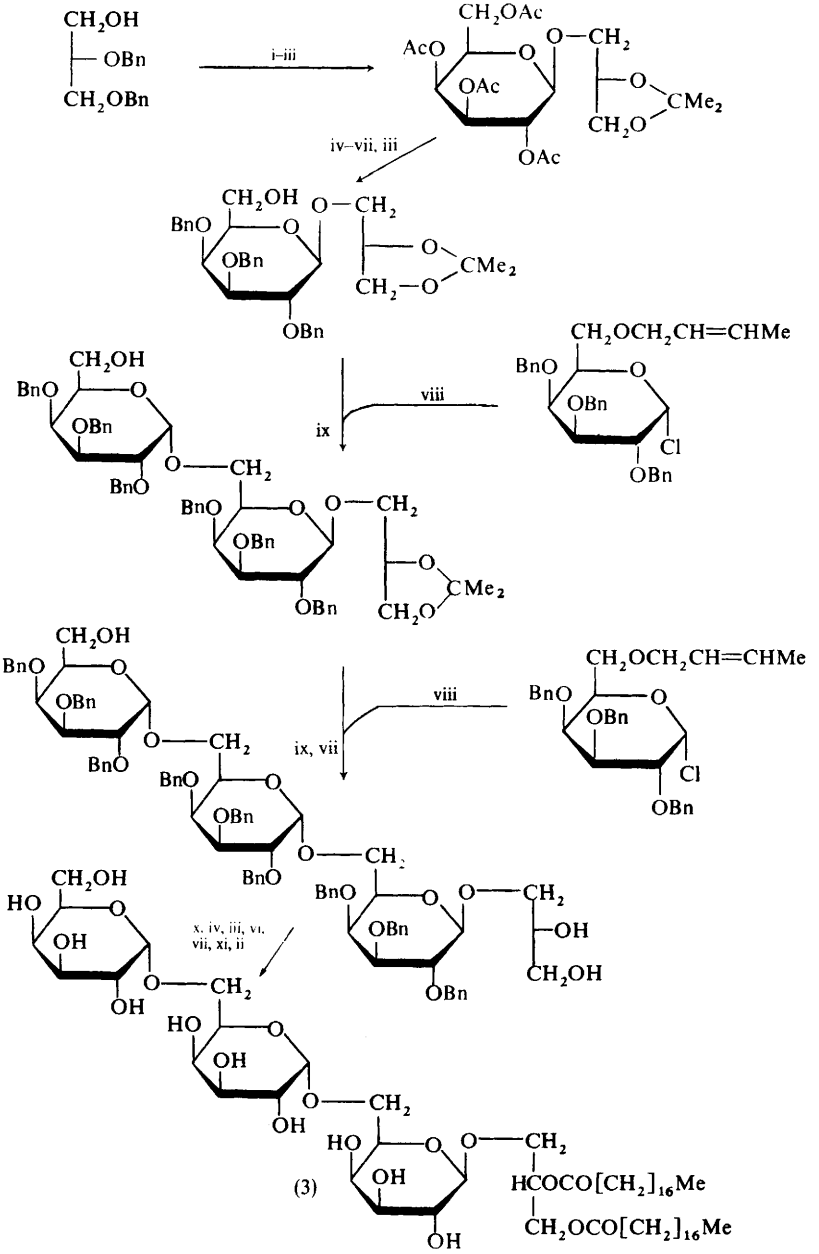
The importance of modified polysaccharides has been commented on briefly,[20] and the uses of derivatized polysaccharides in the purification of biologically active molecules have been discussed.[21] Polysaccharide derivatives used as affinity adsorbents and for immobilizing proteins have been prepared by the routes shown in Scheme 2.[22]

The quantitative effect of diffusion control on the rate of radiation-initiated graft polymerization has been studied theoretically for systems in which the diffusion-free reaction may show various dependences of rate on the concentration of monomer other than the usual first-order dependence.[23] The study is very general and could be applied to systems involving a variety of modes of initiation and termination.

The oxidation of polysaccharides with periodate is inhibited, to varying extents, by the formation of hemiacetals, so that further oxidation can be performed only after the removal (by reduction with sodium borohydride) of the protecting hemiacetals.[24] Theoretical analysis of the kinetics of such repeated oxidations predicts that, for complete protection, the oxidation limit of the second oxidation, as a function of the extent of the first oxidation, lies between 41.1 and 43.2%. This prediction is supported by values found experimentally for alginate.

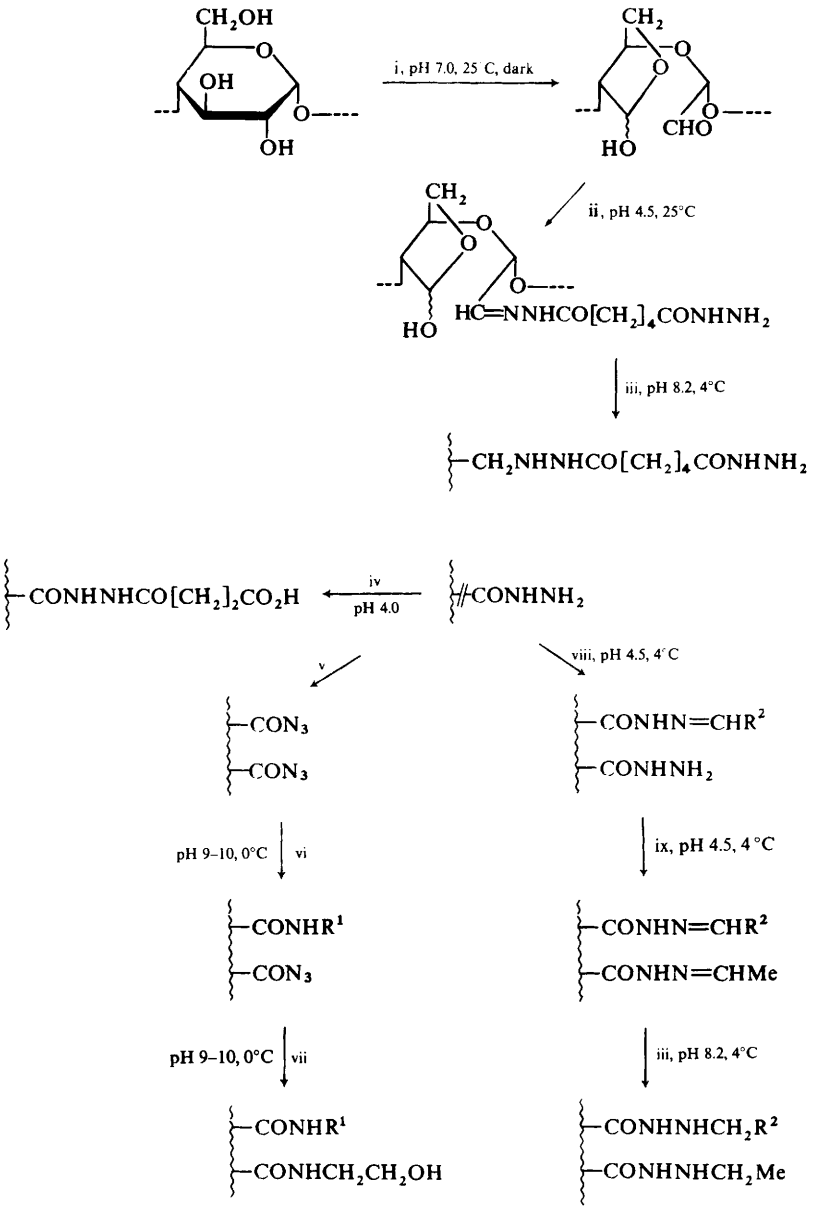
Agarose.—Macroporous agarose has been used in the purification of D-galactose oxidase[25] and of D-galactose-binding lectins from castor beans (*Ricinus communis*)[26] by affinity chromatography. Microscopic examination has shown that beaded

[15] A. R. Rees and R. E. Offord, *Biochem. J.*, 1976, **159**, 487.
[16] M. Z. Atassi, J. Koketsu, and A. F. S. A. Habeeb, *Biochim. Biophys. Acta*, 1976, **420**, 358.
[17] P. A. Gent and R. Gigg, *J. C. S. Perkin I*, 1975, 1779.
[18] D. A. Rees, 'Outline Studies in Biology: Polysaccharide Shapes', Chapman and Hall, London, 1977.
[19] 'Thermal Uses and Properties of Carbohydrates and Lignins', ed. F. Shafizadeh, K. V. Sarkanen, and D. A. Tillman, Academic Press, New York, 1976.
[20] W. G. Overend, *Chem. and Ind.*, 1976, 61.
[21] M. Wilchek and C. S. Hexter, in 'Methods of Biochemical Analysis', vol. 23, ed. D. Glick, Wiley, Chichester, 1976, p. 347.
[22] E. Junowicz and S. E. Charm, *Biochim. Biophys. Acta*, 1976, **428**, 157.
[23] K. Imre, G. Odian, and A. Rabie, *J. Polymer Sci., Polymer Chem.*, 1976, **14**, 3045.
[24] P. C. Hemmer and J. J. González, *J. Polymer Sci., Polymer Phys.*, 1977, **15**, 321.
[25] M. W. C. Hatton and E. Regoeczi, *Biochim. Biophys. Acta*, 1976, **438**, 339.
[26] H. W. Hsu, D. S. Davis, C. H. Wei, and W. K. Yang, *Analyt. Biochem.*, 1976, **73**, 513.



Reagents: i, 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide; ii, Pd–H_2; iii, Me_2CO–H^+; iv, KOH; v, TrCl–py; vi, BnCl–NaH–DMF; vii, H_3O^+; viii, Et_3N–$Et_4N^+Cl^-$; ix, KOBu^t–DMSO; x, *p*-$NO_2C_6H_4COCl$–py; xi, $Me[CH_2]_{16}COCl$–py

Scheme 1



R^1 = $[CH_2]_nNH_2$, $[CH_2]_nCO_2H$, $[CH_2]_2OPO_3^{2-}$, or protein;
R^2 = ribonucleotide, pyridoxal 5′-phosphate, RNA, or glycoprotein

Reagents: i, IO_4^-; ii, adipic acid bishydrazide; iii, $NaBH_4$; iv, succinic anhydride; v, $NaNO_2$–HCl; vi, R^1NH_2; vii, $H_2NCH_2CH_2OH$; viii, R^2CHO; ix, MeCHO

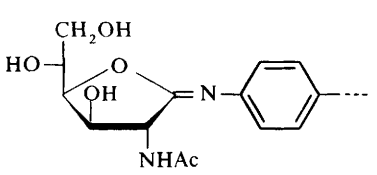
Scheme 2

agarose (Sepharose) and its derivatives deviate from a normal bead structure; such deviations may influence the chromatographic properties of agaroses.[27]

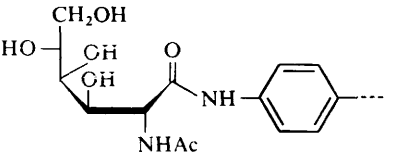
The amount of AMP or ATP coupled to agarose can be determined by combustion and assay of the phosphate content of the ash.[28]

Agarose has been modified for use in affinity chromatography by the reactions outlined in Scheme 2.[22] The reaction of periodate-oxidized, macroporous agarose with 1,6-diaminohexane and reduction ($NaBH_4$) gave a derivative to which glycine carboxypeptidase could be attached with retention of the enzymic activity.[29] Peptides were also coupled to periodate-oxidized agarose.

In the coupling of monosaccharide 1,4-lactones to benzidine-substituted agarose, it is not known whether an imino-lactone [*e.g.* (4)] or an amide [*e.g.* (5)] is formed.[30]



(4)



(5)

The use of butane-1,4-diol diglycidyl ether in coupling 2-acetamido-2-deoxy-D-glucose and -galactose, short oligomers of 2-amino-2-deoxy-D-glucose, methyl α-D-mannopyranoside, methyl β-D-galactopyranoside, lactose, stachyose, yeast mannan, and dextrin to agarose has been examined.[31] All but the reducing sugars withstand the alkaline conditions used in the coupling reaction, which primarily involves O-6 of saccharides and the amino-group of hexosamines. The agarose derivatives obtained in this way are useful affinity matrices or adsorbents for phytohaemagglutinins (lectins) and glycoside hydrolases.

Cyclic imidocarbonate derivatives of agarose continue to be used extensively for the immobilization of biologically active macromolecules and for affinity chromatography. Alkylamines react with agarose cyclic imidocarbonate to form *N*-substituted isoureas, which, being good nucleophiles, attack 4-nitrophenyl acetate (a chromogenic ester) with the release of an equivalent amount of 4-nitrophenol.[32] This reaction forms a basis for direct determination of the extent of derivatization of agarose.

Recent references to the preparation of active immobilized forms of enzymes (for use as immobilized enzymes) and immunologically active macromolecules (for use as immunoadsorbents) and of various affinants (for use as affinity-chromatography matrices) using agarose cyclic imidocarbonate are summarized in the Tables that follow (pp. 451–457), the separate categories being the subject of

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Table 1 *Use of agarose cyclic imidocarbonates for the preparation of active immobilized enzymes*

Enzyme coupled to agarose cyclic imidocarbonate	*E.C. No.*	*Ref.*
Alcohol dehydrogenase	1.1.1.1	33[b], 34
Carboxylesterase	3.1.1.1	35
Chorismate mutase[a]	5.4.99.5	36[c]
Chymotrypsin	3.4.21.1	37, 38
β-D-Galactosidase	3.2.1.23	39
D-Glucose oxidase	1.1.3.4	40
α-D-Glucosidase	3.2.1.20	39
β-D-Glucosidase	3.2.1.21	39
D-Glucose 6-phosphate dehydrogenase	1.1.1.49	41[d]
Lactate dehydrogenase	1.1.1.27	33[b], 34, 40, 42[e]
Lipoamide dehydrogenase isoenzymes[a]	1.6.4.3	43[f]
Peptidase (from human intestinal mucosa)	—	39
Peroxidase	1.11.1.7	40
Phosphatase	3.1.3.1/2	39
Ribonuclease	3.1.4.22/23	44[b]
Ribonuclease I	3.1.4.22	38, 45
αα-Trehalase	3.2.1.28	39
Trypsin	3.4.21.4	46, 47

[a] See (6), Scheme 3; [b] see entry in Table 3; [c] hydrophobic immobilization; [d] also co-immobilized with amino-acids; [e] using cross-linked agarose; [f] affinity coupling of the enzyme.

Table 2 *Use of agarose cyclic imidocarbonates for the preparation of immunoadsorbents*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Anti-(α-foetoprotein) antibody	Purification of rat α-foetoprotein by immunoadsorption	48
Anti-(*Azotobacter vinelandii* component I) antibody	Purification of the nitric-oxide reductase from *Azotobacter vinelandii* by immunoadsorption	49
Anti-(*Bacillus Calmette-Guérin*) antibody	Purification of tuberculin-active components by immunoadsorption	50
Anti-dinitrophenyl antibodies	Immunoadsorption of dinitrophenyl-labelled ribonuclease	37
Anti-(egg albumin) antibody	Isolation of CI-esterase from human sera by immunoadsorption	51
Anti-(*Phaseolus vulgaris* α-D-mannosidase) antibody	Purification of two forms of α-D-mannosidase from *Phaseolus vulgaris* by affinity chromatography	52
Anti-(*Phaseolus vulgaris* α-D-mannosidase I) antibody immunoglobulin IgG		53
Anti-(rat β-D-glucuronidase) antibody	Purification of rat-liver β-D-glucuronidase by immunoadsorption	54
Lipopolysaccharides from *Shigella sonnei* (blood-group O-specific)	Immunoadsorption of anti-(blood-group O-specific polysaccharide) antibodies and specific phages	55

Table 2 *(cont.)*

Immunologically active component coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Rabbit anti-enterotoxin antibody[a]	Purification of *Clostridium perfringens* enterotoxin by immunoadsorption	56
Rabbit immunoglobulin IgG anti-(*Phaseolus vulgaris* α-D-mannosidase) antibody	Investigation of the reversible binding of T-cell factor with immobilized immunoglobulins	52, 53
Rabbit immunoglobulin IgG anti-(rat β-D-glucuronidase) antibody	Purification of rat-liver β-D-glucuronidase by immunoadsorption	54
Ribonuclease I	Demonstration of the cell-free synthesis of ribonuclease-specific immunoglobulin	45

[a] See (7), Scheme 3.

Table 3 *Use of agarose cyclic imidocarbonates for the preparation of affinity-chromatography materials without linkage extension*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Abrus precatorius phytohaemagglutinin	Purification of membrane glycoproteins from human-blood platelets	57
Adenosine 2′-phosphate / Adenosine 5′-phosphate	Purification of the pyridine nucleotide transhydrogenase from *Pseudomonas aeruginosa*	58[a]
Alcohol dehydrogenase	Separation of enzyme cofactors	33
1-Aminoalkanes	Amphiphilic gels for the purification of *Escherichia coli* enzymes	59
	Purification of a threonine-sensitive, aspartokinase–homoserine dehydrogenase complex: comparisons of the adsorptivities of agarose derivatives as a function of the length of the alkyl chain	60
1-Aminobutane	Affinity chromatography of histones on a hydrophobic matrix	61
3-(2-Aminoethyl)thiorifamycin	Investigation of the interaction of RNA nucleotidyltransferase with rifamycin	62
1-Aminohexane	Affinity chromatography of histones on a hydrophobic matrix	61
	Use of alkyl-substituted matrices for the separation and purification of clostripain and collagenase	63
2-(6-Aminohexanoyl)amino-2-deoxy-L-fucose	Purification of α-L-fucosidase; demonstration that the enzyme is a glycoprotein	64

[a] Presumed to be this derivative.

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
4-(6-Aminohexanoyl)$_2$aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside 4-(6-Aminohexanoyl)$_2$aminophenyl 1-thio-β-D-galactopyranoside 4-(6-Aminohexanoyl)$_2$aminophenyl 1-thio-β-D-glucopyranoside 4-(6-Aminohexanoyl)$_2$aminophenyl 1-thio-α-D-mannopyranoside	Affinity chromatography of the glycoside hydrolases in Takadiastase preparations and from soybeans	65
4-[3-(6-Aminohexanoyl)amino-propyl]pyrazole and related pyrazole derivatives	Affinity chromatography of double ternary complex; purification of alcohol dehydrogenases	66
6-Aminohexanoylglycine methyl ester hydrobromide 6-Aminohexanoylglycylglycine methyl ester hydrobromide	Affinity chromatography of 'acid' proteinases, including porcine pepsin, and studies of the ion-exchange and hydrophobic interactions	67
6-Aminohexanoyl-D-phenylalanylamide	Affinity chromatography of chymosin	44
6-Aminohexanoyl-D-phenylalanine	Affinity chromatography of 'acid' proteinases, including porcine pepsin, and studies of the ion-exchange and hydrophobic interactions	67
6-Aminohexanoyl-D-phenylalanine methyl ester	Affinity chromatography of chymosin	44
6-Aminohexanoyl-L-phenylalanyl-D-phenylalanine	Affinity chromatography of 'acid' proteinases, including porcine pepsin, and studies of the ion-exchange and hydrophobic interactions	67
6-Aminohexanoyl-D-tryptophan methyl ester	Assessment of the relative suitabilities of agarose cyclic imidocarbonate and other agarose derivatives as affinity-chromatography matrices	37[a]
N^6-(6-Aminohexyl)adenosine 2′,5′-diphosphate	Affinity chromatography of $NADP^+$-dependent dehydrogenases	68
	Ligand affinity chromatography of succinate thiokinase	69
	Purification of malate enzyme	70
N^6-(6-Aminohexyl)adenosine 3′,5′-diphosphate	Affinity chromatography of $NADP^+$-dependent dehydrogenases	68
	Ligand affinity chromatography of succinate thiokinase	69
N^6-(6-Aminohexyl)adenosine 5′-phosphate	Affinity chromatography of mammalian phosphofructokinase on immobilized adenine dinucleotides	28
	Affinity chromatography of $NADP^+$-dependent dehydrogenases	68

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
N^6-(6-Aminohexyl)adenosine 5′-phosphate (*continued*)	Ligand affinity chromatography of succinate thiokinase	69
	Purification of glyceraldehyde 3-phosphate dehydrogenase	71
	Purification of glycogen phosphorylase	72
8-(6-Aminohexyl)aminoadenosine 2′,5′- and 3′,5′-diphosphates	Purification of cofactor-dependent enzymes	73
8-(6-Aminohexyl)aminoadenosine 5′-phosphate	Purification of human lactate dehydrogenases	74
8-(6-Aminohexyl)aminoadenosine 5′-triphosphate	Purification of adenosine cAMP-dependent protein kinase holoenzyme	75
	Purification of cofactor-dependent enzymes	73
N^{α}-(6-Aminohexyl)-D-asparagine	Purification of microbial asparaginases	76
N^6-[(6-Aminohexyl)carbamoylmethyl]-adenosine 5′-triphosphate	Affinity chromatography of mammalian phosphofructokinase on immobilized adenine dinucleotides	28
8-(6-Aminohexyl)desulphato-coenzyme A	Purification of cofactor-dependent enzymes	73
6-Aminohexyl 1-thio-β-D-galactopyranoside	Purification of human-liver β-D-galactosidases	77
1-Amino-octane	Affinity chromatography of histones on a hydrophobic matrix	61
4-Aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside	Purification of β-D-2-acetamido-2-deoxyglucosidase (*endo*-β-D-2-acetamido-2-deoxyglucanase)	78
3-(Aminopyridine)nicotinamide adenine dinucleotide (oxidized)	Purification of cAMP-dependent protein kinase holoenzyme	75[a]
11-Amino-undecanoic acid	Affinity chromatography of alcohol dehydrogenases on a hydrophobic matrix	79
Antithrombin	Separation of heparin into fractions of high and low anticoagulant activities	80
Arginyl oligosaccharides	Purification of trypsin; studies of the characteristics of the adsorbent	81
1,6-Bis-(2,4-dinitroanilino)hexane	Affinity chromatography of chymosin	44
Blue Dextran 2000	Purification of interferon	82
	Purification of isoleucyl transfer-RNA synthase	83
Bovine submaxillary glycoprotein	Purification of limulin	84
Cephalosporin C and related compounds	Isolation of β-lactamases	85
6-Chloro-9-β-D-ribofuranosylpurine 5′-phosphate	Purification of phosphofructokinase	71

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Collagen	Affinity chromatography of platelets; studies of the interaction between platelets and collagen	86
Collagen (denatured, citrate-soluble)	Affinity chromatography of collagen glycosyltransferases and the purification of specific fractions	87
Concanavalin A	Affinity chromatography of alkaline phosphatase, 5'-nucleotidase, and cAMP phosphodiesterase	88
	Affinity chromatography of the β-D-galactosidase from bovine testes	89
	Matrix for the conjugation of an adsorbed enzyme with another enzyme to give an immobilized, soluble derivative	90
	Fractionation of glycopeptides; studies of the structural requirements for the binding of glycopeptides to concanavalin A	91
	Purification of the glycogen-debranching enzyme (amylo-1,6-glucosidase) from dogfish muscle	92
	Purification of acetylcholinesterase	93
	Purification of glycosulphatases; demonstration that glycosulphatases are glycoproteins	94
	Purification of horseradish peroxidase	95
	Purification of human-liver β-D-galactosidases	77
	Purification of human placental β-D-2-acetamido-2-deoxyhexosidases A and B	96
	Purification of pepsin glycopeptides from human immunoglobulin IgA myeloma glycoprotein	97
	Purification of porcine haptoglobin	98
	Purification of the serum prohormone of angiotensin	99
	Purification of the αα-trehalase from *Saccharomyces cerevisiae*	100
	Separation of different populations of membrane vesicles from thymocytes	101
	Separation of acidic and neutral glycopeptides from rat brain	102
	Studies of the interactions of carcinoma-produced and other amylases with concanavalin A	103

[a] Presumed to be this derivative

Table 3 *(cont.)*

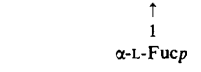
Ligand or affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Concanavalin A *(continued)*	Studies of the interaction of concanavalin A with *Trypanosoma equiperdum* and isolation of the antigenic glycoprotein	104
	Studies of the dissociation of α-amylase–immunoglobulin complexes	105
21-Deoxy-21-(ω-aminohexyl)-aminocortisol	Purification of transcortin	106
α,ω-Diaminoalkanes	Attempted hydrophobic immobilization of chorismate mutase	36
	Purification of clostripain and collagenase	63
	Purification of maltodextrin phosphorylase	107
	Purification of rabbit-liver glycogen phosphorylase *b*	108
	Purification of a threonine-sensitive, aspartokinase–homoserine dehydrogenase complex; comparisons of the adsorptivities of the agarose derivatives as a function of the length of the alkyl chains	60
1,4-Diaminobutane	Purification of clostripain	63
1,10-Diaminodecane	Hydrophobic immobilization of chorismate mutase	36
1,7-Diaminoheptane	Purification of collagenase	63
1,6-Diaminohexane	Purification of malate dehydrogenase	109
	Purification of pullulanases	110
DNA (double- and single-stranded)	Affinity chromatography and purification of enzymes, including DNA polymerase I and RNA polymerase; hybridization of nucleic acids	111
Enterotoxin	Purification of rabbit anti-enterotoxin antibodies by immunoadsorption	56
Ferredoxin	Purification of assimilatory nitrite reductase [NAD(P)H]	112
Glycylglycylarginine[a]	Quantitative analysis of trypsin; investigations of protein–ligand interactions	113
Gramicidin C	Affinity chromatography of chymosin	44
Growth hormone	Analysis of the rates of release of ligands and the characterization of soluble, radiolabelled products containing the growth hormone	114
6-Guanidinohexylamine	Purification of clostripain and collagenase	63
Heparin	Purification of antithrombins from plasma	80[a]

Table 3 *(cont.)*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Insulin B-chain	Purification of the peptidase active against insulin B-chain, *etc.*, from rat kidneys	115
Lactate dehydrogenase	Separation of enzyme cofactors	33
Lacto-*N*-difucohexaose I[b] (secondary amine derivatives)	Affinity chromatography of anti-(lacto-*N*-difucohexaose I) antibody immunoglobulin	116
Lens culinaris phytohaemagglutinin	Purification of membrane glycoproteins from human-blood platelets	57
Lipopolysaccharides from *Shigella sonnei* (blood-group O-specific)	Affinity chromatography of specific phages	55
Maltodextrins (*N*-4-sulphamoylphenyl glycosides)	Purification of maltodextrin phosphorylase	107
Modular protein–phosphodiesterase stimulant (from bovine brain)	Purification of a cyclic nucleotide phosphodiesterase	117
Oligosaccharides (secondary amine derivatives)	Affinity chromatography of carbohydrate-specific immunoglobulins	116
Pepsin inhibitor from roundworms (*Ascaris lumbricoides*)	Purification of chicken pepsin	118
Peptidophosphogalactomannan (from *Penicillium charlesii*)	Purification of (*exo*-)β-D-galactofuranosidase from *P. charlesii*	119
Poly(adenylic acid) Poly(uridylic acid)	Fractionation of proteins from rat-liver nuclear 30*S*-particles	120
Protamine peptides	Purification of trypsin; studies of the affinity characteristics of the adsorbent	81
Red-cell glycoprotein (desialylated)	Purification of the anti-(blood-group N) lectin from *Vicia graminea* seeds	121
Ribonuclease	Separation of pepsin and chymosin	44
Ricinis communis agglutinin	Demonstration of the heterogeneity of rat α-foetoprotein	48
Thymidine 3′-(4-aminophenyl phosphate) 5′-phosphate	Comparison with kinetic and equilibrium dialysis methods for analysis of the binding of nucleotides to staphylococcal nuclease	122
Transferrin (^{125}I- and ^{59}Fe-labelled)	Investigations of iron-transport by immobilized transferrin and of the permeability of cells to transferrin	123
Wheat-germ agglutinin	Affinity chromatography of the β-D-galactosidase from bovine testes	89
Wheat-germ phytohaemagglutinin	Purification of membrane glycoproteins from human-blood platelets	57

[a] Presumed to be this derivative;

[b] α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 3)-β-D-Glc*N*Ac*p*-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc*p*



Tables 1,[33-47] 2,[48-56] and 3,[57-123] respectively. The derivatives are listed according to the uses prescribed, since, for example, the insoluble form of an enzyme used for affinity chromatography may not be enzymically active.

The effect of the reaction conditions on the cationic charge introduced and the extent of substitution of agarose by ligands containing amino-groups have been examined.[124] A spectrophotometric method for monitoring the amount of protein coupled to agarose cyclic imidocarbonate has been reported.[40]

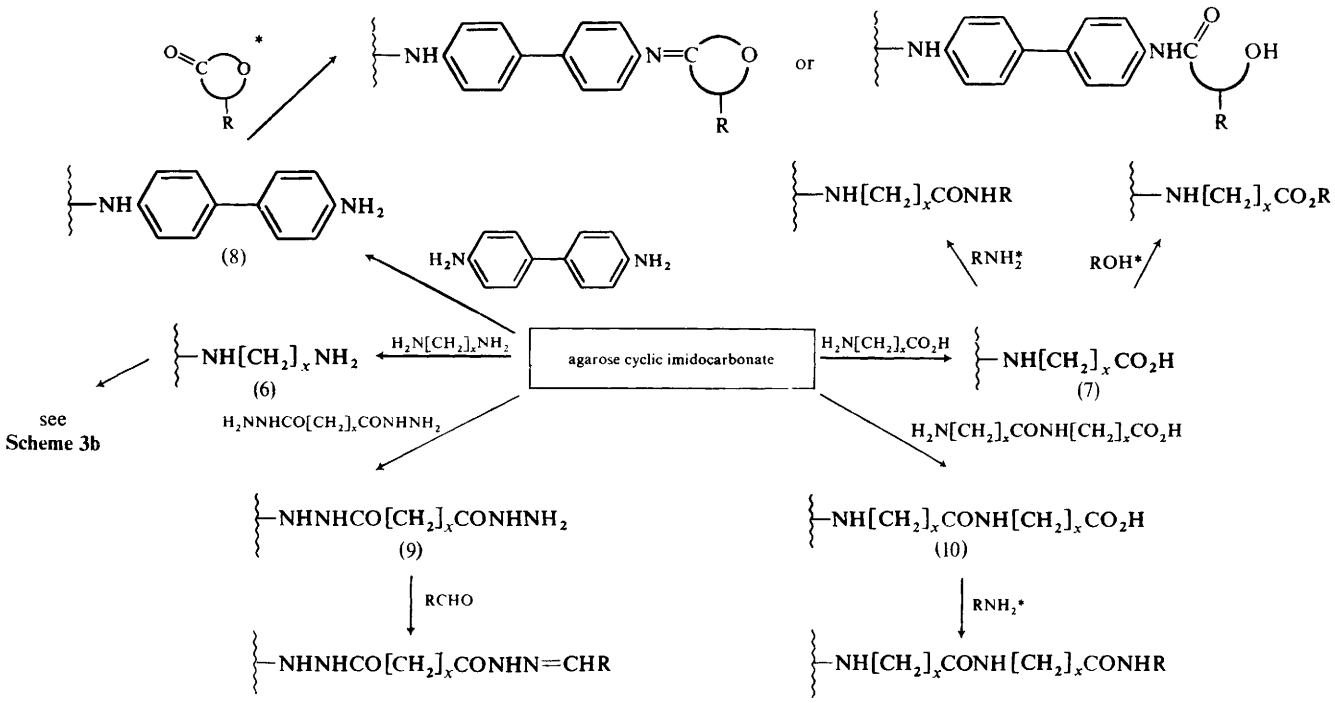
Concanavalin A immobilized on agarose cyclic imidocarbonate has been succinylated so that β-D-fructofuranosidase could be adsorbed onto the matrix.[90] Subsequent reaction with glutaraldehyde gave an activated derivative of the enzyme that could be coupled to another enzyme.

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DNA has been covalently linked to agarose cyclic imidocarbonate to give a derivative that is stable at high temperatures and to prolonged storage and, in the case of single-stranded DNA, to washing with sodium hydroxide solution in order to destroy any nuclease activity and to release digested oligo- or mononucleotides.[111]

The direct attachment of an affinant or an enzyme to agarose cyclic imidocarbonate has frequently been unrewarding, since approach to the bound ligand

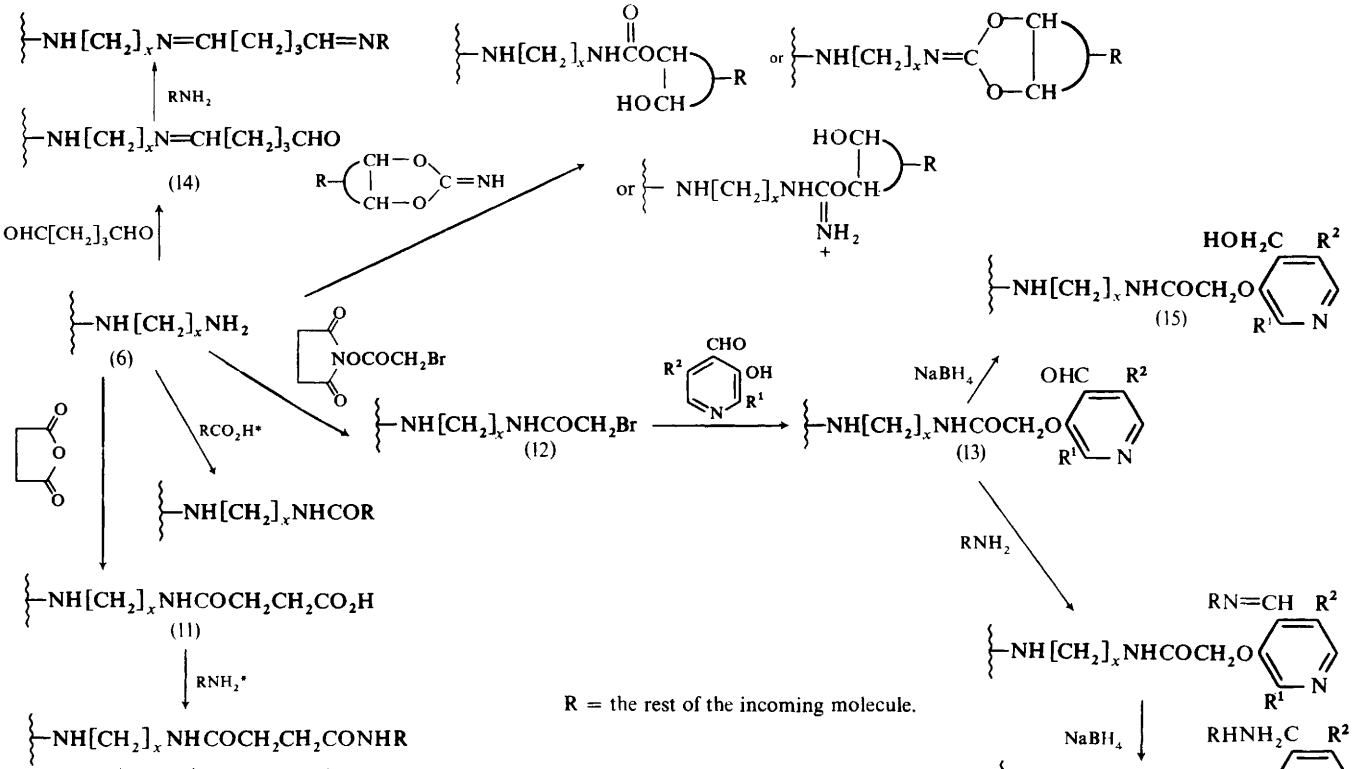
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R = the rest of the incoming molecule.

* = reaction involving carbodi-imide. In subsequent references to this Scheme, the suffixes A and B denote the use of $H_2N[CH_2]_3NH[CH_2]_3NH_2$ and $H_2N[CH_2]_3NH[CH_2]_4NH[CH_2]_3NH_2$, respectively, instead of $H_2N[CH_2]_xNH_2$ in the preparation of (6), *etc.*

Scheme 3a



R = the rest of the incoming molecule.

* = reaction involving carbodi-imide. In subsequent references to this Scheme, the suffix A denotes the use of $H_2N[CH_2]_3NH[CH_2]_3NH_2$ instead of $H_2N[CH_2]_3NH_2$ in the preparation of (6), *etc.*

Scheme 3b

Table 4 *Use of agarose cyclic imidocarbonates for the preparation of affinity-chromatography materials* via *linkage extension*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Agarose intermediate*	*Use of product*	*Ref.*
2-Acetamido-2-deoxy-D-galactono-1,4-lactone 2-Acetamido-2-deoxy-D-glucono-1,4-lactone 2-Acetamido-2-deoxy-D-mannono-1,4-lactone	(8)	Purification of β-D-2-acetamido-2-deoxyhexosidase	30
Adenosine 5'-triphosphate (periodate-oxidized)	(9)	Affinity chromatography of mammalian phospho-fructokinase on immobilized adenine nucleotides	28
Alkylsuccinic anhydrides	(6)	Studies of the effect of the chain-length on the adsorption of albumin; removal of albumin from sera	125
4-Aminobenzamidine	(7)	Purification of commercial preparations of urokinase	126
4-Aminobenzyl 2-acetamido-2-deoxy-1-thio-β-D-gluco-pyranoside	(11)	Purification of *Solanum tuberosum* agglutinin (sta-lectin)	127
	(11)	Purification of wheat-germ agglutinin	128
2-Amino-2-deoxy-D-galactose hydrochloride	(7)	Isolation of soybean agglutinin	129
4-Aminophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside	(11A)	Purification of hen egg-white lysozyme	130
4-Aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside	(10)	Competitive affinity chromatography of glycoside hydrolases from soybeans and in Takadiastase preparations	65
4-Aminophenyl di-*N*-acetyl-β-chitobioside	(11)	Purification of *Solanum tuberosum* agglutinin (sta-lectin)	127
4-Aminophenyl 1-thio-β-D-galactopyranoside	(10)	Competitive affinity chromatography of glycoside hydrolases from soybeans and in Takadiastase preparations	65
	(7)	Purification of β-D-galactosidase	131
3-Carboxymethylflavin derivative	(6)	Isolation of the apo-form of ovoflavoprotein	132
Cephalosporin C and related compounds	(7)[a]	Isolation of β-lactamases	85
4-Chloromercuribenzoate	(6)	Purification of a cysteine-containing histone	133
	(6), (6A)	Separation of the principal glycoproteins in human red-cell membranes from other proteins	134
Cortisol hemisuccinate	(6A)	Purification of corticosteroid-binding globulin	135
	(6), (6A), (6B)	Purification of transcortin	106
Cytidinediphosphate-1,2-diacyl-*sn*-glycerol (oxidized)	(9)	Separation of membrane-bound enzymes	136
Decylsuccinic anhydride	(6)	Studies of the effect of the chain-length on the adsorption of albumin; removal of albumin from sera	125

Table 4 *(cont.)*

NO-Dibenzyloxycarbonyltyrosine	(6)	Purification of the 'acid' proteinase from *Aspergillus saitoi*	137
Estradiol-17β-hemisuccinate	(6)	Affinity chromatography of α-foetoprotein; studies of the estrogen-binding properties of various α-foeto-proteins	138
Estrone hemisuccinate	(6), (6A), (6B)	Purification of transcortin	106
N^{10}-Formylaminopterin	(6)	Purification of difolate reductase from soybean seedlings	139
β-D-Glucopyranosylamine	(10)	Competitive affinity chromatography of glycoside hydrolases from soybeans and in Takadiastase preparations	65
Glycocholic acid	(6)	Purification of 3α-hydroxysteroid:NAD^+-oxidoreductase from *Pseudomonas testosteroni*	140
Glycogen cyclic imidocarbonate	(6)	Isolation of α-amylase	141
Hippurylhistidyl-leucine	(6)	Purification of angiotensin-I-converting enzyme (peptidyldipeptide hydrolase)	142
25-Hydroxycholecalciferol 3β-hemisuccinate (^{125}I-labelled)	(6)	Purification of 25-hydroxycholecalciferol-binding proteins from chickens	143
Nicotinamide adenine dinucleotide phosphate ($NADP^+$)	(7)	Affinity chromatography of $NADP^+$	68
Nicotinamide adenine dinucleotide phosphate ($NADP^+$) (periodate-oxidized)	(9)	Purification of NADPH–cytochrome P-450 reductase	144
N-(4-Nitrophenyl)oxamic acid	(6)	Purification of the neuraminidase from *Vibrio cholerae*	145
Octadecylsuccinic anhydride Octylsuccinic anhydride	(6)	Studies of the effect of the chain-length on the adsorption of albumin; removal of albumin from sera	125
Ovomucoid	(11)	Purification of *Solanum tuberosum* agglutinin (sta-lectin)	127
Pyridoxal 5′-phosphate	(12) → (13)	Purification of tryptophanase and tyrosine phenol-lyase	146[b]
Pyridoxine 5′-phosphate	(13) → (15)		
Succinyl nicotinamide adenine dinucleotide	(6)	Studies of the recycling of the cofactor in an enzyme reactor; comparison of reactions using free or immobilized nicotinamide adenine dinucleotide	147
Sulphanilic acid	(7)	Investigations of the role of carbonic anhydrases *in vivo*	148[c]
Tryptophan	(13) followed by reduction	Purification of tryptophanase and tyrosine phenol-lyase	146
Xylitol	(11)	Purification of D-glucose isomerase	149

[a] Using an agarose-acrylamide copolymer; [b] product also reduced, as shown in Scheme 3b; [c] presumed to be this derivative.

is sterically impeded. This difficulty can be overcome by inserting a covalent bridge between the matrix and the ligand. Bridging is also used when functional groups on the ligand do not react with those on the matrix, since it allows a new type of functionality to be introduced. Modifications recently used to provide bridges in the preparation of affinity-chromatography matrices, *etc.*, from agarose cyclic imidocarbonate are summarized in Schemes 3a and 3b and Table 4.[125-149] Where bridging has been used to immobilize antibodies and antigens, details are given in Table 2. The products obtained by coupling various substances to agarose cyclic imidocarbonate for diverse uses are given in Table 5.[50-155]

DEAE-agarose has been used in the purification of laminarinase[156] and β-1,6-D-glucanases.[157] Macroporous agarose activated with 1,4-bis-(2,3-epoxypropyloxy)butane reacted with cyclohepta-amylose in the presence of alkali to give a material that was used in the affinity chromatography of α-amylase.[158] β-Ecdysone reacted with epoxy-activated agarose to give a material suitable for use in the affinity chromatography of β-ecdysone-binding proteins.[159] Epoxy-activated agarose has been derivatized with acriflavin in a study of the continuous regeneration of $NAD(P)^+$ by immobilized flavins,[42] with adenosine 3',5'-diphosphate in studies on succinate thiokinase,[69] and with blood-group O-specific

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Table 5 *Use of agarose cyclic imidocarbonates for the preparation of miscellaneous derivatives*

Species coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
Acriflavin	Continuous regeneration of oxidized $NADP^{+}$ [a–c]	42
Alkylamines	Determination of the number of alkylamino-groups in substituted agaroses	32
α_1-Antitrypsin	Sulphide–disulphide interchange chromatography in the separation of plasma proteins	150
Collagen	Investigation of the binding of chondroitin sulphate to collagen	151
Concanavalin A	Investigation of the structure and the function of bovine testicular β-D-galactosidase	89
	Structural studies of saracen corn (*Fagopyrum esculentum*) α-L-rhamnosidase	152
Cysteine Cysteine *S*-methyl ether	Sulphide–disulphide interchange chromatography in the separation of plasma proteins	150
Diamines (various)	Determination of the number of alkylamino side-chains coupled to agarose by use of radiometric, spectroscopic, and elemental analyses	153
1,6-Diaminohexane	Control in the affinity chromatography of neuraminidase on agarose derivatives	145
Elastin (^{3}H- or ^{125}I-labelled)	Assay of elastase	154
Flavins (and derivatives thereof)	Continuous regeneration of oxidized $NADP^{+}$ [b, d]	42
Glutathione 2-Hydroxy-3-mercapto-propylamine[e] Homocysteine thiolactone	Sulphide–disulphide interchange chromatography in the separation of plasma proteins	150
Insulin	Continuous monitoring of the uptake of protein by the gel during immobilization	40
α-Lactalbumin	Fluorometric studies of the interactions of immobilized human α-lactalbumin with D-galactosyltransferase and with anti-(α-lactalbumin) antibodies	155
Penicillamine	Sulphide–disulphide interchange chromatography in the separation of plasma proteins	150
Wheat-germ agglutinin	Investigation of the structure and the function of bovine testicular β-D-galactosidase	89
X-Type immunoglobulin chains	Sulphide–disulphide interchange chromatography in the separation of plasma proteins	150

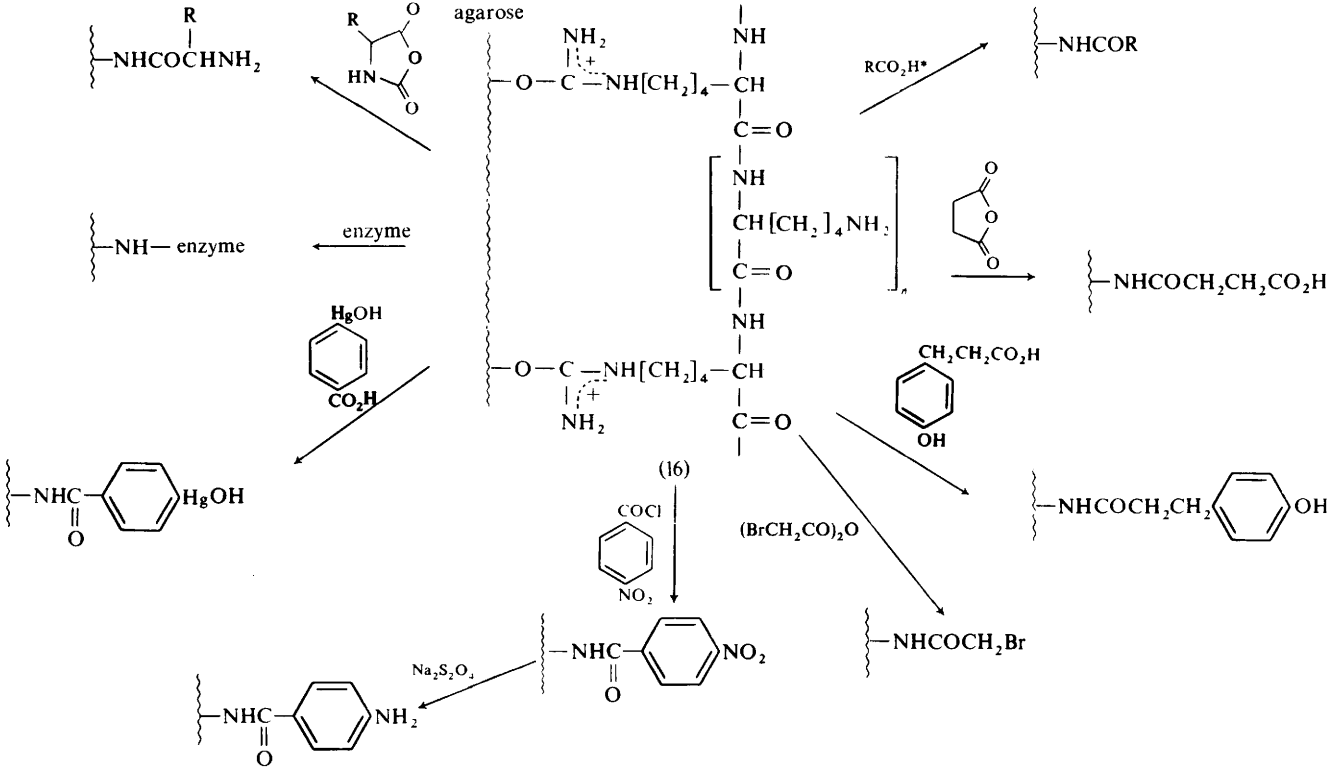
[a] Cross-linked agarose; [b] using the agarose derivative (7); [c] using the agarose derivative (14); [d] using the agarose derivative (6); [e] presumed to be this derivative.

lipopolysaccharides (from *Shigella sonnei*) to produce matrices for immunoadsorption or affinity chromatography.[55]

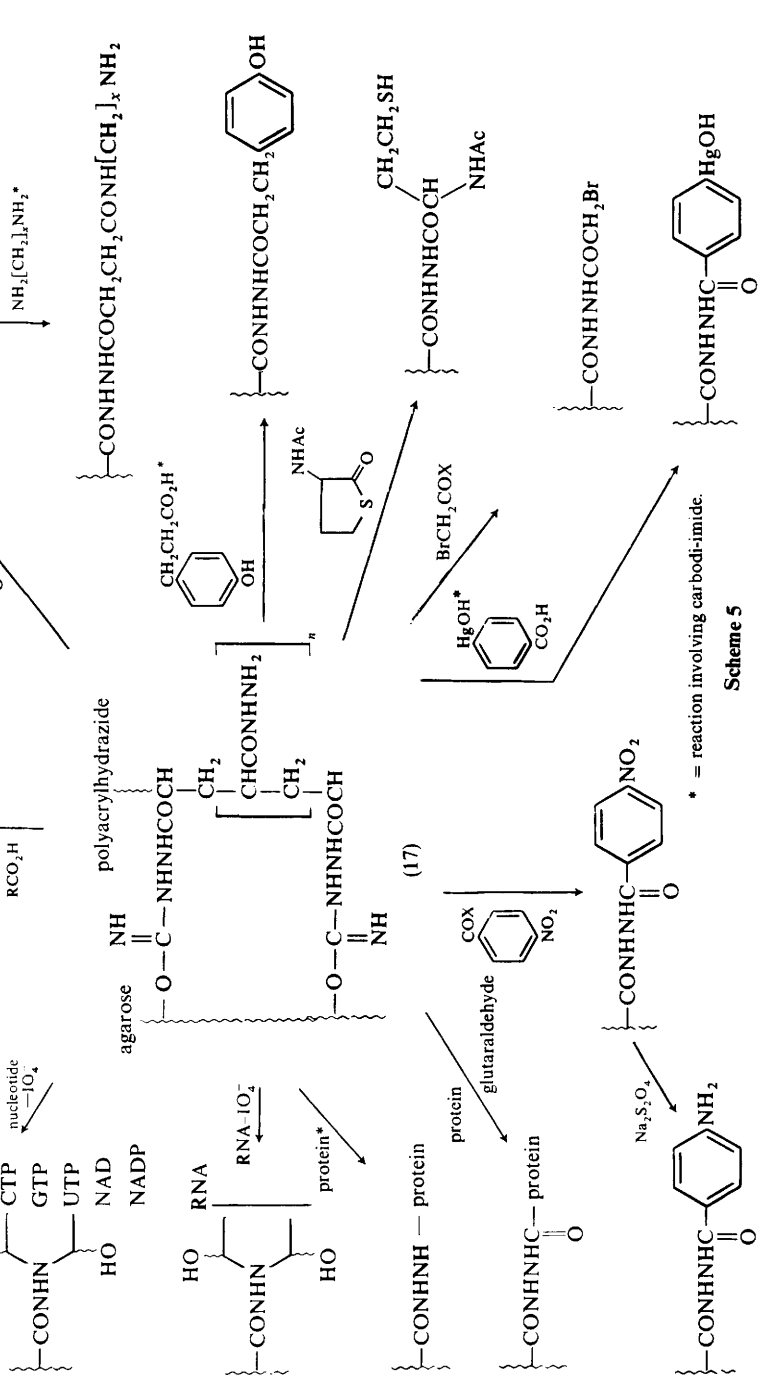
The derivative obtained when macroporous agarose activated with phloroglucinol and divinylsulphone reacted with 1,2-diaminoethane is suitable for the attachment of such affinants as methicillin (see p. 468), in which case the product was used in the purification of β-lactamase.[160]

Agarose has been converted into polylysine (16), poly-(DL-alanyl-lysine), and polyacrylhydrazide (17) derivatives, which have been used to prepare other

[160] R. G. Coombe and A. M. George, *Analyt. Biochem.*, 1976, **75**, 652.


agarose
enzyme
(16)
RCO2H*
(BrCH2CO)2O
Na2S2O4
• = reaction involving carbodi-imide

Scheme 4


AMP
ADP
ATP
CTP
GTP
UTP
NAD
NADP
nucleotide
$-IO_4^-$
RNA
$RNA\text{-}IO_4^-$
protein*
protein
glutaraldehyde
RCO_2H
polyacrylhydrazide
agarose
(17)
$NH_2[CH_2]_x NH_2$*
$BrCH_2COX$
$HgOH^*$
CO_2H
$Na_2S_2O_4$
* = reaction involving carbodi-imide.

Scheme 5

derivatives (Schemes 4 and 5) suitable for affinity chromatography[161] and for the immobilization of enzymes, antibodies, and lectins.[37] The polyacrylhydrazide derivative (17) of agarose combines the properties of agarose and polyacrylamide in one matrix.

The suitability of macroporous agarose derivatized with diverse Procion dyes or Cibachron Blue for substrate-elution chromatography of carboxypeptidase G has been investigated.[162] A mercapto-derivative of macroporous agarose has been used in separating the principal glycoproteins from other proteins in human red-cell membranes.[134]

Blue Sepharose 6B (a dyed derivative of cross-linked agarose) has been used in isolating alcohol dehydrogenase from extracts of cotton seeds in a single-step procedure.[163]

Agarose cross-linked with epichlorohydrin reacted with 1-bromohexane to give a derivative that adsorbed β-amylase in an active form.[164] Hexyl- and octyl-substituted, epichlorohydrin-cross-linked agaroses containing mercapto-groups on the side-chains have been used for the reversible immobilization of β-amylase and glucoamylase.[165]

Derivatives of agarose (Sepharose) have been used to immobilize methicillin (Scheme 6) and proteins (Scheme 7).[166] The products were used in the affinity chromatography of penicillinase and for the preparation of active immobilized forms of α-chymotrypsin, respectively.

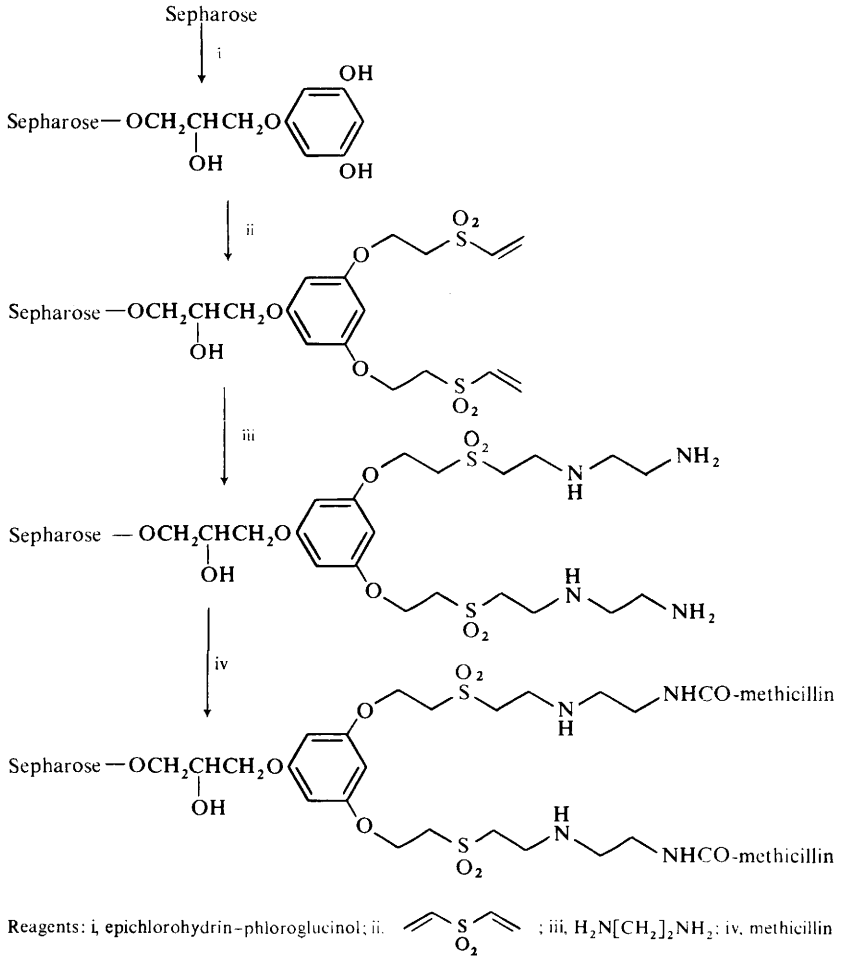
Beads containing a small proportion of silica are formed when agarose is mixed with colloidal silica; the beads can be stabilized with poly(ethylene glycol) and polyvinylpyrrolidone for use in the chromatography of proteins and polysaccharides.[167]

Alginic Acids.—Theoretical and practical aspects of the oxidation of alginates with periodate have been discussed.[24]

Alginic acid has been coupled to NAD^+ to give (18), or possibly (19), whose solubility depends on pH.[168] Possible uses of (18) [or (19)] as a coenzyme were discussed. A covalently bound form of NAD^+ and alginic acid has been found to undergo electrochemical reduction without significant loss of enzymic activity.[169] The resulting NADH complex could be oxidized again to the enzymically active NAD^+ complex by an enzymic reaction. It is now possible to carry out redox reactions in which NADH is enzymically oxidized to NAD^+, with the simultaneous electrochemical regeneration of NADH from NAD^+.

Microbial cells, subcellular organelles, and enzymes have been trapped in gels of calcium alginate, giving active immobilized enzymes, *etc.*[170]

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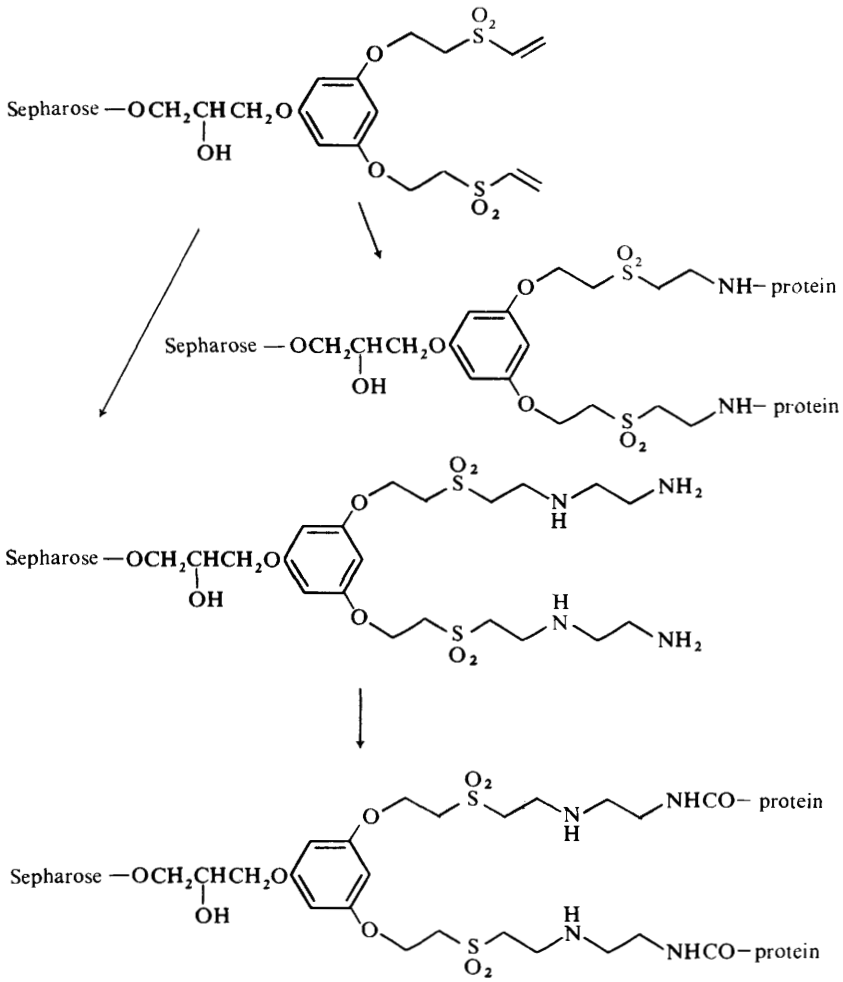


Scheme 6

Amyloses.—Various parameters and techniques have been used to define the morphology of amylose acetates.[171] Chemical modification of maltotriose has been reported.[172] Periodate-oxidized and reduced (sodium borohydride) amylose has been used as the substrate in an assay of α-amylase.[173] The susceptibility of amylose–Cibachron Blue 3G-A to α-amylase and the use of this dyed amylose derivative in assays of α-amylase have been discussed.[174, 175]

Maltoheptaose has been oxidized (hypoiodite) and coupled to ethylenediamine

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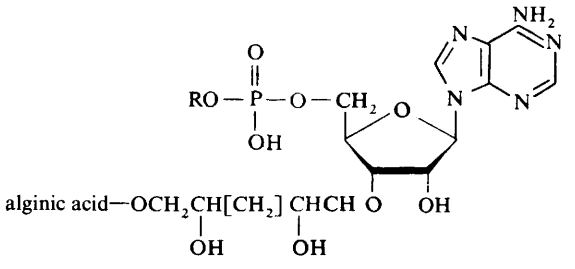


Scheme 7

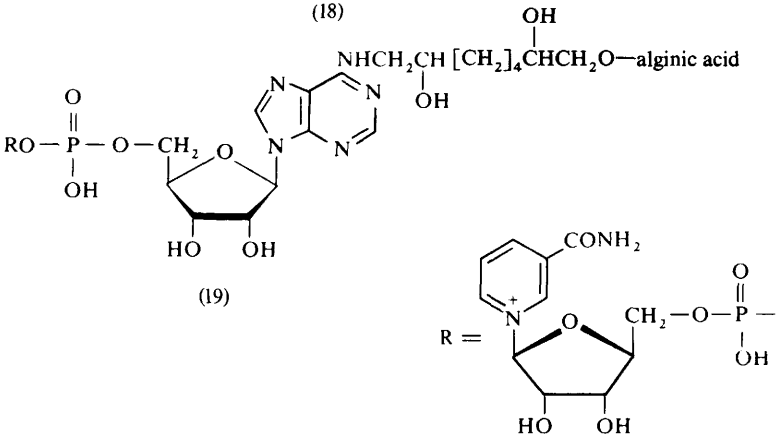
to give an 'amino-oligosaccharide' that condensed with the 4-nitrophenyl esters of mono-, di-, tetra-, and poly-carboxylic acids to form derivatives containing 1, 2, 4, and approximately 52 maltodextrin chains, respectively.[176] Similar derivatives were prepared from maltodextrins of average DP 11.7. The products were used to study the properties of glycogen phosphorylase *a*.

6-*O*-Tritylamylose reacted with 1.1 molar equivalents of benzoyl chloride to give an *O*-benzoyl-*O*-tritylamylose, which on oxidation (DMSO–P_2O_5), reduction (sodium borohydride), and detritylation furnished a polymer composed of residues of D-glucose (73%), D-allose (26%), and D-mannose (2%).[177]

[176] H. Y. Hu and A. M. Gold, *Biochemistry*, 1975, **14**, 2224.
[177] Y. Kondo and K. Takeo, *Carbohydrate Res.*, 1976, **52**, 232.



(18)



(19)

Amylose cross-linked with epichlorohydrin has been used to distinguish between the α- and β-amylase activities of amylolytic preparations (see p. 397).[178]

Celluloses.—Papers presented at a symposium on the thermal uses and properties of polysaccharides and lignins have discussed the pyrolysis of cellulosic materials.[19]

Phenols[179] and carboxylic acids[180] present in condensates of the smoke of cigarettes containing a cellulosic tobacco substitute have been identified and measured. The solubility of holocelluloses in DMSO—paraformaldehyde has provided the basis for a rapid laboratory method for predicting the dry-matter digestibility of cellulosic materials.[181] The amount of dry matter solubilized by this chemical system correlated with that digested by rumen fluid *in vitro* or by cellulolytic bacteria.

The technology and applications of enzymic conversions of cellulosic materials[182] and the production of single-cell protein from cellulosic wastes[183] have been dis-

[178] M. A. Mateescu, H. D. Schell, F. Mihailescu, and I. Cornoiu, *Biochimie*, 1976, **58**, 875.
[179] H. Sakuma, M. Kusama, S. Sato, and S. Sugawara, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 2013.
[180] H. Sakuma, M. Kusama, S. Ishiguro, N. Shimojima, and S. Sugawara, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 2021.
[181] L. C. Chiang and C. E. Dunlap, *Biotechnol. and Bioeng.*, 1977, **19**, 143.
[182] 'Enzymatic Conversion of Cellulosic Materials: Technology and Applications', ed. E. L. Gaden, jun., M. H. Mandels, E. T. Reese, and L. A. Spano, Biotechnol. and Bioeng. Symposium No. 6, Wiley, New York, 1976.
[183] 'Continuous Culture: Applications and New Fields', ed. A. C. R. Dean, D. C. Ellwood, C. G. T. Evans, and J. Melling, Wiley, Chichester, 1977.

cussed at recent symposia. The enzymic conversion of cellulosic materials into ethanol and single-cell protein has been described.[184] An integrated process for converting newsprint into sugars and then into ethanol has been reported.[185] Unconverted solids were burned to produce energy for the process and electrical power.

Photodegradation of cellulose acetate fibres by u.v.-irradiation *in vacuo* at 77 or 293 K resulted in deacetylation and chain-scission.[186]

The hydrolysis of *O*-methylcellulose in solutions of hydrochloric, sulphuric, and perchloric acids and the acetolysis of cellulose triacetate have been shown to follow the mechanisms established earlier for glycosides of low molecular weight.[187] Degradation of *O*-ethylcellulose films in hydrochloric acid vapour occurred in the kinetic region. The light-scattering behaviour and the elasticity of thermally reversible gels of cellulose acetate in benzyl alcohol have been studied over a range of temperatures and polymer concentrations.[188] The small-angle light scattering appears to originate from the non-random character of cross-linking in the gels.

The interactions of cellulose, cellulose triacetate, carboxymethyl-, phosphato-, DEAE-, trimethylaminoethyl-, and ECTEOLA-celluloses with aflatoxins B_1 and G_1 have been studied by means of equilibrium dialysis.[189]

Cellulose has been converted into affinity-chromatography materials by the reactions shown in Scheme 2.[22]

Aminoethylcellulose (by way of glutaraldehyde coupling) and activated DEAE-cellulose have been derivatized with alcohol dehydrogenase or lactate dehydrogenase or both to give affinity matrices that were used in the isolation of enzyme cofactors.[33] The reaction of a conjugate of pneumococcal SIII polysaccharide and azo-(bovine serum albumin) with *O*-(bromoacetyl)cellulose afforded an immunoadsorbent that was used in the purification of anti-SIII antibodies.[190]

Carboxymethylcelluloses also containing either 2,4,6-trinitrophenyl or fluorescamine groups have been used as chromophoric or fluorescent substrates, respectively, in sensitive assays of cellulases.[191]

A poly(uridylic acid)–cellulose resin has been prepared by coupling poly(uridylic acid) oxidized with periodate at one end to a hydrazide derivative of carboxymethylcellulose.[192] The resin was used as a column-bound ribosome system in studies of translocation in ribosomes.

Cellulose cyclic imidocarbonate can be coupled to bovine serum albumin and ^{125}I-labelled human luteinizing hormone.[193] A second antibody coupled to the cellulose derivative was used to separate antibody-bound and free hormones in radioimmunoassays of gonadotrophins in plasma and sera.

[184] G. R. Cysewski and C. R. Wilke, *Biotechnol. and Bioeng.*, 1976, **18**, 1297.
[185] C. R. Wilke, G. R. Cysewski, R. D. Yang, and U. von Stockar, *Biotechnol. and Bioeng.*, 1976, **18**, 1315.
[186] N.-S. Hon, *J. Polymer Sci., Polymer Chem.*, 1977, **15**, 725.
[187] Y. V. Moiseev, N. A. Khalturinskii, and G. E. Zaikov, *Carbohydrate Res.*, 1976, **51**, 39.
[188] K. D. Goebel and G. C. Berry, *J. Polymer Sci., Polymer Phys.*, 1977, **15**, 555.
[189] A. O. Uwaifo and O. Bassir, *Biochem. Pharmacol.*, 1977, **26**, 863.
[190] J. C. Jaton, H. Huser, Y. Blatt, and I. Pecht, *Biochemistry*, 1975, **14**, 5308.
[191] J. S. Huang and J. Tang, *Analyt. Biochem.*, 1976, **73**, 369.
[192] N. V. Belitsina, M. A. Glukhova, and A. S. Spirin, *F.E.B.S. Letters*, 1975, **54**, 35.
[193] P. Koninckx, R. Bouillon, and P. DeMoor, *Acta Endocrinol.*, 1976, **81**, 43.

DNA adsorbed onto cellulose has been used in the purification of three DNA-polymerases from *Escherichia coli*.[194]

A process for obtaining cellulose ethers directly from defibred hardwoods has been reported.[195] Some aspects of the determination of the distribution of substituents in *O*-ethylcelluloses have been investigated.[196] Microcapsules of *O*-ethylcellulose containing hydrous magnesium aluminium hydroxide have been prepared by a phase-separation procedure that uses solutions of the polymer in an organic solvent.[197]

Cellulose reacted with hexamethylene di-isocyanate to give a carbamate derivative that yielded (20) following hydrolysis and treatment with *O*-methylisourea.[198] The ionic character of the derivative (20) was used to immobilize



(20)

β-D-fructofuranosidase. A cellulose isothiocyanate has been prepared by treating cellulose with 2,4-di-isocyanatotoluene, followed by hydrolysis and reaction of the resulting amine with thiophosgene.[199] The cellulose isothiocyanate was characterized by its binding capacity with respect to [^{14}C]glycine, ^{131}I-labelled human serum albumin, and 2-mercaptoethanol. A reliable analytical method for determining the binding capacity of cellulose isothiocyanates, based on reaction with ^{35}S-labelled toluene-α-thiol, was developed. The interaction between dimethylurea and water-soluble *O*-methylcellulose has been studied.[200]

The thermo-oxidative degradation of nitrocellulose in aprotic solvents has been examined.[201] Cellulose nitrate membranes have been used to encapsulate β-D-galactosidase.[202] Characteristics of the semi-permeable membranes obtained from the system cellulose nitrate–methanol–dioxan–barium perchlorate have been investigated.[203] These membranes may find use in the construction of artificial kidneys.

Oligo(deoxythymidylic acid) adsorbed onto cellulose can be used in the analysis and preparation of poly(adenylic acid) RNA.[204]

Phosphatocellulose has been used in the purification of CDPdiglyceride: serine *O*-phosphatidyltransferase by affinity chromatography.[205]

[194] O. I. Khlebalina and V. G. Debabou, *Biochemistry* (*U.S.S.R.*), 1976, **41**, 533.
[195] D. F. Durso, *Svensk. Papperstidn.*, 1976, **79**, 50.
[196] L. B. Ivannikova, I. A. Mikhailopulo, M. I. Prigozhin, V. A. Tropina, A. I. Fanar, and M. V. Prokofieva, *Cellulose Chem. Technol.*, 1976, **10**, 673.
[197] S. Kasai and M. Koishi, *Chem. and Pharm. Bull.* (*Japan*), 1977, **25**, 314.
[198] P. A. Dickensheets, L. F. Chen, and G. T. Tsao, *Biotechnol. and Bioeng.*, 1977, **19**, 365.
[199] P. Gemeiner, J. Augustin, and L. Drobnica, *Carbohydrate Res.*, 1977, **53**, 217.
[200] G. A. Petropavlovski, L. K. Yakovenko, and G. G. Vasilieva, *Cellulose Chem. Technol.*, 1976, **10**, 323.
[201] V. N. Syutkin, B. I. Dudrov, N. I. Klenkova, and S. N. Danilov, *Cellulose Chem. Technol.*, 1976, **10**, 595.
[202] D. T. Wadiak and R. G. Carbonell, *Biotechnol. and Bioeng.*, 1975, **17**, 1157.
[203] T. Uragami, M. Tamura, and M. Sugihara, *Angew. Makromol. Chem.*, 1976, **55**, 59.
[204] J. A. Bantle, I. H. Maxwell, and W. E. Hahn, *Analyt. Biochem.*, 1976, **72**, 413.
[205] T. J. Larson and W. Dowhan, *Biochemistry*, 1976, **15**, 5212.

Poly(uridylic acid) is firmly adsorbed by cellulose, furnishing a derivative that was used in the purification of eukaryotic mRNAs.[206]

Active immobilized enzymes were obtained when a cellulose isothiocyanate reacted with glucoamlyase and when cellulose cross-linked with formaldehyde and treated with 2-chlorotriethylamine reacted with D-glucose isomerase.[207] Alcohol dehydrogenase and lactate dehydrogenase have been immobilized, without loss of the enzymic activities, on *s*-triazinylated DEAE-cellulose.

Micropores that are accessible to toluene but not to decalins can be found in viscose rayon (produced as cellulose xanthate).[208]

Cellulose fibres (in the form of viscose) have been used to immobilize enzymes, including trypsin and chymotrypsin, by allowing amino-groups of the enzymes to react with aldehydo-groups of the viscose.[209]

Microcrystalline cellulose treated in turn with epichlorohydrin and di-*N*-ethyl-2,3-epoxypropylamine afforded an *O*-(3-diethylamino-2-hydroxypropyl) derivative that can be used in the fractionation of polysaccharides.[210]

The reactions of macroradicals formed on the surface of regenerated cellulose (rayon) by the action of an argon plasma have been investigated.[211]

The effects of diffusion control on the rate of radiation-initiated graft polymerization of cellulose have been studied theoretically.[23] The effects of such variables as the initiator concentration, temperature, and monomer:polymer ratio on the graft copolymerization of acrylamide and *O*-methylcellulose have been investigated.[212] The thermal properties and behaviour of graft copolymers of formaldehyde-cross-linked *O*-cyanoethylcellulose and acrylates have been studied.[213]

'Copolymerization' of periodate-oxidized cellulose (aldehydocellulose) with glycidyl methacrylate in the presence of an enzyme (*e.g.* peroxidase) afforded a means of immobilizing the enzyme without loss of activity.[214]

The radiation-induced grafting of 2- and 4-vinylpyridines and 2-methyl-5-vinylpyridine onto cellulose by mutual irradiation has been examined in a wide range of solvents, which must contain a nitrogen atom or an oxygen atom or both for high grafting efficiency.[215] The two most favourable groups are NH_2 and OH. Inclusion of both groups at suitable positions in the molecule (*e.g.* the amino-alkanols) gave the maximum effect in grafting. Other variables affecting grafting were the p*K* and the swelling, wetting, and dielectric properties of the solvent and the surface area of cellulose. A novel ionic mechanism for the grafting reaction, involving association between the solvent and the monomer, was proposed.

A book on the physical chemistry of dye adsorption has dealt with the adsorp-

[206] A. K. Popov, A. R. Fedotov, and G. A. Dvorkin, *Biochemistry (U.S.S.R.)*, 1975, **30**, 37.
[207] L. F. Chen and G. T. Tsao, *Biotechnol. and Bioeng.*, 1976, **18**, 1507.
[208] H. G. Shinouda and M. M. Abdel Moteleb, *J. Polymer Sci., Polymer Chem.*, 1977, **15**, 719.
[209] A. M. Klibanov, G. P. Samokhin, K. Martinek, and I. V. Berezin, *Biochim. Biophys. Acta*, 1976, **438**, 1.
[210] M. Antal and R. Toman, *J. Chromatog.*, 1976, **123**, 434.
[211] C. I. Simionescu, M. M. Macoveanu, and N. Olaru, *Cellulose Chem. Technol.*, 1976, **10**, 197.
[212] K. Bardhan, S. Mukhopadhyay, and S. R. Chatterjee, *J. Polymer Sci., Polymer Chem.*, 1977, **15**, 141.
[213] D. S. Varma and V. Narasimhan, *Cellulose Chem. Technol.*, 1976, **10**, 293.
[214] B. Focher, P. Cremonesi, L. d'Angiuro, G. Veccio, and G. Mazzola, *Cellulose Chem. Technol.*, 1975, **9**, 135.
[215] J. L. Garnett and E. C. Martin, *Austral. J. Chem.*, 1976, **29**, 2591.

tion of dyes by cellulosic substrates.[216] Differences in the porous and crystalline structures of the cell walls of never- and nature-dried cottons have been examined by imbibing such liquids as water and glycerol, by decrystallization of the fibrillar structure with aqueous sodium hydroxide solutions, and by equilibrium dye adsorption.[217] With a view to preserving the porous, strain-free organization of fibrillar surfaces existing in never-dried cotton, certain chemical treatments (*e.g.* solvent exchange, acetylation, and dyeing) were performed on never-dried cotton, and the extent of structural collapse, which was always less than that of drying never-dried fibres, was assessed using optical microscopy, *X*-ray diffraction, and liquid retention. Changes in the crystal lattice of cellulose were observed following the formation of a complex between cellulose and ethylenediamine.[218, 219] Water dissociated the complex by displacing ethylenediamine.[220]

An effective means of activating cellulose has been devised for the removal of carbohydrates from solution.[221] Cellulose coated with hydrous titanium(IV) oxide readily adsorbed branched polysaccharides (*e.g.* glycogen) but had little affinity for monosaccharides. The hydrous oxide, as a dried powder, had a lower maximum adsorption, showing the beneficial effect of its coating onto cellulose.

Chitins.—^{14}C-Labelled chitopentasaccharide has been prepared by lysozyme-catalysed transglycosylation reactions on the unlabelled pentasaccharide in the presence of an excess of 2-acetamido-2-deoxy-D-[^{14}C]glucose.[222]

D-Glucose isomerase has been immobilized on chitin by direct adsorption and by coupling *via* glutaraldehyde.[223] Chitin has been used as an affinity matrix in the purification of wheat-germ agglutinin.[89]

Chitosans.—Chitosan adsorbs chymotrypsin and acid phosphatase, presumably by non-covalent interactions, to give active immobilized derivatives of the enzymes.[224]

Cycloamyloses.—Starch has been converted into cyclohepta-amylose by soluble, immobilized forms of cyclodextrin D-glucanotransferase.[225]

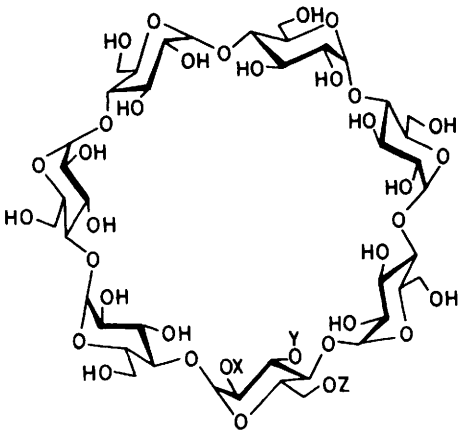
Mild acidic hydrolysis (5 mM HCl at 100 °C) of cyclohepta-amylose gave an 8% yield of maltoheptaose.[176] The first-order rate constant increased during hydrolysis because the α-(1 → 4)-linkages of cyclohepta-amylose and linear dextrins are cleaved at different rates.[226] Various thermodynamic parameters and the dependence of the rate of hydrolysis on pH and on complexation of cyclohepta-amylose with toluene were determined.

[216] I. D. Rattee and M. M. Breuer, 'The Physical Chemistry of Dye Adsorption', Academic Press, London and New York, 1974.
[217] A. K. Kulshreshtha, A. R. Patel, N. T. Baddi, and H. C. Srivastava, *J. Polymer Sci., Polymer Chem.*, 1977, **15**, 165.
[218] J. J. Creely, *J. Polymer Sci., Polymer Chem.*, 1977, **15**, 521.
[219] H. T. Lockhande, S. R. Shukla, P. K. Chidambareswaran, and N. B. Patil, *J. Polymer Sci., Polymer Letters*, 1976, **14**, 747.
[220] H. T. Lockhande, S. R. Shukla, P. K. Chidambareswaran, and N. B. Patil, *J. Polymer Sci., Polymer Letters*, 1977, **15**, 97.
[221] J. F. Kennedy, S. A. Barker, and C. A. White, *Carbohydrate Res.*, 1977, **54**, 1.
[222] P. van Eikeren and H. McLaughlin, *Analyt. Biochem.*, 1977, **77**, 513.
[223] W. L. Stanley, G. G. Watters, S. H. Kelly, B. G. Chan, J. A. Garibaldi, and J. E. Schade, *Biotechnol. and Bioeng.*, 1976, **18**, 439.
[224] R. A. A. Muzzarelli, G. Barontini, and R. Rocchetti, *Biotechnol. and Bioeng.*, 1976, **18**, 1445.
[225] N. Nakamura and K. Horikoshi, *Biotechnol. and Bioeng.*, 1977, **19**, 87.
[226] J. Szejtli and Z. Budai, *Acta Chim. Acad. Sci. Hung.*, 1976, **91**, 73.

D-Glucose and malto-oligosaccharides (DP 2—6) are released on γ-irradiation of cyclohepta-amylose; a mechanism was proposed for the ring-cleaving processes that occur in oxygen-free, aqueous solution.[227] E.s.r. measurements during the oxidation of cyclohepta-amylose with Ti^{3+} and hydrogen peroxide indicated that the radical-induced reactions are initiated by a radical centred at C-5 of the pyranose ring.

Cyclohepta-amylose couples to macroporous agarose activated with 1,4-bis-(2,3-epoxypropyloxy)butane to give a material that can be used in the purification of α-amylase.[158]

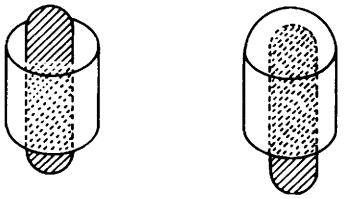
Treatment of cyclohepta-amylose with 2.2 molar equivalents of benzoyl chloride afforded an *O*-benzoylcyclohepta-amylose that consisted mainly of 2,6-di-*O*-benzoyl-D-glucopyranosyl residues with a degree of substitution by benzoyl groups of 52%.[177] Oxidation (DMSO–P_2O_5) and reduction (sodium borohydride), with concomitant cleavage of the benzoyl groups, gave a cyclic oligomer containing residues of D-allose (57%), D-glucose (41%), and D-mannose (2%).



(21) X = PO_3H_2, Y = Z = H
(22) Y = PO_3H_2, X = Z = H
(23) Z = PO_3H_2, X = Y = H

Cyclohepta-amylose has been converted into the phosphates (21)—(23), which were examined as catalysts for the hydrolysis of 4-nitrophenyl tetrahydropyranyl ether at low pH and for the exchange of 4-t-butylphenacyl alcohol (tritiated in the α-methylene group) at high pH.[228] All three isomers, as the phosphate dianions, were effective catalysts for the latter reaction in which the functional group assists enolization of the bound ketone. Only the 3-phosphate (22) showed net catalysis of the hydrolysis of the ether, which was bound and hydrolysed with the assistance of a monoanionic phosphoric acid group.

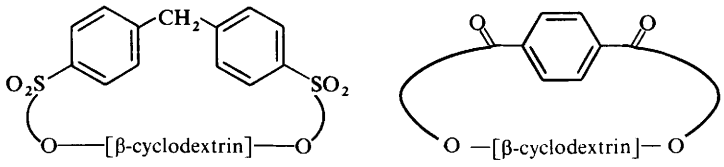
[227] T. Komiya, T. Yamada, and S. Nara, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1917.
[228] B. Siegel, A. Pinter, and R. Breslow, *J. Amer. Chem. Soc.*, 1977, **99**, 2309.



binding by a common cylindrical host *binding by a capped cylindrical host*

Schematic representation of hydrophobic binding. Obliquely lined areas are exposed in water and do not contribute to binding, whereas obliquely dotted-lined areas are contributing to binding.

Scheme 8



(24) (25)

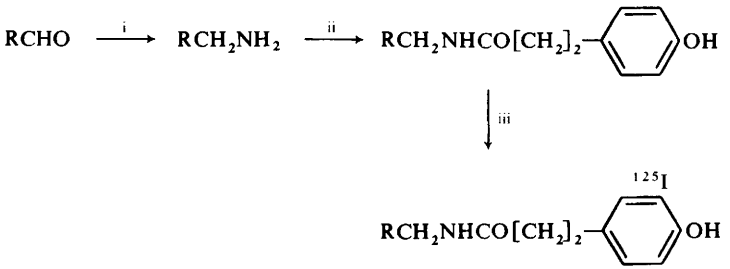
The hydrophobic binding of guest molecules to cycloamyloses can be represented schematically as shown in Scheme 8.[229] The cyclohepta-amylose derivatives (24) and (25), which are capped by hydrophobic entities, bound sodium 1-anilinonaphthalene-8-sulphonate more effectively than did cyclohepta-amylose.

^{1}H N.m.r. measurements have shown that each of the D-glucopyranosyl residues of cyclohexa-amylose maintains a $^4C_1$ conformation in aqueous solution.[230] The formation of an aromatic guest–cyclohexa-amylose complex was revealed by the influence of the guest molecule on the magnetic shielding of hydrogen nuclei located on the inner surface of the torus. The inclusion of aromatic molecules (4-nitrophenol and DL-phenylalanine) affected neither the pucker of the D-glucopyranose rings nor the rotamer population about the C-5–C-6 bonds.

The molecular structure of a 1:1 complex of cyclohepta-amylose and 2,5-diiodobenzoic acid has been determined by *X*-ray crystallography.[231] The cyclohepta-amylose molecules form channels in the crystal by means of head-to-head and tail-to-tail stacking. Details of the crystal structures of cyclohexa-amylose hexahydrate and cyclohexa-amylose–propan-1-ol hydrate have been summarized.[232]

Dextrans.—A ^{125}I-labelled derivative of isomaltotetraose has been prepared (Scheme 9) and used in assays of dextran-specific antibodies.[233]

[229] I. Tabushi, K. Shimokawa, N. Shimizu, H. Shirakata, and K. Fujita, *J. Amer. Chem. Soc.*, 1976, **98**, 7855.
[230] D. J. Wood, F. E. Hruska, and W. Saenger, *J. Amer. Chem. Soc.*, 1977, **99**, 1735.
[231] J. A. Hamilton, M. N. Sabesan, L. K. Steinrauf, and A. Geddes, *Biochem. Biophys. Res. Comm.*, 1976, **73**, 659.
[232] G. A. Jeffrey and M. Sundaralingam, *Adv. Carbohydrate Chem. Biochem.*, 1976, **32**, 353.
[233] K. Himmelspach, H. Geyer, G. Hoyer, and G. Schepers, *F.E.B.S. Letters*, 1977, **75**, 154.



R = rest of isomaltotetraose molecule

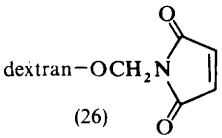
Reagents: i, $NaBH_3CN$–NH_4OAc; ii, 3-(4-hydroxyphenyl)propionic acid; iii, $^{125}I_2$–chloramine T

Scheme 9

Blue Dextran 2000 (Cibachron Blue F3GA–dextran), immobilized on agarose cyclic imidocarbonate, has been used in studies of the molecular interactions of interferons[82, 234] and in the purification of isoleucyl transfer-RNA synthase.[83]

Dextran cyclic imidocarbonate has been coupled to α-amylase[235] and catalase[236] to give water-soluble, active, immobilized derivatives of the enzymes.

The maleimide derivative (26), obtained by treating dextran with *N*-chloromethylmaleimide, has been used as an oriented, impermeant probe in studies of the sulphydryl-containing polypeptides of human erythrocyte membranes.[237] Base-catalysed condensation of periodate-oxidized dextran with nitromethane gave a 'nitrodextran' that yielded an 'aminodextran' on reduction.[238] The 'nitrodextran' was shown to contain residues of 3-deoxy-3-nitro-D-manno-, -gluco-, -galacto-, and -talo-pyranose in the molar proportions 6:5:1:2.



(26)

Substituents in dextrans containing palmitoyl or phosphate groups have been located by the Smith degradation procedure.[239] These derivatives did not inhibit the growth of sarcoma 180 ascites tumour in mice, even in combination therapy with mitomycin C, indicating that both fatty-acid and phosphate groups must be present in the same molecule for anti-tumour activity. However, a water-insoluble *O*-palmitoyldextran phosphate showed a significant growth-inhibition against the ascites tumour, while a related water-soluble compound revealed a marked synergistic effect against the same tumour in combination therapy with mitomycin C.

[234] W. J. Jankowski, W. von Muenchhausen, E. Sulkowski, and W. A. Carter, *Biochemistry*, 1976, **15**, 5182.
[235] J. J. Marshall, *Carbohydrate Res.*, 1976, **49**, 389.
[236] J. J. Marshall and M. L. Rabinowitz, *Biotechnol. and Bioeng.*, 1976, **18**, 1325.
[237] R. E. Abbott and D. Schachter, *J. Biol. Chem.*, 1976, **251**, 7176.
[238] K. Maekawa, Y. Miyoshi, and K. Tsuru, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1951.
[239] M. Suzuki, T. Mikami, T. Matsumoto, and S. Suzuki, *Carbohydrate Res.*, 1977, **53**, 223.

Sephadexes (dextrans cross-linked with epichlorohydrin) have been modified (Scheme 2) for use as affinity-chromatography matrices.[22] Ligands containing amino-groups are often linked to polysaccharides that have been activated with cyanogen bromide.[124] However, the cationic charge introduced into the insoluble derivatives may interfere with their use as affinity-chromatography matrices. Studies with cyanogen-bromide-activated Sephadex have attempted to measure and reduce this potential artefact; conditions that result in maximal incorporation of the ligand with minimal cationic charge on the product can be selected.

D-Glucose 6-phosphate dehydrogenase reacted with Sephadex cyclic imidocarbonate, either in the presence or in the absence of amino-acids, to give an active immobilized form of the enzyme.[41] Sephadex activated with either cyanogen bromide or trichloro-1,3,5-triazine reacted with trypsin to give an active immobilized form of the enzyme.[46]

The use of Procion-dyed Sephadexes and Blue Dextran 2000 as matrices in substrate-elution chromatography of carboxypeptidase G has been investigated.[162] Sephadexes containing either fluorescamine or 2,4,6-trinitrophenyl groups have been used as fluorescent or chromophoric substrates, respectively, in assays of dextranase.[191]

Intact cells adsorbed onto concanavalin-A-coated Sephadex or a form of Sephadex cross-linked with glutaraldehyde were used as specific, immobilized, cell-surface carbohydrates in the purification of immunoglobulins by immunoadsorption.[240]

Glycogens.—The 4-nitrophenyl glycosides of 4-*O*-α-D-[^{14}C]glucopyranosyl-β-D-glucopyranose, 4-*O*-α-[^{14}C]maltosyl-β-D-glucopyranose, and 4-*O*-α-[^{14}C]-maltotriosyl-β-D-glucopyranose were obtained when α-amylases acted on [^{14}C]-glycogen in the presence of 4-nitrophenyl β-D-glucopyranoside.[241]

An immobilized form of glycogen resulted when glycogen cyclic imidocarbonate reacted with a 6-aminohexyl derivative of agarose; the immobilized glycogen derivative was used in the isolation of α-amylase by competitive affinity chromatography.[141] Glycogen is adsorbed by magnetic iron oxide or cellulose coated with hydrous titanium(IV) oxide.[221]

Glycosaminoglycans.—Experiments with immobilized collagen have suggested that chondroitin sulphate binds to three sites on the collagen molecule, the binding affinity decreasing with increasing ionic strength.[151]

Studies of the interaction of dermatan sulphate and Acridine Orange in 1:1 mixtures have shown that not all the dye binds at low concentrations.[242] Binding of the dye is highly co-operative, as evidenced by strong interactions between adjacent molecules of the bound dye.[243]

The chromatography of bovine serum albumin on gels of hyaluronic acid cross-linked with diepoxybutane has been investigated.[244] A very sensitive spectrophotometric assay of hyaluronidase activity is based on the binding of a carbocyanine dye to the undegraded substrate–this results in a spectral shift

[240] B.-A. Sela and G. M. Edelman, *J. Exp. Med.*, 1977, **145**, 443.
[241] M. Takeshita and E. J. Hehre, *Arch. Biochem. Biophys.*, 1975, **169**, 627.
[242] S. S. West, R. E. Hurst, and J. M. Menter, *Biopolymers*, 1977, **16**, 685.
[243] J. M. Menter, R. E. Hurst, and S. S. West, *Biopolymers*, 1977, **16**, 695.
[244] M. Shaw and A. Schy, *Biophys. J.*, 1977, **17**, 47.

which is proportional to the amount of hyaluronic acid.[245] Hyaluronic acid forms a 2:1 complex with Cu^{2+} ions, the binding involving D-glucopyranosyluronic acid residues but not 2-acetamido-2-deoxy-D-glucopyranosyl residues.[246] The absorption band in the region 238 nm was attributed to a charge-transfer involving ligand to metal ion.

Selective *N*-acylation of *N*-desulphated heparin and of *N*-deacetylated glycosaminoglycans can be accomplished with acid anhydrides in formamide at room temperature.[247] Heparin coupled to agarose cyclic imidocarbonate has been used as the affinity matrix in the purification of antithrombins.[80]

Guarans.—The bead form of a cross-linked guaran (a galactomannan composed of a chain of (1 → 4)-linked β-D-mannopyranosyl residues having single α-D-galactopyranosyl residues attached to O-6 of roughly half of the D-mannopyranosyl residues) is suitable for the affinity chromatography of, for example, D-galactose-binding lectins.[248] Guar gum trapped in a polyacrylamide gel has been used in the purification of peanut lectin by affinity chromatography.[13]

Lipopolysaccharides.—The blood-group O-specific lipopolysaccharides from *Shigella sonnei* reacted either directly, or after treatment with hydroxylamine, with epoxy and cyclic imidocarbonate derivatives of macroporous agarose to give materials suitable for use in immunoadsorption and affinity chromatographies.[55]

D-**Mannans.**—Yeast D-mannan bound to macroporous agarose with the aid of butane-1,4-diol diglycidyl ether can be used as an affinity adsorbent,[31] and D-mannans from *Candida utilis* and *Saccharomyces cerevisiae* have been absorbed and immobilized on magnetite.[249]

Miscellaneous Polysaccharides, Oligosaccharides, and Glycopeptides

The reducing-end residue of several oligosaccharides has been labelled by reduction with sodium borotritiide,[91] and oligosaccharides have been coupled to proteins, to give pseudoglycoproteins, following oxidation to the aldonic acids.[11]

A simple method of attaching oligosaccharides to agarose cyclic imidocarbonate has been reported.[116] The oligosaccharide is first treated with 2-(4-aminophenyl)-ethylamine to form the alkyl *N*-glycoside, which is then reduced (sodium borohydride) and coupled through its arylamino-group to the imidocarbonate. Lacto-*N*-difucohexaose I attached to agarose by this procedure was used in the purification of antibodies directed against it. Lactose reacted with a hydrazide derivative of Bio-Gel P-300 to give an affinity matrix that was used in the purification of wheat-germ β-D-galactosidase.[250] A tritiated and dansylated derivative of (27), prepared by partial oxidation of the peripheral D-mannopyranosyl residues with periodate, reduction (sodium borotritiide), and dansylation, has been used as the substrate in a specific assay for *endo*-β-D-2-acetamido-2-deoxyglucanases.[251]

[245] L. C. Benchetrit, S. L. Pahuja, E. D. Gray, and R. D. Edstrom, *Analyt. Biochem.*, 1977, **79**, 431.
[246] N. Figueroa, B. Nagy, and B. Chakrabarti, *Biochem. Biophys. Res. Comm.*, 1977, **74**, 460.
[247] S. Hirano and W. Onashi, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 2501.
[248] J. Lönngren, I. J. Goldstein, and R. Bywater, *F.E.B.S. Letters*, 1976, **68**, 31.
[249] M. Horisberger, *Biotechnol. and Bioeng.*, 1976, **18**, 1647.
[250] H. Hamazaki and K. Hotta, *F.E.B.S. Letters*, 1977, **76**, 299.
[251] A. L Tarentino and F. Maley, *Analyt. Biochem.*, 1977, **77**, 185.

$$(\text{D-Man}p)_6\text{-}(\text{D-Glc}N\text{Ac}p)_2\text{-Asn}$$
(27)

$$\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 3)\text{-D-Glc}p$$
(28)

$$\alpha\text{-D-GlcUA}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 3)\text{-D-Glc}q$$
(29)

$$\begin{array}{c} \alpha\text{-D-GlcUA}p \\ \uparrow \\ 3 \end{array}$$
$$\alpha\text{-D-GlcUA}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 3)\text{-D-Glc}p$$
(30)

Some ^{125}I-labelled derivatives of α-nigerosyl-(1 → 3)-nigerose (28) and the single (29) and double (30) repeating-units of *Klebsiella pneumoniae* B 5055 (O1:K2) capsular polysaccharide (prepared as shown in Scheme 9) have been used in radioimmunoassays.[233]

A peptidophosphogalactomannan (from *Penicillium charlesii*) immobilized by reaction with agarose cyclic imidocarbonate has been used as the affinity matrix in the purification of (*exo*-)β-D-galactofuranosidase.[119]

Oligosaccharide fragments have been obtained from pneumococcal SIII polysaccharide by mild acid hydrolysis.[190,252] Pneumococcal SIII polysaccharide reacted with azo-(bovine serum albumin) to give a conjugate which, following immobilization on *O*-bromoacetylcellulose, was used as an immunoadsorbent in the purification of anti-SIII antibodies.[190]

Pachymans.—Carboxymethylated pachyman[253] and pachyman dyed with Cibachron Blue[254] have been used as substrates in assays for *endo*-β-1,3-D-glucanases.

Pectic and Polygalacturonic Acids.—The effects of cross-linking of pectic acid on the cation-exchange properties, on the binding of *endo*-polygalacturonase, and on the biodegradability have been examined.[255]

Poly(D-galacturonic acid) cross-linked with epichlorohydrin has been used in the purification of polygalacturonase by affinity chromatography.[256]

Starches.—A dextrin attached to macroporous agarose with the aid of butane-1,4-diol diglycidyl ether has been used as the affinity adsorbent in the purification of bovine pancreatic α-amylase.[31] The determination of diverse substituents in starch derivatives by means of i.r. spectroscopy has been described.[257]

The conversion of starch into cyclohepta-amylose by soluble or immobilized cyclodextrin D-glucanotransferases[225] and the ability of α-amylases to convert starch into some products by a non-hydrolytic (transglycosylation) pathway have been reported.[241] The production of single-cell protein from starchy wastes has been discussed.[183]

The preparation of benzoylated carboxymethylstarches has been reported.[258] The relation between the conditions for carboxymethylation of starch and the properties of the resulting carboxymethylstarches has been investigated.[259] The sodium salt of carboxymethylstarch is readily converted into the free-acid form, which could not be washed effectively because of swelling. The sodium salt of starch glycolate has been used as the substrate in an assay of α-amylase.[173]

A novel preparation of *O*-hydroxyethylstarch has been reported.[260] By retaining the granular structure of the starch throughout a series of heterogeneous reactions, the *O*-hydroxyethylstarch produced contains low levels of salts and other impurities

[252] J. C. Jaton, H. Huser, D. G. Braun, D. Givol, I. Pecht, and J. Schlessinger, *Biochemistry*, 1975, **14**, 5312.
[253] G. M. Ballance and W. O. S. Meredith, *J. Inst. Brewing*, 1976, **82**, 64.
[254] W. A. Philpott and J. M. Chapman, *Analyt. Biochem.*, 1977, **79**, 257.
[255] R. Kohn, L'Rexová-Benkova, O. Luknár, and L. Kuniak, *Coll. Czech. Chem. Comm.*, 1976, **41**, 1879.
[256] M. J. Foglietti, H. Girerd, and F. Percheron, *Biochimie*, 1975, **57**, 667.
[257] H. Klaushofer, E. Berghofer, and L. Diesner, *Stärke*, 1976, **28**, 298.
[258] L. Colombe, R. A. Schutz, and I. Abd-el-Thalouth, *Stärke*, 1977, **29**, 126.
[259] T. Ogawa, *J. Jap. Soc. Starch Sci.*, 1976, **23**, 49.
[260] C. T. Greenwood, D. D. Muir, and H. W. Whitcher, *Stärke*, 1975, **27**, 109.

and is a good cryoprotective agent for human red-blood cells. *O*-Hydroxyethylstarches with a range of useful properties could be prepared by modification of this procedure.

Starch cross-linked with epichlorohydrin has been used as an affinity matrix in the purification of α-amylases[261] and, following reaction with *NN*-diethyl-2,3-epoxypropylamine, in the fractionation of polysaccharides.[210]

Dextrin graft copolymers containing about 50% of polyacrylamide by weight have been assessed as retention aids in the production of filled bond paper.[262] Their effectiveness increased as the molecular weight of the graft increased and they were more effective than mixtures of the individual polymers. Graft copolymers produced by reaction of starch with cationic or anionic or non-ionic acrylic monomers show considerable promise as flocculating agents for the treatment of industrial and municipal waste-waters, *etc.*[263] The effectiveness of the graft copolymers depends on the system being treated and on the structure of the graft.

Polyacrylonitrile has been located in the granules of two wheat-starch graft copolymers containing 22 or 44% of polyacrylonitrile.[264] Micrographs suggested that grafting onto granules containing 24% of graft copolymer occurs largely on the surface, whereas it takes place throughout most of the interior of granules containing 44% of graft copolymer. Starch in starch–vinyl graft copolymers can be degraded and removed by oxidation with perchloric acid in glacial acetic acid, thus providing an effective means for determining the content of starch graft copolymers with little or no degradation of the grafted polymer.[265]

The interactions of starch with aflatoxins B_1 and G_1 have been studied.[189]

Modification of Glycoproteins and Uses of Modified Glycoproteins

Albumins.—A conjugate of an azo derivative of bovine serum albumin and pneumococcal SIII polysaccharide has been used as the immunoadsorbent in the purification of anti-SIII antibodies.[190]

Bifunctional chelating agents, which are structurally similar to H_4edta, have been used to attach stable chelates of terbium and europium to human serum albumin, permitting the lanthanide fluorescence of the products to be studied at micromolar concentrations using standard instrumentation.[266] The use of these metal complexes in energy-transfer experiments should greatly reduce the uncertainty in estimating the separation between the donor and the acceptor.

Bovine serum albumin reacted with the 4-isothiocyanatophenyl glycosides of *Salmonella*-derived disaccharides to give immunogens that are suitable for eliciting specific anti-*Salmonella* O antisera.[10]

Antibodies.—Antisera (raised against the red-cell membranes of mice) and goat immunoglobulins (raised against the red-cell membranes of rabbits) have been

[261] M. Weber, M. J. Foglietti, and F. Percheron, *Biochimie*, 1976, **58**, 1299.
[262] H. D. Heath, G. F. Fanta, A. J. Ernst, B. T. Hofreiter, and R. C. Burr, *Svensk. Papperstidn.*, 1975, **78**, 488.
[263] R. C. Burr, G. F. Fanta, W. M. Doane, C. R. Russell, and D. A. Jones, *Stärke*, 1975, **27**, 155.
[264] G. F. Fanta, F. L. Baker, R. C. Burr, W. M. Doane, and C. R. Russell, *Stärke*, 1973, **25**, 157.
[265] R. J. Dennenberg and T. P. Abbott, *J. Polymer Sci., Polymer Letters*, 1976, **14**, 693.
[266] C. S.-H. Leung and C. F. Meares, *Biochem. Biophys. Res. Comm.*, 1977, **75**, 149.

treated with biotinyl-*N*-hydroxysuccinimide ester and used to locate antibody receptors on erythrocytes.[267]

Circulatory Glycoproteins.—A series of closely related glycoproteins containing only 2-acetamido-2-deoxy-α-D-galactose in their carbohydrate components was obtained by fragmentation of anti-freeze glycoproteins from the sera of the Antarctic fish *Trematomus borchgrevinki*.[268] The Ala-Ala-Thr sequences of these glycoproteins were substituted with the hexosamine in ways that affected the amount of α-helix, the density of the acceptor sites, and, possibly, the rigidity, polarity, and hydrophobicity of the polypeptide chain. Of these alterations, only the density of the acceptor sites had a statistically significant effect on the ability of the 2-acetamido-2-deoxy-α-D-galactopyranosyl—threonine unit to act as a substrate for the D-galactosyltransferase from rat-liver microsomes.

Collagens.—Collagen containing pendant acyl azide groups has been coupled to enzymes.[269]

Erythrocyte Glycoproteins.—A glycoprotein from human blood-group O erythrocytes could be desialylated by hydrolysis in 0.025M-sulphuric acid at 60°C.[121]

Fibrinogens.—The 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid residues were released with 0.05 M-sulphuric acid at 80 °C from glycopeptides derived from bovine fibrinogen.[270] After *N*-acetylation, the modified glycopeptides were treated with *N*-ethyl-5-phenylisoxazolium-3′-sulphonate and attached to lysozyme. This route could be applied generally to the formation of conjugates of glycopeptides and proteins.

Hormonal Glycoproteins.—Tritium-labelling of hormonal glycoproteins containing terminal residues of 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid can be accomplished by selective oxidation with periodate ion and reduction with sodium borotritiide.[271] Such modification, which removes C-8 and C-9 from terminal 5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid residues, did not appreciably alter the biological or immunological properties of human follicle-stimulating hormone or of human chorionic gonadotrophin and its subunits.

Immunoglobulins.—Immunoglobulin IgA myeloma glycoprotein from human sera has been fragmented by the action of pepsin.[97] 5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid residues in this glycoprotein and in glycopeptides derived from it were labelled by oxidation with periodate and reduction with sodium borotritiide.

The sulphydryl groups of K-type immunoglobulins and of α_1-antitrypsin have been modified by reaction with 5,5′-dithiobis(2-nitrobenzoic acid).[150]

Orosomucoids.—Orosomucoids without some of their non-reducing, terminal sugar residues have been used as antagonists in studies of the clearance of lysosomal glycoside hydrolases from the circulation of rats.[272]

[267] E. A. Bayer, M. Wilchek, and E. Skutelsky, *F.E.B.S. Letters*, 1976, **68**, 240.
[268] W. T. Shier and G. J. Roloson, *Canad. J. Biochem.*, 1977, **55**, 886.
[269] J. M. Brillouet, P. R. Coulet, and D. C. Gautheron, *Biotechnol. and Bioeng.*, 1976, **18**, 1821.
[270] E. Moczar and C. Sepulchre, *Biochimie*, 1975, **57**, 1241.
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[272] P. Stahl, P. H. Schlesinger, J. S. Rodman, and T. Doebber, *Nature*, 1976, **264**, 86.

Ovalbumins.—Inhibition of the interaction between anti-(D-mannan) antibodies and yeast D-mannan by a glycopeptide from ovalbumin has been examined.[273]

Phytohaemagglutinins.—[^{3}H-Acetyl]concanavalin A has been shown to bind to, and partially inhibit the enzymic activity of, proline 2-oxoglutarate dioxygenase.[274]

The products obtained when concanavalin A is labelled with 4-azidophenyl α-D-mannopyranoside under u.v.-irradiation have been separated by affinity chromatography on Sephadex G-100 at pH 5.[275] One of the products is a monovalent dimer at pH 5 and a divalent tetramer at pH 7, indicating that photoaffinity labelling did not alter the quaternary structure of concanavalin A, although the haemagglutinating activity of the derivatized lectin was reduced.

Concanavalin A and peanut agglutinin have been treated with biotinyl-*N*-hydroxysuccinimide ester and used to locate lectin-receptor sites on erythrocytes.[267] Fluoresceinyl derivatives of concanavalin A and of the agglutinins from wheat germ (*Canavalia ensiformis*), peanuts (*Arachis hypogaea*), soybeans (*Glycine max*), lotus (*Lotus tetragonolobus*), and waxbeans (*Phaseolus vulgaris*) have been used in studies of the hyphal walls of lichens.[276]

The non-covalent binding of phytohaemagglutinins to derivatives of agarose has been investigated.[31]

Immobilized Derivatives of Glycoproteins.—The modification of glycoproteins to give biologically active, immobilized derivatives continues to attract attention. These derivatives and their uses are summarized in Table 6.[277–281]

Studies with labelled oligosaccharides have shown that, at least, two α-D-mannopyranosyl residues with unsubstituted hydroxy-groups at C-3, C-4, and C-6 are required for oligosaccharides to be retained by agarose-bound concanavalin A.[91] Neutral glycopeptides (derived from rat-brain glycoproteins) have been separated from acidic glycopeptides by affinity chromatography on agarose-bound concanavalin A, using a concentration gradient of methyl α-D-glucopyranoside for elution.[102]

Modification of Enzymes and Uses of Modified Enzymes

A book has dealt with the spin-labelling of enzymes and the results of studies using spin-labelled enzymes.[282]

β-D-2-Acetamido-2-deoxyhexosidases.—Dissociation of human placental β-D-2-acetamido-2-deoxyhexosidase A with 4-chloromercuribenzoate afforded an alkylated fragment (mol. wt. 5.0×10^4) that was converted into the S form of the enzyme by 1,4-dithiothreitol.[283] It appears that β-D-2-acetamido-2-deoxyhexosid-

[273] N. Itoh and I. Yamashina, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 840.
[274] N. A. Guzman, R. A. Berg, and D. J. Prockop, *Biochem. Biophys. Res. Comm.*, 1976, **73**, 279.
[275] M. Beppu, T. Terao, and T. Osawa, *J. Biochem. (Japan)*, 1975, **78**, 1013.
[276] M. Galun, A. Braun, A. Frendsdorff, and E. Galun, *Arch. Mikrobiol.*, 1976, **108**, 9.
[277] I. Karube, T. Suganuma, and S. Suzuki, *Biotechnol. and Bioeng.*, 1977, **19**, 301.
[278] T. Sato, T. Mori, T. Tosa, I. Chibata, M. Furui, K. Yamashita, and A. Sumi, *Biotechnol. and Bioeng.*, 1975, **17**, 1797.
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[282] G. I. Likhtenshtein, 'Spin Labelling Methods in Molecular Biology', Wiley, Chichester, 197
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Table 6 *Modification of glycoproteins by coupling to insoluble matrices and other macromolecules*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*[a]	*Use of product*	*Ref.*
Abrus precatorius phytohaemagglutinin		Purification of membrane glycoproteins from human-blood platelets	57
Anti-(α-foetoprotein) antibody	Reaction with agarose cyclic imidocarbonate	Purification of rat α-foetoprotein	48
Anti-(*Azotobacter vinelandii*) antibody		Purification of the nitric-oxide reductase from *Azotobacter vinelandii*	49
Anti-dinitrophenyl antibody		Comparison with other agarose matrices used in affinity chromatography	37
	Reaction with (17) *via* glutaraldehyde-coupling (see Scheme 5)	Affinity chromatography; comparison with agarose cyclic imidocarbonate	37
Anti-(*Phaseolus vulgaris* α-D-mannosidase) antibody		Purification of two forms of α-D-mannosidase from *Phaseolus vulgaris* by affinity chromatography	52
Anti-(*Phaseolus vulgaris* α-D-mannosidase I) antibody immunoglobulin IgG		Purification of two forms of α-D-mannosidase from *Phaseolus vulgaris* by immunoadsorption	53
Anti-(rat β-D-glucuronidase) antibody	Reaction with agarose cyclic imidocarbonate	Purification of rat-liver β-D-glucuronidase	54
α_1-Antitrypsin		Sulphide–disulphide interchange chromatography in the separation of plasma proteins	150
Bacillus Calmette-Guérin antibody		Purification of tuberculin-active components	50
Bovine serum albumin	Reaction with cellulose cyclic imidocarbonate	Investigation of the binding capacity of the matrix	193
Bovine serum albumin (azo derivative)	Reaction with pneumococcal SIII polysaccharide	Production of an immunoadsorbent for anti-SIII antibodies	190
Bovine serum albumin (pneumococcal SIII polysaccharide derivative)	Reaction with bromoacetylcellulose		
Bovine submaxillary glycoprotein	Reaction with agarose cyclic imidocarbonate	Purification of limulin	84
Cells (diverse)	Glutaraldehyde coupling with Sephadex G-50 beads treated with concanavalin A	Matrix for the adsorption of cells to give immobilized cell-surface carbohydrates for use in the purification of immunoglobulins	240

Collagen	Entrapment of *Achromobacter lunatus* bacteriolytic enzyme	Active immobilized enzyme	277
	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of platelets; studies of the interaction between platelets and collagen	86
		Investigation of the binding of collagen to chondroitin sulphate	151
Collagen (derivatized)	Reaction with glucoamylase	Active immobilized enzyme	269
Concanavalin A	Adsorption onto magnetite	Selective binding of micro-organisms	249
	Coating onto Sephadex G-50 beads	Matrix for the adsorption of cells to give immobilized cell-surface carbohydrates for the purification of immunoglobulins	240
	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of alkaline phosphatase, 5′-nucleotidase, and cAMP phosphodiesterase	88
		Fractionation of glycopeptides; studies of the structural requirements for binding glycopeptides	91
		Purification of acetylcholinesterase from the head-tissues of house flies	93
		Purification of the glycogen-debranching enzyme (amylo-1,6-glucosidase) from dogfish muscle	92
		Purification of glycosulphatase	94
		Purification of horseradish peroxidase	95
		Purification of human-liver β-D-galactosidases	77
		Purification of β-D-2-acetamido-2-deoxy-hexosidases A and B from human placenta	96
		Purification of pepsin glycopeptides from human immunoglobulin IgA_1 myeloma glycoprotein	97
		Purification of porcine haptoglobin	98
		Purification of the serum prohormone of angiotensin	99

[a] Covalent coupling may be assumed, unless otherwise indicated.

Table 6 *(cont.)*

Glycoprotein	*Macromolecule or matrix coupled and mode of coupling*[a]	*Use of product*	*Ref.*
Concanavalin A *(continued)*		Purification of the αα-trehalase from *Saccharomyces cerevisiae*	100
		Separation of different populations of membrane vesicles from thymocytes	101
		Separation of acidic and neutral glycopeptides from rat brain	102
		Structural investigations of saracen corn (*Fagopyrum esculentum*) α-L-rhamnosidase	152
		Studies of the amylases from human carcinomas	103
		Studies of the interaction of concanavalin A with *Trypanosoma equiperdum* and for isolation of the antigenic glycoproteins	104
		Studies of the dissociation of α-amylase–immunoglobulin complexes	105
		Matrix for the adsorption of a glycoprotein enzyme (subsequently coupled to another enzyme)	90
Escherichia coli cells	Entrapment in a polyacrylamide gel	Active immobilized aspartase in the continuous production of L-aspartic acid by immobilized *E. coli* cells	278
Gluconobacter melanogenus cells	Entrapment in a polyacrylamide gel	Active immobilized enzyme for the conversion of L-sorbose into L-sorbosone	279
D-Glucose isomerase	As heat-treated whole cells of *Streptomyces phaeochromogenus*	Active immobilized enzyme	280
Goat anti-dinitrophenyl antibody	Reaction with agarose cyclic imidocarbonate	Comparison with other agarose matrices	37
	Reaction with (17) *via* glutaraldehyde-coupling	Comparison with agarose cyclic imidocarbonate	37

Goat immunoglobulin anti-(rabbit immunoglobulin) antibody	Reaction with cellulose cyclic imidocarbonate	Immobilized second antibody used for the separation of bound and free hormones in radioimmunoassays of gonadotrophin	193
Goat immunoglobulin IgG anti-(*Azotobacter vinelandii*) antibody	Reaction with agarose cyclic imidocarbonate	Purification of the nitric-oxide reductase from *Azotobacter vinelandii*	49
Human [^{125}I]luteinizing hormone	Reaction with cellulose cyclic imidocarbonate	Investigation of the binding capacity of the matrix	193
Lens culinaris phytohaemagglutinin	Reaction with agarose cyclic imidocarbonate	Purification of membrane glycoproteins from human-blood platelets	57
Microbial cells	Attachment to a collagen membrane by impregnation	Investigation of the attachment of microbial cells to collagen	281
Peanut lectin	Reaction with (17) *via* glutaraldehyde-coupling	Matrix for affinity chromatography	37
Rabbit anti-enterotoxin	Reaction with agarose cyclic imidocarbonate or (7)	Purification of *Clostridium perfringens* enterotoxin	56
Rabbit immunoglobulin *Bacillus Calmette-Guérin* antibody		Purification of tuberculin-active components	50
Rabbit immunoglobulin IgG anti-(*Phaseolus vulgaris* α-D-mannosidase) antibody		Purification of two forms of α-D-mannosidase from *Phaseolus vulgaris* by affinity chromatography	52
Rabbit immunoglobulin IgG anti-(*Phaseolus vulgaris* α-D-mannosidase I) antibody		Purification of two forms of α-D-mannosidase from *Phaseolus vulgaris* by immunoadsorption	53
Rabbit immunoglobulin IgG anti-(rat β-D-glucuronidase) antibody	Reaction with agarose cyclic imidocarbonate	Purification of rat-liver β-D-glucuronidase	54
Red-cell glycoprotein (desialylated)		Purification of *Vicia graminea* anti-(blood-group N) lectin	121
Ricinis communis agglutinin		Investigation of the heterogeneity of rat α-foetoprotein	48
Type-K immunoglobulin chains		Sulphide–disulphide interchange chromatography in the separation of plasma proteins	150
Wheat-germ phytohaemagglutinin		Purification of membrane glycoproteins from human-blood platelets	57

[a] Covalent coupling may be assumed, unless otherwise indicated.

ase A is composed of two α- and two β-subunits, each with a molecular weight of about 2.7×10^4, and that the correct structures of β-D-2-acetamido-2-deoxyhexosidases B and S are β_4 and α_4, respectively.

β-D-Galactosidases.—The β-D-galactosidase from *Curvularia inaequalis* adsorbed two molecules of Bright Orange KH; the six sulphate groups introduced by the dye had little effect on the isoelectric point or catalytic activity of the enzyme.[284]

β-D-Galactosidase coupled to insulin showed no loss of enzymic activity, so that the conjugated form could be used in an enzyme-linked immunoassay for insulin.[285]

α-Amylases.—A glycopeptide derived from Taka-amylase A strongly inhibited the interaction of anti-(*Saccharomyces cerevisiae* D-mannan) antibodies with yeast D-mannan.[273] The results were compared with those obtained using glycopeptides derived from ovalbumin.

Photo-control of the enzymic activity has been achieved by attaching a photochromic spiropyran to α-amylase.[286] The derivative obtained when *Bacillus subtilis* α-amylase reacted with 1-(2-carboxyethyl)-3,3-dimethyl-6′-nitrospiro[indoline-2,2′-2*H*-benzopyran] anhydride exhibited a reversible photochromism in aqueous solution: it is yellow in the dark and colourless under u.v.-irradiation. The enzymic activity of the colourless derivative is much less than that of the coloured derivative, due, most probably, to a change in affinity for the substrate (amylose).

Kinetic studies of porcine pancreatic α-amylase derivatized with 5,5′-dithiobis-(2-nitrobenzoic acid) have shown that V_m is decreased by a factor of four whereas K_m is unchanged.[287] The derivatized enzyme binds to polydextran gel as effectively as the native enzyme.

Treatment of 4-phenylazobenzoylated Taka-amylase A with hydroxylamine released some of the substituent groups and restored the activity of the enzyme.[288] This suggests that one of the substituent groups is attached to the tyrosyl residue located near the active site of the enzyme. Moreover, the derivatized enzyme did not catalyse the transfer of maltose from phenyl α-maltoside to maltotri-itol, indicating that a 4-phenylazobenzoyl group attached near one of the aglycone-binding subsites impedes transglycosylation.

β-Amylases.—Five half-cystine residues in soybean β-amylase can be titrated with 4-chloromercuribenzoate after denaturing the enzyme with guanidinium hydrochloride.[289] The reactivities of these groups towards 4-chloromercuribenzoate differ and are pH-dependent. Differential titrations at different pH values and in the presence of maltose showed that reaction of only one sulphydryl group with 4-chloromercuribenzoate results in loss of enzymic activity and a conformational change.

Amylo-1,6-D-glucosidases.—The amylo-1,6-D-glucosidase from dogfish muscle has been completely carboxymethylated.[92]

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Cellulases.—During the hydrolysis of insoluble, amorphous cellulose by *Trichoderma viride* cellulase in a batch reactor, the enzyme was adsorbed onto the substrate and gradually returned to solution as the reaction progressed.[290] A kinetic model was proposed to account for these observations.

Lysozymes.—The steady-state and transient fluorescence of derivatives of hen egg-white lysozyme have been compared with those of derivatives of other lysozymes and α-lactalbumin.[291]

Hen egg-white lysozyme has been *N*-acetylated and the positions of the *N*-acetylated amino-groups in the protein have been estimated.[292] Six amino-groups in lysozyme were acetylated and one amino-group (that of lysine-33) remained free.

The binding constants for 2-acetamido-2-deoxy-D-glucopyranose and its α- and β-glycosides with hen and turkey egg-white lysozymes have been determined at various pH values by measuring changes in the c.d. band at 295 nm.[293]

Tryptophan-62 of hen egg-white lysozyme has been selectively (2-nitrophenylsulphenyl)ated.[294] The pH-dependence of the extrinsic c.d. band at 375 nm of this lysozyme derivative was interpreted in terms of the participation by glutamic acid-35 (p*K* 6.2) and aspartic acid-101 (p*K* 4.6) and -66 (p*K* 1.5). The fact that ionization of glutamic acid-35 affects the extrinsic c.d. band of the chromophore attached to tryptophan-62 confirms that there is a relation between the state of tryptophan-62 and the ionization state of one of the catalytic groups in hen egg-white lysozyme.

Hen egg-white lysozyme has been converted into a carboxyethylated derivative, which was used in delineation of the reactive site around the disulphide bridge linking residues 6 and 127.[16] Carboxymethylated, carboxamidomethylated, and ethyltriphenylphosphonium derivatives of reduced hen egg-white lysozyme have been prepared.[295] The proportions of protein chain present as α-helix, β-structure, and disordered structure in these derivatives were estimated by means of a computer-adapted curve-fitting program. The fluorescence of hen egg-white lysozyme derivatized with 1-anilinonaphthalene-8-sulphonate has been investigated.[296] Rat lysozyme has been reduced and carboxymethylated prior to sequencing.[297]

Triclinic crystals of hen egg-white lysozyme cross-linked with glutaraldehyde can be analysed by *X*-ray methods, even after significant conformational changes are introduced by the actions of denaturants.[298] Crystals of the cross-linked enzyme are isomorphous with those of the native form, and *E*-maps showed that intermolecular cross-linking does not cause any appreciable change in the molecular conformation. The results suggest that detergent-like reagents disrupt the hydrophobic interactions joining the two wings of the molecule of hen egg-white lysozyme, whereas hydrophilic denaturants interact primarily with groups on the

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surface. Cross-linked triclinic, denatured lysozyme assumes a conformation similar to, but not identical with, that of the native enzyme on re-folding after removal of the denaturant (sodium dodecyl sulphate).[299] Analysis of *E*-maps for two stages of the re-folding pathway revealed that trapping of the denaturant in the interior of the protein forces apart the two wings of the molecule.

Polygalacturonases.—The (*endo*-)polygalacturonase from *Pseudomonas cepacia* has been labelled with fluorescein isocyanate so that its penetration into onion (*Allium cepa*) tissues could be followed.[300]

D-Galactose Oxidases.—Although four tryptophanyl residues in the holoenzyme of a fungal D-galactose oxidase can be readily oxidized with NBS, only one of them need be oxidized to abolish almost completely the enzymic activity.[301] C.d. and e.s.r. measurements suggested that inactivation of D-galactose oxidase with NBS converts the pseudo-square-planar copper complex into a more distorted, tetrahedral-like complex. Since inactivation can be accomplished without affecting the binding of the sugar substrate, oxidation of a single tryptophanyl residue must affect the catalytic activity. The fluorescence intensity of a fungal D-galactose oxidase was reduced by 23% on oxidation (NBS) of the equivalent of 0.85 of the tryptophanyl residues and it was further reduced by 25% following oxidation of two of the tryptophanyl residues. Fluorescence experiments showed that the modified enzyme contains, at least, one tryptophanyl residue which is unreactive towards NBS but which interacts with the Cu^{II} atom and the substrate.[302] This and other data support the proposal that one or more tryptophanyl residues, the Cu^{II} atom, and the sugar substrate mutually interact within the enzyme.

Alkylation of a histidyl residue of D-galactose oxidase with [^{14}C]iodoacetamide resulted in loss of the catalytic activity, although the binding of methyl β-D-galactopyranoside was not affected by alkylation.[303] The protein-bound copper atom was not lost during alkylation, but its optical activity and optical absorbance were almost completely abolished. Apo-D-galactose oxidase did not react with iodoacetamide. Alkylation of D-galactose oxidase could be inhibited by oxidation (NBS) of a tryptophanyl residue at the active site–the fraction of catalytic activity remaining after such oxidation corresponded to the extent of alkylation that could be achieved.

D-Glucose Oxidases.—Oxidation of the D-glucose oxidase from *Aspergillus niger* with periodate decreased its carbohydrate content, but the catalytic activity, immunological reactivity, and gross secondary and quaternary structures were not affected.[304] The periodate-oxidized derivative was stable to heat treatment, although its stability was reduced in the presence of denaturants.

Cyclodextrin D-Glucanotransferases.—A succinyl derivative has been prepared from the cyclodextrin D-glucanotransferase isolated from an alkalophilic *Bacillus* species.[225]

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Bromelains.—The reactive sulphydryl groups of stem and juice bromelains have been blocked with phenylmercuriacetate during purification of the enzymes, which were further modified by reaction with 2-hydroxy-5-nitrobenzyl bromide or tetranitromethane or hydrogen peroxide.[305] The activities of the modified enzymes towards substrates of high or low molecular weight were examined.

α-Lactalbumins.—The chemical modification of α-lactalbumins has been reviewed.[306] Fluorescence methods have been used in studies of the interactions of bovine α-lactalbumin with 1-anilinonaphthalene-8-sulphonate[296] and of immobilized human α-lactalbumin, which had been dyed with 2-(4-toluidinyl)-naphthalene-6-sulphonate, with D-galactosyltransferase and anti-(α-lactalbumin) antibodies.[155]

Immobilized Derivatives of Enzymes.—The latest volume in the series 'Methods in Enzymology' is devoted to such aspects of immobilized enzymes as immobilization techniques, the applications of immobilized enzymes to biochemistry and organic chemistry, and immobilized coenzymes, *etc.*[307] Reviews, *etc.*, on diverse aspects of modified enzymes have dealt with the design of flow reactors incorporating immobilized enzymes,[308] the chemistry of immobilization,[309,310] and industrial and other applications of immobilized enzymes and immobilized microbial cells.[311,312]

A simple and rapid method for finding the best conditions for immobilization of proteinaceous molecules on agarose cyclic imidocarbonate is based on continuous spectrophotometric monitoring of the unbound protein as it is recycled through a flow reactor containing the activated gel.[40]

A book on biomedical applications of immobilized enzymes has reviewed current research on the uses of immobilized enzymes and immobilized proteins in diagnosis and therapy (*e.g.* in the construction of artificial kidneys and livers, detoxification, and fuel cells).[313] The uses of immobilized enzymes in clinical and biochemical analyses have also been reviewed.[314]

Kinetic parameters and mass-transfer effects of an immobilized enzyme in a packed-bed reactor[315] and non-isothermal, heterogeneous reactions in a denaturable, immobilized enzyme catalyst have been examined from theoretical viewpoints.[316] It appears that thermal inactivation (denaturation) has not been considered in previous investigations of the effectiveness factor of an immobilized enzyme catalyst. It was pointed out that it is important to consider the transient

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state, rather than the steady state, when investigating the heterogeneous reactions in an immobilized enzyme catalyst.

A short review on the preparation and uses of immobilized microbial cells has tabulated information that has appeared in the literature.[317] The potential use of magnetic supports in separations of biologically active proteins has been discussed.[318]

Many preparations of immobilized enzymes and of enzymes coupled to other macromolecules to give water-soluble or -insoluble forms have been reported during the past year. These derivatives and their uses are summarized in Table 7.[319–382] Immobilized enzymes are referred to in this Table, whether they be enzymically active or not.

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Table 7 *Modification of enzymes by coupling to insoluble matrices and other macromolecules*

Enzyme	*E.C No.*	*Matrix or macromolecule coupled and mode of coupling*[a]	*Use of product*	*Ref.*
Acetylcholinesterase substrate	3.1.1.7	Immobilization of acetylcholine hydrochloride on an ion-exchange resin	Assay of cholinesterase	319
Alcohol dehydrogenase	1.1.1.1	Adsorption onto magnetite	Active immobilized enzyme	249
		Adsorption onto alumina		320
		Adsorption onto alumina coated with denatured bovine serum albumin		320
		Glutaraldehyde-coupled to a dimethylsilicone membrane with a polyester-fibre backing on the solution side	Active immobilized enzyme used in chemical assays with mass spectrometry	321
		Glutaraldehyde-coupled to aminoethylcellulose	Affinity chromatography; separation of enzyme cofactors	33
		Reaction with agarose cyclic imidocarbonate	Studies on the conformations of soluble, immobilized enzymes using differential scanning calorimetry, and of the thermal stabilities of NAD^+-linked dehydrogenases	34
		Reaction with a 2-amino-4,6-dichloro-*s*-triazinyl derivative of DEAE-cellulose	Affinity chromatography; separation of enzyme cofactors	33
		Reaction with *s*-triazinylated DEAE-cellulose	Active immobilized enzyme; investigation of the recycling of the cofactor in an enzyme reactor using the immobilized enzyme and the immobilized cofactor	147
Amine oxidase (flavin-containing)	1.4.3.4	Glutaraldehyde-coupled to a derivatized, zirconia-clad porous glass	Application of an insolubilized microsomal oxidase to the continuous production of *N*-oxides	322
L-Amino-acid oxidase	1.4.3.2	Cross-linked with glutaraldehyde to a protein on an electrode	Construction of electrodes incorporating cross-linked enzymes	323

[a] Covalent coupling may be assumed, unless otherwise indicated.

Table 7 *(cont.)*

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*[a]	*Use of product*	*Ref.*
Aminoacylase	3.5.1.14	Adsorption onto alumina Adsorption onto alumina coated with denatured bovine serum albumin	Active immobilized enzyme	320
α-Amylase	3.2.1.1	Reaction with dextran cyclic imidocarbonate	Active immobilized enzyme (water-soluble form)	235
β-Amylase	3.2.1.2	Hydrophobic adsorption onto hexyl-substituted, epichlorohydrin-cross-linked agarose	Active immobilized enzyme	164
			Active immobilized enzyme; studies of the relation between the enzymic activity retained and the extent of substitution of the matrix	324
		Hydrophobic attachment to hexyl- or octyl-substituted, epichlorohydrin-cross-linked agarose containing mercapto-groups on the side-chains	Demonstration of the reversible immobilization of enzymes	165
Arginase (monomer)	3.5.3.1	Coupled with glutaraldehyde to nylon	Active immobilized enzyme; studies of the organization and tetramerization of the arginase monomer	325
L-Asparaginase	3.5.1.1	Embedded in fibrin polymer	Active immobilized enzyme; investigations of blood coagulation	326
Aspartate ammonia-lyase (as *Escherichia coli* cells)	4.3.1.1	Entrapment in a polyacrylamide gel	Continuous production of L-aspartic acid by immobilized *E. coli* cells in fixed beds	278
Aspartate aminotransferase	2.6.1.1	Covalent reaction with activated forms of silk	Active immobilized enzyme	327
Bacteriolytic enzyme from *Achromobacter lunatus*	—	Entrapment in collagen membranes	Active immobilized enzyme used for the lysis of bacterial cells	277
Carboxyesterase	3.1.1.1	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; evaluation of the kinetics of the immobilized enzyme in single enzyme–agarose beads by microfluorometry	35

Catalase	1.11.1.6	Adsorption onto magnetite	Active immobilized enzyme	249
		Attachment to collagen by impregnation	Investigation of the mechanism of enzyme attachment to collagen	281
		Glutaraldehyde-cross-linked with a protein on an electrode	Construction and study of an electrode using cross-linked enzymes	323
		Glutaraldehyde-linked to silanized, nickel-impregnated silica–alumina containing free amino-groups	Active immobilized enzyme; comparison of the relative efficiency of a soluble, immobilized two-enzyme system of D-glucose oxidase and catalase	328[b]
			Investigation of the parameters operating in the construction of an immobilized two-enzyme system	329[b]
		Reaction with dextran cyclic imidocarbonate	Water-soluble, active immobilized enzyme; stabilization of the enzymic activity	236
		Reaction with various forms of diazotized 1,4-diaminobenzene	Active immobilized enzyme	330
Catalase (free or glutaraldehyde-cross-linked)	1.11.1.6	Reaction with derivatives of glass, silica, *etc.*	Active immobilized enzyme; for increasing the catalytic activity by the immobilization of enzyme conjugates	331
Cellulase	3.2.1.4	Adsorption onto cellulose	Active immobilized enzyme used in kinetic studies of an insoluble cellulose–cellulase system	290
		Reaction with pendant methylimidate groups on a modified polyacrylonitrile resin	Active immobilized enzyme; investigations of the catalytic activity on immobilization	332

[a] Covalent coupling may be assumed, unless otherwise indicated; [b] immobilized together with D-glucose oxidase.

Table 7 *(cont.)*

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*[a]	*Use of product*	*Ref.*
Cephalosporin acetylesterase	—	Containment within an ultrafiltration device	Active immobilized enzyme; time–course studies on immobilized cephalosporin acetylesterase	333
Chorismate mutase	5.4.99.5	Hydrophobic binding to (6)	Active immobilized enzyme; preparation of prephenate from chorismate	36
Chymotrypsin	3.4.21.2	Coupling to methacrylate rubber Covalent attachment to nylon fibres derivatized with polyalanine by glutaraldehyde coupling Covalent attachment to viscose fibres containing aldehydo-groups	Active immobilized enzyme; investigation of the change in enzymic activity on reversible deformation of the matrix	209
		Entrapment in, and mechanochemical attachment to, partially hydrolysed nylon fibres	Active immobilized enzyme; demonstration of a new mechanochemical method for immobilization	334
		Glutaraldehyde-coupled to human hair	Active immobilized enzyme; investigation of the change in enzymic activity on reversible deformation of the matrix	209
		Glutaraldehyde-cross-linked to partially hydrolysed caprone (poly-ε-caproamide, nylon 6) thread		335
		Immobilized within a poly(methacrylic acid) gel	Active immobilized enzyme	336
		Microencapsulation in semipermeable nylon membranes	Active immobilized enzyme; investigation of the effects of microencapsulation on the properties of the enzyme	337
		Non-covalent interaction with chitosan		224
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme	37

R*		Reaction with cross-linked agarose cyclic imidocarbonate	Studies on the conformations of soluble, immobilized enzymes by differential scanning calorimetry and of the thermal stability and specific activity of the immobilized enzyme	38
		Reaction with cross-linked, phloroglucinol-modified agarose (*see* Scheme 7)		166
		Reaction with, or glutaraldehyde-coupled to, non-porous magnetic materials	Active immobilized enzyme	338
		Reaction with (17) *via* glutaraldehyde-coupling	Active immobilized enzyme; comparison of the properties of the matrix with those of agarose cyclic imidocarbonate	37
Creatinine deiminase	3.5.4.21	Immobilized within a cellophane membrane in an Orion model 95-10 ammonia-gas sensing electrode	Enzyme electrode used in the determination of creatinine	339
Cyclodextrin D-glucano-transferase	2.4.1.19	Adsorption onto a vinylpyridine copolymer	Active immobilized enzyme used in the production of cyclohepta-amylose	225
Deoxyribonuclease II (oxidized)	3.1.4.6	Reaction with hydrazide derivatives of poly-saccharides (Scheme 2)	Affinity chromatography	22
Dextranase	3.2.1.11	Chelation to porous titanium(IV) oxide	Active immobilized enzyme; investigation of the reversibility of immobilization	340
β-D-Fructofuranosidase	3.2.1.26	Adsorption onto Amberlite IRA resin	Active immobilized enzyme used for continuous hydrolysis of sucrose	341
		Covalent attachment or chelation to activated derivatives of pyroxene, quartz, hornblende, and porous glass	Comparisons of the suitability of mineral and mineralized supports for immobilization	342
		Diffusion into hollow-fibre cartridges	Active immobilized enzyme for use in hollow-fibre reactors	343
		Entrapment in poly(2-hydroxyethyl acrylate) or in polydimethylacrylamide during γ-irradiation	Active immobilized enzyme	344

[a] Covalent coupling may be assumed, unless otherwise indicated.

Table 7 *(cont.)*

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*[a]	*Use of product*	*Ref.*
β-D-Fructofuranosidase *(continued)*		Ionic interaction with a guanidino derivative of cellulose	Active immobilized enzyme; investigations of the diffusion of substrates	198
		Reaction of an immobilized, glutaraldehyde-activated form with urease (see p. 510)	Active, soluble immobilized enzyme	90
		Reaction with Enzacryl-ALO and -TIO	Comparisons of the suitability of mineral and mineralized supports for immobilization	342
D-Galactose oxidase	1.1.3.9	Reaction with derivatized porous glass	Determinations of D-galactose and lactose in biological fluids	345
			Active immobilized enzyme; investigation of pore diffusion with a two-substrate reaction	346
α-D-Galactosidase	3.2.1.22	Diffusion into hollow-fibre cartridges	Active immobilized enzyme used in hollow-fibre reactors	343
		Enzyme-containing spherical pellets formed in a submerged culture of *Mortierella vinacea*	Active immobilized enzyme; kinetic studies of the immobilized enzyme	347
β-D-Galactosidase	3.2.1.23	Adsorption of a dyed derivative of the enzyme on ion-exchange resins	Active immobilized enzyme	284
		Adsorption onto carbon and cross-linked with glutaraldehyde	Demonstration of the unsuitability of the method for the immobilization of β-D-galactosidase	348
		Attachment to collagen by impregnation	Investigations of the mechanism of attachment of the enzyme to collagen	281
		Attachment to hydrolysed and etched nylon tubing	Active immobilized enzyme used for the hydrolysis of β-D-galactosides in an open tubular reactor	349
		Encapsulation in microcapsules of cellulose nitrate	Studies of the kinetic properties of microencapsulated β-D-galactosidase	202

		Entrapment in poly(2-hydroxyethyl acrylate) or in polydimethylacrylamide during γ-irradiation	Active immobilized enzyme	344
		Entrapment in polyvinylpyrrolidone during γ-irradiation	Active immobilized enzyme used for the continuous hydrolysis of lactose in acid whey	350
		Glutaraldehyde-coupled to beads of derivatized porous glass	Active immobilized enzyme; immobilized two-enzyme system for activation of the lactoperoxidase system in milk	351[b]
		Glutaraldehyde-linked to granular feather-meal protein	Active immobilized enzyme	352
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; hydrolysis of synthetic disaccharides	39
		Reaction with granulated pumice coated with a copolymer of phenylenediamine and glutaraldehyde	Active immobilized enzyme used in the preparation of columns for the hydrolysis of lactose	353
Glucoamylase	3.2.1.3	Adsorption onto acid-activated molecular sieves and on alumina	Active immobilized enzyme	354
		Entrapment in poly(2-hydroxyethyl acrylate) or in polydimethylacrylamide during γ-irradiation		344
		Glutaraldehyde-coupled to aminoalkylsilylated glass	Active immobilized enzyme; heat-transfer study of packed reactors incorporating immobilized glucoamylase	355
		Glutaraldehyde-coupled to, and cross-linked on, hornblende and activated hornblendes	Active immobilized enzyme	356
		Glutaraldehyde-linked to an alkylamine derivative of porous glass	Active immobilized enzyme used in the pilot-plant production of D-glucose from dextrins	357

[a] Covalent coupling may be assumed, unless otherwise indicated; [b] immobilized together with D-glucose oxidase.

Table 7 *(cont.)*

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*[a]	*Use of product*	*Ref.*
Glucoamylase *(continued)*		Hydrophobic adsorption onto hexyl-substituted, epichlorohydrin-cross-linked agarose	Active immobilized enzyme	358
		Hydrophobic attachment to hexyl-substituted, epichlorohydrin-cross-linked agarose or to octyl-substituted, epichlorohydrin-cross-linked agarose containing mercapto-groups on the side-chains	Demonstration of the reversible immobilization of enzymes	165
		Reaction with derivatized collagen	Active immobilized enzyme used in a helicoidal reactor	359
			Active immobilized enzyme used in a thin-layer reactor	269
		Reaction with various forms of diazotized 1,4-diaminobenzene	Active immobilized enzyme	330
		Reaction with a polyacrylonitrile resin by amidination	Active immobilized enzyme; investigations of the change in the catalytic activity on immobilization	360
		Reaction with tolylene 2,4-di-isocyanate derivatives of cellulose beads	Active immobilized enzyme; studies of the suitability of cellulose beads for immobilization	207
D-Glucose isomerase	5.3.1.18	Adsorption onto DEAE-cellulose	Active immobilized enzyme used for the continuous isomerization of D-glucose to D-fructose on a commercial scale	361
		As intact bacterial cells	Derivation of a model for the enzymic isomerization of D-glucose to D-fructose in a batch reactor	362
		Glutaraldehyde-coupled to aminoalkylsilylated glass	Active immobilized enzyme	363
		Glutaraldehyde-linked to chitin or adsorbed onto chitin		223

		Reaction with pendant methylimidate on a modified polyacrylonitrile resin	Active immobilized enzyme	364
D-Glucose oxidase	1.1.3.4	Reaction with beads of derivatized non-porous glass	Active immobilized enzyme; studies of the effects of immobilization on the properties of the enzyme	365
		Entrapment in gels of calcium alginate	Active immobilized enzyme	170
		Glutaraldehyde-coupled to beads of derivatized porous glass	Active immobilized enzyme; immobilized two-enzyme system for activation of the lactoperoxidase system in milk	351[c]
		Glutaraldehyde-cross-linked on manganese (III) oxide	Active immobilized enzyme with inbuilt pseudoperoxidase	366
		Glutaraldehyde-cross-linked with a protein on an electrode	Construction and study of electrodes incorporating the cross-linked enzyme	323
		Glutaraldehyde-linked to silanized, nickel-impregnated silica–alumina containing free amino-groups	Active immobilized enzyme; comparisons of the relative efficiencies of soluble and immobilized two-enzyme systems consisting of D-glucose oxidase and catalase	328[d]
			Active immobilized enzyme; investigations of the construction of an immobilized, two-enzyme system	329[d]
		Reaction with agarose cyclic imidocarbonate	Continuous monitoring of the amount of enzyme immobilized	40
		Reaction with various forms of diazotized 1,4-diaminobenzene	Active immobilized enzyme	330
		Reaction with a derivative of formaldehyde-cross-linked DEAE-cellulose beads	Active immobilized enzyme; studies of the suitability of cellulose beads for immobilization	207

[a] Covalent coupling may be assumed, unless otherwise indicated; [b] immobilized together with D-glucose oxidase; [c] immobilized together with β-D-galactosidase; [d] immobilized together with catalase.

Table 7 *(cont.)*

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*[a]	*Use of product*	*Ref.*
D-Glucose oxidase (free or glutaraldehyde-cross-linked)	1.1.3.4	Reaction with derivatives of glass, silica, *etc.*	Active immobilized enzyme; immobilization of enzyme conjugates	331
D-Glucose 6-phosphate dehydrogenase	1.1.1.49	Reaction with the cyclic imidocarbonate derivatives of agarose or Sephadex in the presence, or absence, of amino-acids	Active immobilized enzyme; comparison of the properties of the soluble and insoluble enzymes	41
α-D-Glucosidase β-D-Glucosidase	3.2.1.20 3.2.1.21	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; hydrolysis of synthetic disaccharides	39
		Reaction with various forms of diazotized 1,4-diaminobenzene		330
Glycine carboxypeptidase	3.4.12.8	Reaction with periodate-oxidized agarose that had been treated with 1,6-diaminohexane and reduced	Active immobilized enzyme	29
Glycyl-tRNA synthase	6.1.1.14	Reaction with activated forms of silk		327
Hexokinase	2.7.1.1	Reaction with, and glutaraldehyde-linked to, various derivatives of controlled-porosity glass		367
Inulinase	3.2.1.7	Entrapment in gels of calcium alginate		170
α-Lactalbumin	—	Reaction with agarose cyclic imidocarbonate	Fluorometric studies of the interactions of immobilized α-lactalbumin with D-galacto-syltransferase and with anti-(α-lactalbumin) antibodies	155
Lactate dehydrogenase	1.1.1.27	Reaction with agarose cyclic imidocarbonate	Affinity chromatography: separation of enzyme cofactors	33
			Continuous monitoring of the amount of enzyme immobilized	40
			Studies of the conformations of soluble and immobilized enzymes by differential scanning calorimetry and of the thermal stability of NAD^+-linked dehydrogenases	34

		Reaction with a 2-amino-4,6-dichloro-*s*-triazinyl derivative of DEAE-cellulose	Affinity chromatography; separation of enzyme cofactors	33
		Reaction with cross-linked agarose cyclic imidocarbonate	Active immobilized enzyme; studies of the use of immobilized flavins in the regeneration of a coenzyme	42
		Reaction with a *s*-triazinyl derivative of DEAE-cellulose	Active immobilized enzyme; studies of the recycling of a cofactor in an enzyme reactor incorporating the immobilized enzyme and the immobilized cofactor	147
Lipoamide dehydrogenase (NADH) isoenzymes	1.6.4.3	Reaction with (6)	Studies of the behaviour of lipoamide dehydrogenase isoenzymes bound to immobilized aminoalkanes	43
Lysozyme	3.2.1.17	Attachment to collagen by impregnation	Investigations of the mechanism of attachment to collagen	281
Microbial cells	—		Investigation of the mechanism of attaching intact cells to collagen	281
Monophenol mono-oxygenase	1.14.18.1	Entrapment in polyacrylamide gels during polymerization	Active immobilized enzyme used for the detection of phenol	368
Nitrate reductase	1.7.99.4	Entrapment in polyacrylamide gels cast around platinum gauze	Active immobilized enzyme used for the detection of nitrate ions	369
Pectinesterase	3.1.1.11	Reaction with derivatized porous glass	Active immobilized enzyme; investigation of the properties of the immobilized enzyme	370

[a] Covalent coupling may be assumed, unless otherwise indicated; [b] immobilized together with D-glucose oxidase; [c] immobilized together with β-D-galactosidase; [d] immobilized together with catalase.

Table 7 *(cont.)*

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*[a]	*Use of product*	*Ref.*
Pepsin A	3.4.23.1	Glutaraldehyde-coupled to a protein-coated, activated alkylamine derivative of porous glass	Active immobilized enzyme; investigations of the stability of immobilized pepsin in the continuous coagulation of milk	371
Peptidase (from human intestinal mucosa)	—	Reaction with agarose cyclic imidocarbonate	Evaluation of the digestibility of dipeptides	39
Peroxidase	1.11.1.7	Copolymerization of periodate-oxidized cellulose with glycidyl methacrylate in the presence of the enzyme	Active immobilized enzyme	214
		Embedding in a polyacrylamide gel by way of photo-induced polymerization of acrylamide	Active immobilized enzyme; studies of the catalytic properties of immobilized peroxidase	372
		Reaction with agarose cyclic imidocarbonate	Continuous monitoring of the amount of enzyme immobilized	40
		Reaction with various forms of diazotized 1,4-diaminobenzene		330
Phosphatase	3.1.3.1/2	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme	39
Phosphatase (acid)	3.1.3.2	Non-covalent interaction with chitosan		224
Phosphatase (alkaline)	3.1.3.1	Covalent reaction with activated forms of silk		327
Ribonuclease	3.1.4.22/23	Reaction with agarose cyclic imidocarbonate	Separation of pepsin from chymosin	44
Ribonuclease I	3.1.4.22		Demonstration (by immuno-adsorption) of the cell-free synthesis of ribonuclease I-specific immunoglobulin	45
		Reaction with cross-linked agarose cyclic imidocarbonate	Studies of the conformations of soluble and immobilized enzymes by differential scanning calorimetry and of the thermal stability and specific activity of the immobilized enzyme	38

L-Sorbose dehydrogenase (as *Gluconobacter melanogenus* cells)	1.1.99.12	Covalent reaction with activated forms of silk Entrapment of *Gluconobacter melanogenus* cells in a polyacrylamide gel	Active immobilized enzyme	327 279
Subtilisin BNP′	(3.4.21.14)	Coupling to an isothiocyanate derivative of glass beads	Active immobilized enzyme; investigation of the change in pH optimum on immobilization	373
Thrombin	3.4.21.5	Reaction with a glass derivative carrying an active ester of *N*-hydroxysuccinimide	Active immobilized enzyme; studies of the interactions of immobilized thrombin	374
αα-Trehalase	3.2.21.8	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; hydrolysis of synthetic disaccharides	39
Trypsin	3.4.21.4	Adsorption onto magnetite	Active immobilized enzyme	249
		Carbodi-imide-mediated coupling to methacrylate rubber	Active immobilized enzyme; investigations of the change in enzymic activity on reversible deformation of the fibres	209
		Covalent attachment to modified nylon fibres with glutaraldehyde		
		Covalent attachment to viscose fibres containing aldehydo-groups		
		Entrapment in partially hydrolysed nylon fibres	Active immobilized enzyme; demonstration of a new mechanochemical method for immobilization	334
		Glutaraldehyde-coupled to human hair	Active immobilized enzyme; investigations of the change in enzymic activity on deformation of the fibres	209

[a] Covalent coupling may be assumed, unless otherwise indicated; [b] immobilized together with D-glucose oxidase; [c] immobilized together with β-D-galactosidase; [d] immobilized together with catalase.

Table 7 *(cont.)*

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled and mode of coupling*[a]	*Use of product*	*Ref.*
Trypsin (*continued*)		Glutaraldehyde-linked to a fibrin film	Active immobilized enzyme; kinetic studies of immobilized trypsin	375
		Mechanochemically attached to partially hydrolysed nylon fibres	Active immobilized enzyme	336
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; histological dissociation of neuronal cell-bodies from rat brain	47
			Comparative study of the immobilization of trypsin on diverse macromolecular supports	46
		Reaction with copolymers of acrolein and acrylic acid	Formation of a soluble ⇋ insoluble, active immobilized enzyme	376
		Reaction with silanized porous glass	Active immobilized enzyme; studies on the activity of immobilized trypsin at high concentrations of organic solvents	377
		Reaction with a macromolecular inhibitor of trypsin	Active immobilized enzyme	378
		Reaction with a cyclic imidocarbonate derivative of Sephadex Reaction with a trichloro-*s*-triazinyl derivative of activated Sephadex Reaction with synthetic-polymer supports	Comparative study of the immobilization of trypsin on diverse macromolecular supports	46
Urate oxidase	1.7.3.3	Entrapment in a cross-linked gel of poly(2-hydroxyethyl methacrylate)	Active immobilized enzyme; kinetic studies of the trapped enzyme	379

		Reaction with various forms of diazotized 1,4-diaminobenzene	Active immobilized enzyme	330
		Reaction with glutaraldehyde-activated, papain-treated elastin	Active immobilized enzyme	380
Urease	3.5.1.5	Coupling to a dimethylsilicone membrane with a polyester-fibre backing on the solution side	Active immobilized enzyme in biochemical assays using mass spectrometry	321
		Covalent reaction with activated derivatives of non-porous glass beads	Active immobilized enzyme; studies of the effects of immobilization on the properties of the enzyme	381
		Enzgel urease coated onto Teflon-coated bar magnets	Active immobilized enzyme used in a specific assay for urea	382
		Glutaraldehyde-cross-linked with a protein on an electrode	Construction of electrodes incorporating cross-linked enzymes	323
		Reaction with a derivative of glass coated onto Teflon-coated bar magnets	Active immobilized enzyme used in a specific assay for urea	382
		Reaction with glutaraldehyde-activated, immobilized β-D-fructofuranosidase (*see* p. 510)	Soluble immobilized enzyme	90

[a] Covalent coupling may be assumed, unless otherwise indicated; [b] immobilized together with D-glucose oxidase; [c] immobilized together with β-D-galactosidase; [d] immobilized together with catalase.

Some aspects of immobilized glycoside and polysaccharide hydrolases, carbohydrate isomerases, and carbohydrate oxidases, *etc.*, are worthy of comment. β-D-Fructofuranosidase adsorbed onto an ion-exchange resin was not desorbed on washing the resin with 0.01 M-sodium acetate solution at 20°C, although desorption occurred at higher temperatures and higher molarities.[341] The pH optimum of the immobilized enzyme depended on the resin used for immobilization. Continuous hydrolysis of sucrose was possible when the resin-bound enzyme was incorporated into a tubular reactor. A comparison of the suitability of mineral and mineralized supports for the immobilization of β-D-fructosidase showed that hornblende gave a derivative possessing good physical, thermal, and operational stabilities.[342]

β-D-Fructofuranosidase attached to a cellulose derivative by ionic interactions was more stable than the free enzyme, and its activation energy was less than that of the free enzyme.[198] The use of controlled diffusion to reduce the concentration of substrate in the vicinity of the enzyme was studied. β-D-Fructofuranosidase adsorbed onto immobilized, succinylated concanavalin A has been activated (with glutaraldehyde) and coupled to urease.[90] An active, immobilized, water-soluble conjugate containing both enzymes was obtained following desorption from the matrix.

The leakage of immobilized β-D-fructofuranosidase, β-D-galactosidase, and glucoamylase from gels of poly(2-hydroxyethyl acrylate) or polydimethylacrylamide has been studied.[344] β-D-Fructofuranosidase and α-D-glucosidase immobilized within hollow fibres showed no detectable leakage and, when the fibres were pretreated with albumin, the stabilities of the immobilized enzymes approached those of the free enzymes.[343]

α-D-Galactosidase can be immobilized within the pellets formed in certain cultures.[347] The immobilized enzyme was inhibited less by the products of its action than was the free enzyme.

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β-D-Galactosidase of *Escherichia coli* coupled (by way of *NN'-o*-phenylenedimaleimide) to mercaptosuccinylated insulin exhibited competitive binding in the reaction between insulin and anti-insulin antibodies, and could be used in an enzyme-linked immunoassay of insulin.[383]

The coupled reaction of β-D-galactosidase (lactase) and D-glucose oxidase immobilized on glass beads is highly efficient at the neutral pH of milk, although the individual enzymes exhibit optimal activity at much lower pH values.[351] The coupled reaction can be used to inactivate bacteria present in milk. The catalytic properties of *Aspergillus niger* β-D-galactosidase attached to a copolymer of phenylenediamine and glutaraldehyde have been compared with those of the free enzyme.[353] The immobilized enzyme is stable at 50°C.

The kinetics of hydrolyses catalysed by *Escherichia coli* β-D-galactosidase immobilized within microcapsules of cellulose nitrate have been examined in continuously stirred tank and packed-bed reactors.[202] By using 2-nitrophenyl β-D-galactopyranoside and lactose in aqueous solution and lactose in whole milk, it was possible to determine the kinetics of the microencapsulated enzyme under a wide range of values of kinetic rate constants and substrate concentrations. From experimental measurements of the effectiveness factors, it was possible to estimate the membrane permeabilities of the substrates.

Evidence has been obtained in support of an earlier suggestion that the attachment of β-D-galactosidase and lysozyme to collagen membranes by an impregnation process involves multiple ionic and van der Waals' interactions and hydrogen bonds.[281]

The continuous hydrolysis of lactose in acid whey has been carried out at 50°C, using a column packed with a mixture of sawdust (to prevent clogging) and β-D-galactosidase trapped in a polyvinylpyrrolidone gel.[350] The use of a polyvinylpyrrolidone gel for immobilizing the enzyme has several advantages, including simplicity of the procedure, stability, and the fact that it is not toxic.

Adsorption onto carbon and cross-linking with glutaraldehyde is unsuitable for the immobilization of β-D-galactosidase, since it results in loss of enzymic activity.[348] β-D-Galactosidase immobilized on nylon tubing can be used for the continuous hydrolysis of lactose.[349] An activity half-life of 100 days has been reported for β-D-galactosidase immobilized on feather-meal protein.[352]

Bacillus amyloliquefaciens α-amylase can be attached to dextran after activation of the polysaccharide by using a modification of the cyanogen bromide method.[235] The soluble dextran–amylase conjugate has greater stability than the unmodified enzyme at low pH values, during heat treatment, and after removal of calcium ions with a chelating agent. The conjugate probably consists of a cross-linked aggregate of many dextran and many enzyme molecules, some of which are unable to express their activity until the polysaccharide is partly hydrolysed with dextranase.

Reversible immobilization of β-amylase[164, 165, 324] and glucoamylase[165, 358] by hydrophobic interactions appears to be a viable alternative to conventional methods. Hydrophobic immobilization of β-amylase, for example, on hexyl-substituted agarose gels was optimal with a hexyl:D-galactose ratio of around 0.5 in the carrier gel.[324]

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Immobilization of *Aspergillus niger* cellulase on a polyacrylonitrile derivative raised the pH optimum of the enzyme by 0.4 units and lowered the temperature optimum by 2 °C.[332] The pH–activity profile of the enzyme also narrowed on immobilization.

The amount of dextranase attached to porous titanium(IV) oxide by chelation increased in the presence of ammonia, although more enzyme specific activity was retained in the absence of ammonia.[340] Properties of the immobilized enzyme include a pH-dependence and reversibility of the coupling between the enzyme and the matrix.

A novel enzyme reactor uses glucoamylase attached to sheets of a derivatized collagen, which can be stacked to give a large surface area in a comparatively small volume.[269] The pH and temperature optima, activation energy, and K_m for the hydrolysis of maltose by glucoamylase were not affected by coupling, which increased the thermal stability of the enzyme.[359] The membranes were also incorporated into a helicoidal reactor used for converting starch into D-glucose. Since porous-glass particles are poor thermal conductors, the temperature distribution in a packed-bed reactor of glucoamylase immobilized on porous-glass beads may not be uniform.[355] Procedures for examining the possible effects of heat on the performance of packed-bed reactors have been described.

The pH optimum (*ca.* 4.5) of glucoamylase increased by 0.7 units and the temperature optimum dropped by 5 °C on immobilization of the enzyme on a polyacrylonitrile carrier, and a slight shift in the pH optimum occurred on storing the immobilized enzyme in distilled water at 4 °C.[360] Glucoamylase immobilized on derivatized porous silica proved to be extremely stable in laboratory and pilot-plant operations.[357] When the dextrin feed had previously been only lightly hydrolysed, pore diffusion limitation caused an appreciable decrease in the rate of production of D-glucose, since long oligosaccharide chains diffused much more slowly into the pores of the carrier than did shorter chains.

Glucoamylase adsorbed onto acid-activated molecular sieves and onto alumina exhibited 50—100% of the original enzymic activity and good thermal stability.[354] Stable immobilized forms of glucoamylase have been obtained by attaching the enzyme to hornblende supports.[356]

The use of immobilized D-glucose isomerase in a multi-bed reactor is economic for producing D-fructose in a continuous process.[361] Immobilized D-glucose isomerase has been used in the United States to produce more than 500000 metric tons of corn syrups containing a high proportion of D-fructose, which are widely used as high-quality sweeteners. D-Glucose isomerase immobilized on polyacrylonitrile retained its pH–activity characteristics and 44% of its enzyme specific activity, although progressive loss of activity occurred owing to denaturation and release of the enzyme.[364] D-Glucose isomerase has been immobilized on porous glass (half-life 200 days),[363] on chitin,[223] and in the form of heat-treated whole cells.[280]

A method for the analysis of a two-substrate enzymic reaction taking place inside a porous support has been described.[346] The resulting equations were applied to D-galactose-oxidase-catalysed oxidation of D-galactose when the enzyme is immobilized on porous-glass particles. The physical constants of the system were obtained from theoretical predictions, while the concentration of the

enzyme in the porous medium was derived from the experimental results. The theoretically calculated and experimental effectiveness factors were found to be in agreement. The model was extended in order to study the effects of external mass-transfer coefficients and the concentration of the enzyme in the porous medium on the effectiveness factor.

D-Glucose oxidase has been immobilized on manganese(III) oxide, which decomposed hydrogen peroxide formed in the enzymic reaction, so preventing inhibition of the enzyme.[366] By using glutaraldehyde as the cross-linking agent, soluble conjugates of D-glucose oxidase and catalase have been prepared and immobilized on inorganic supports.[331] The use of enzyme conjugates, rather than non-conjugated enzymes, increased the specific activity of the immobilized catalyst, especially where the number of binding sites available on the support is limited. Dual catalysts of D-glucose oxidase and catalase have been prepared by immobilization of the enzymes on an inorganic support.[328] In general, the dual catalysts were superior to mixed catalysts (each enzyme immobilized on separate particles) and soluble, homogeneous systems at equal total activities.

Pectinesterase has been attached covalently to porous-glass particles by reaction of the native protein with pendant benzoyl acide groups on the carrier.[370] The immobilized enzyme, unlike the native enzyme, displayed a five-fold increase in the apparent K_m for a high-molecular-weight fraction of citrus pectin relative to that for a low-molecular-weight fraction. A striking difference exists in the low-pH profile of immobilized pectinesterase relative to that of the native enzyme; interactions between the carrier matrix and the polyelectrolyte substrate were invoked to explain this difference.

An immobilized form of cyclodextrin D-glucanotransferase catalysed the conversion of starch into cyclohepta-amylose in a yield of 46%, without significant loss of enzymic activity; a yield of 52% was achieved when pullulanase was also added.[225]

Modification of Gangliosides and Glycolipids, and Uses of Modified Gangliosides and Glycolipids

Ganglioside $G_{D_{1a}}$ labelled with tritium in the sphingosine moiety has been used as the substrate in a new microscale assay for neuraminidase.[384] ^{14}C-Labelled gangliosides G_{M_3} and $G_{D_{1a}}$ have been synthesized by enzyme-catalysed transfer of the sugar residue from CMP-*N*-[^{14}C]acetylneuraminic acid to the appropriate glycolipid acceptor.[385] The labelled gangliosides were used as substrates in measurements of the sialidase activities of HeLa cells. A D-[^{14}C]glucosylceramide has been used to examine the hydrolytic and transferase activities of β-D-glucosidases,[386] while D-galactosyl- and D-glucosyl-[^{3}H]cerebrosides have been used as substrates in assays for D-galacto- and D-gluco-cerebrosidases.[387]

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