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Carbohydrate Chemistry

VOLUME 10

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A Specialist Periodical Report

Carbohydrate Chemistry

Volume 10

A Review of the Literature Published during 1976

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Organic formulae composed by Wright's Symbolset method

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Preface

This Report, the tenth in the series, covers the literature available to us between mid-January 1976 and mid-January 1977. With the publication of this volume, the number of Reports in the series has reached double figures: we hope that this Report maintains the standards set by its predecessors in summarizing the recent literature in carbohydrate chemistry in a concise, lively, and palatable form. Once again the literature coverage is comprehensive, rather than selective, although inevitably a few papers are missed each year – we are grateful when these are brought to our attention for inclusion in a subsequent Report.

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

Dr. J. M. Williams has joined our team of Reporters for Part I.

We thank Dr. L. C. N. Tucker for reading and commenting on the whole of Part I, and Mrs. Alice Duncan, Miss Moira Endersby, and Miss Lynda Esplin for typing almost all of this Report.

Finally, it is a pleasure to acknowledge the invaluable assistance provided by Philip Gardam and his staff at the Chemical Society in the production of this Report.

August 1977 J. S. B.

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Abbreviations

The following abbreviations have been used:

ADP	adenosine diphosphate
ATP	adenosine triphosphate
c.d.	circular dichroism
CDP	cytidine diphosphate
CMP	cytidine monophosphate
DBU	1,5-diazobicyclo[5,4,0]undec-5-ene
DCC	dicyclohexylcarbodi-imide
DEAE	diethylaminoethyl
DMF	*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	hexamethylphosphortriamide
i.r.	infrared
NBS	*N*-bromosuccinimide
n.m.r.	nuclear magnetic resonance
o.r.d.	optical rotatory dispersion
py	pyridine
RNA	ribonucleic acid
THF	tetrahydrofuran
Thp	tetrahydropyranyl
TMS	trimethylsilyl
UDP	uridine 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) and the arrangement of subject matter follows that of previous Reports in this Series.

New methods, including the use of trifluoromethanesulphonic anhydride, have been reported for activating the anomeric centre of sugars for the synthesis of glycosides and higher saccharides, and trialkylstannylation has been found to enhance the nucleophilicity of the hydroxy-groups of simple and complex aglycones in the formation of glycosides from acetylated glycosyl halides (Chapter 3). A new method for the synthesis of 1,2-*trans* alkyl glycosides, which circumvents the use of glycosyl halides, has involved the reaction of peracylated sugars with a DMF dialkyl acetal in the presence of a Lewis acid catalyst (Chapter 3).

Among other interesting developments to be reported during the past year are the use of phase-transfer catalysts in the preparation of fully or partially alkylated sugar derivatives (Chapter 4) and of 2,3-*O*-dibutylstannylene derivatives in the selective esterification of HO-2 of certain methyl α-D-hexopyranosides, without the need to protect the primary hydroxy-group (Chapter 6). Moreover, alkylation of *cis*-2,3- or -3,4-*O*-dibutylstannylenepyranosides has been shown to occur regioselectively at the equatorial oxygen atom (Chapter 18). The knowledge that hydrogenolytic cleavage of the dioxolan ring of alkyl 2,3- and 3,4-*O*-benzylidenepyranosides depends on the configuration of the acetal carbon atom has also opened the way to a number of synthetically useful benzyl ether derivatives (Chapter 5). A new procedure for the synthesis of deoxy-sugars has involved the opening of diol thiocarbonates in a radical fashion with tributyltin hydride (Chapter 13).

Many and varied syntheses of aminoglycoside and nucleoside antibiotics and their analogues are reported in Chapters 20 and 21. Careful labelling studies over the past year or so, particularly by Rinehart's group, have provided information on the biosynthetic pathways that convert D-glucose into the aminocyclitol moieties of streptomycin and spectinomycin (Chapter 20).

Another report has shown the need to use ^{13}C-labels in making unambiguous assignments to resonances in the ^{13}C n.m.r. spectra of carbohydrates (Chapter 20). Earlier assignments to some of the resonances in the natural abundance ^{13}C n.m.r. spectra of several common monosaccharides have had to be revised in the light of coupling data obtained from the spectra of [1-^{13}C]monosaccharides, and it is clear that empirical chemical-shift rules for the effects of derivatization must be carefully reassessed. Differences observed between the proton

spin–lattice relaxation times of the anomeric protons of the reducing and non-reducing residues of disaccharides suggest that the anomeric proton of the non-reducing residue receives relaxation contributions from the protons on both sugar rings — if this is so, a new and powerful method for studying the conformations of small oligosaccharides in solution is to hand.

Sugar derivatives have been used for the total synthesis of the non-carbohydrate pheromones of several species of beetles (Chapter 28).

Some aspects of sucrose chemistry have been reviewed,[1] and several books of general interest have appeared during the past year.[2–4]

The July issue [5] of *Carbohydrate Research* was dedicated to the memory of Professor E. J. Bourne (1922—1974).

[1] A. J. Vlitos, *Chem. and Ind.*, 1976, 255.
[2] *Adv. Carbohydrate Chem. Biochem.*, 1976, vols. 32 and 33.
[3] Methods in Enzymology, vol. XLI, 'Carbohydrate Metabolism', Part B, and vol. XLII, 'Carbohydrate Metabolism', Part C, ed. W. A. Wood, Academic Press, New York and London, 1975; 'Natural Products Chemistry', ed. K. Nakanishi, T. Goto, S. Ito, S. Natori, and S. Nazoe, Kodansha, Tokyo, and Academic Press, New York and London, 1975.
[4] 'Liquid Column Chromatography. A Survey of Modern Techniques and Applications', ed. Z. Deyl, K. Macek, and J. Janák, Elsevier Scientific Publishing Co., Amsterdam, 1975.
[5] *Carbohydrate Res.*, 1976, vol. 49.

2
Free Sugars

A review of the relative reactivities of the hydroxy-groups of carbohydrates has dealt with esterification, etherification, acetalation, halogenation, and oxidation, and with the migration of substituents.[6] Shallenberger's rationale is considered to explain satisfactorily the relative sweetness of sucrose, xylitol, arabinitol, ribitol, D-*galacto*-sucrose, and methylated derivatives of sucrose.[7]

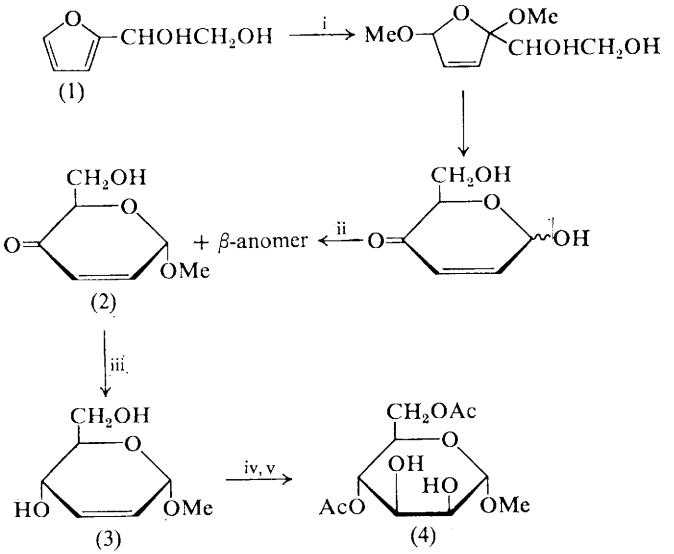
Isolation and Synthesis

6-Deoxy-D-*altro*-heptose is a component of the polysaccharide antigen produced by *Eubacterium saburreum*[8] and D-*glycero*-D-*manno*-octulose has been identified as a minor product (~10%) of the aldolase-catalysed reaction of D-ribose and D-fructose 1,6-diphosphate;[9] the major product of this reaction was previously reported to be D-*glycero*-D-*altro*-octulose. αα-Trehalose has been converted into its D-*allo*- and mono-D-*galacto*-analogues,[10, 11] and lactose has been transformed into its 3-epimer, namely 4-*O*-β-D-galactopyranosyl-D-allopyranose.[12] Isosucrose, which was shown to be β-D-glucopyranosyl α-D-fructofuranoside by ^{1}H n.m.r. measurements at 300 MHz on the octa-acetate, was not hydrolysed by β-D-fructofuranosidase (E.C. 3.2.1.26), β-D-glucosidase (E.C. 3.2.1.21), or α-D-glucosidase (E.C. 3.2.1.20).[13] 1-Deoxy-D-*threo*-pentulose, the first 1-deoxypentulose to be isolated from Nature, has been found as a metabolite of *Streptomyces hygroscopicus*; it exists mainly as the acyclic form in solution.[14]

Derivatives of racemic α-manno-, α-altro-, and β-allo-pyranoses have been synthesized from 1-(2-furyl)-1,2-dihydroxyethane (1).[15] The route used to obtain the α-mannopyranoside derivative (4) is shown in Scheme 1; the isomeric β-allopyranoside was prepared similarly from the β-anomer of (2), and the α-altropyranoside was obtained by ring-opening of the 2,3-anhydromannopyranoside derived from (3). *trans*-5,6-Dihydro-6-hydroxymethyl-2-methoxy-2*H*-pyran (5), an intermediate used in total syntheses of hexoses, has been

[6] A. H. Haines, *Adv. Carbohydrate Chem. Biochem.*, 1976, **33**, 11.
[7] M. G. Lindley, G. G. Birch, and R. Khan, *J. Sci. Food Agric.*, 1976, **27**, 140.
[8] J. Hoffman, B. Lindberg, J. Lönngren, and T. Hofstad, *Carbohydrate Res.*, 1976, **47**, 261.
[9] G. Haustveit, *Carbohydrate Res.*, 1976, **47**, 164.
[10] G. G. Birch, C. K. Lee, A. C. Richardson, and Y. Ali, *Carbohydrate Res.*, 1976, **49**, 153.
[11] C. K. Lee, *Carbohydrate Res.*, 1976, **50**, 152.
[12] R. S. Bhatt, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1976, **51**, 272.
[13] G. R. Newkome, J. D. Sauer, V. K. Majestic, N. S. Bhacca, H. D. Braymer, and J. D. Wander, *Carbohydrate Res.*, 1976, **48**, 1.
[14] H. Hoeksema and L. Baczynskyj, *J. Antibiotics*, 1976, **29**, 688.
[15] O. Achmatowicz, jun., R. Bielski, and P. Bukowski, *Roczniki Chem.*, 1976, **50**, 1535.

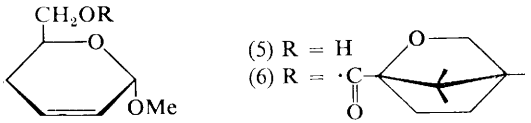
resolved by fractional crystallization of the diastereoisomeric esters (6).[16] The (−)-enantiomer of (5) was converted into methyl 4-deoxy-α-D-*xylo*-hexopyranoside, thus enabling it to be assigned the 2*S*,6*S* configuration. The



Reagents: i, MeOH–Br_2; ii, $HC(OMe)_3$–BF_3; iii, $NaBH_4$; iv, Ac_2O–py; v, OsO_4–H_2O_2

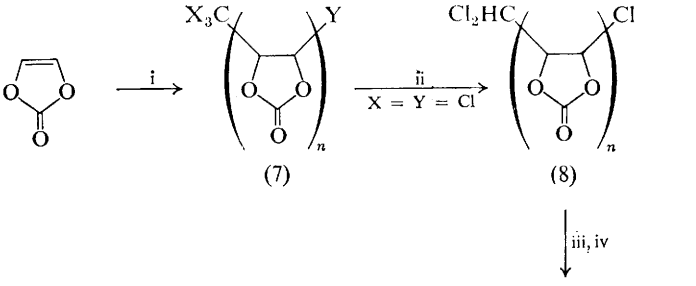
Scheme 1

synthesis of aldoses by free-radical telomerization of vinylene carbonate with polyhalogenomethanes (Scheme 2) has been modified and extended (see Vol. 8, p. 59).[17, 17a] The four crystalline telomers (7; $n = 3$), obtained in 6% yield, were converted into heptoses. Identification of the derived heptitols and of the pentoses produced showed that *trans* addition occurs in the telomerization. Hexoses were also synthesized from (8; $n = 2$) by extension of the carbon chain using sodium cyanide *etc.*



Tritium-labelled sugars possessing high specific activity have been synthesized by using tritium gas in solution in the presence of a metal hydrogen-transfer catalyst; reducing sugars incorporated tritium at C-1, whereas glycosides and D-glucose 1-phosphate were unaffected.[18]

[16] A. Konowak, J. Jurczak, and A. Zamojski, *Tetrahedron*, 1976, **32**, 2957.
[17] H. Takahata, T. Kunieda, and T. Takizawa, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 3017.
[17a] Y. Nii, T. Kunieda, and T. Takizawa, *Tetrahedron Letters*, 1976, 2323.
[18] E. A. Evans, H. C. Sheppard, J. C. Turner, and D. C. Warrell, *J. Labelled Compounds*, 1974, **10**, 569 (*Chem. Abs.*, 1976, **84**, 3862c).



Reagents: i, X_3CY (X = Cl or Br; Y = H, Cl, or Br); ii, *hν* or $Ni(CO)_4$; iii, $NaBH_4$; iv, $AgNO_3$–H_2O

Scheme 2

Physical Measurements

A new method for qualitative and quantitative analyses of mixtures of monosaccharides has used the unique proton-decoupled ^{13}C n.m.r. spectra of the individual sugars in conjunction with computer-storage of the data.[19] The procedure is rapid and does not require derivatization of the sugars, although relatively large samples are required. The formation of a number of complexes between 2,3,4,6-tetra-*O*-methyl-D-glucopyranose and the acid (or acid dimer) has been proposed to account for the kinetics of oxyacid-catalysed mutarotation of this sugar.[20] The polarographic behaviour of D-glucose and maltose in phosphate buffer at pH 8 has been examined,[21] and the kinetics of mutarotation of β-maltose have been investigated polarographically in phosphate buffer at pH 6.8; values for the four rate constants were calculated for the equilibria β-maltose ⇄ *aldehydo*-form ⇄ α-maltose.[22]

The specific volumes and coefficients of refraction have been calculated for aqueous solutions of sucrose at several temperatures.[23] Self-diffusion coefficients have been reported for sucrose,[24] sodium D-glucuronate, and 2-amino-2-deoxy-D-glucose hydrochloride[25] in aqueous solution. The triboluminescence (*i.e.* luminescence caused by mechanical stress) of crystalline mono- and di-saccharides has been studied; this phenomenon was shown by some sugars (*e.g.* sucrose and D-glucose) but not by others (*e.g.* cellobiose and D-mannose).[26] An e.s.r. spectroscopic study of D-glucose, D-mannose, D-galactose, D-fructose, and methyl

[19] J. W. Blunt and M. H. G. Munro, *Austral. J. Chem.*, 1976, **29**, 975.
[20] A. Kergomard, Lê Quang Yáng, and M. F. Renard, *Tetrahedron*, 1976, **32**, 1983.
[21] F. H. Chowdhury, N. B. Fouzder, and S. A. Tarafdar, *J. Indian Chem. Soc.*, 1975, **52**, 1139 (*Chem. Abs.*, 1976, **84**, 165 138y).
[22] M. Bhaduri, F. H. Chowdhury, and N. B. Fouzder, *J. Indian Chem. Soc.*, 1975, **52**, 1141 (*Chem. Abs.*, 1976, **84**, 165 139z).
[23] M. I. Daishev and N. V. Orlova, *Izvest. Vyssh. Uchebn. Zaved., Pishch. Tekhnol.*, 1975, 98 (*Chem. Abs.*, 1976, **84**, 5270g).
[24] F. Schneider, A. Emmerrich, D. Finke, and N. Panitz, *Zucker*, 1976, **29**, 222 (*Chem. Abs.*, 1976, **85**, 177 820y).
[25] H. Uedaira and H. Uedaira, *Zhur. fiz. Khim.*, 1975, **49**, 2306 (*Chem. Abs.*, 1976, **84**, 59 892e).
[26] J. I. Zink, G. E. Hardy, and J. E. Sutton, *J. Phys. Chem.*, 1976, **80**, 248.

α-D-glucopyranoside and their deuteriated analogues showed that radicals are formed by cleavage of carbon–hydrogen bonds.[27] Two reports have discussed dielectric relaxation (frequency range 200 KHz—35 GHz) [28] and n.m.r. relaxation studies [29] on aqueous solutions of sugars, while another report has described how dielectric and n.m.r. relaxation methods can be used to elucidate the molecular dynamics and the extent of hydration of small hydrophilic molecules in aqueous solution.[30]

Dipole moments have been determined for several mono- and di-saccharides, including amino-sugars.[31] The hydrolysis of sucrose in aqueous 1,4-dioxan in the presence of a reversed micelle formed by dodecylbenzenesulphonic acid has been studied; the pseudo first-order rate constant was found to increase with decreasing sucrose concentration and with increasing 1,4-dioxan concentration, with a maximum rate enhancement of 21-fold.[32] The first-order rate constant for the alkaline hydrolysis of 2,4-dinitrochlorobenzene catalysed by micellar cetyltrimethylammonium bromide was increased by the addition of D-glucose, D-arabinose, or D-fructose.[33] A predominance of α-lactose monohydrate was found in samples of commercial lactoses, the α-anomer being converted into the β-anomer at *ca.* 94 °C.[34] Isothermal crystallization of D-glucose and cello-biose, -triose, and -tetraose from the glassy state has been studied by differential scanning calorimetry.[35] Amorphous cello-biose and -tetraose crystallized far more rapidly than amorphous D-glucose and cellotriose, and there appears to be an odd–even effect associated with the crystallization of these sugars. The i.r. spectra of D-glucose and cello-oligosaccharides (up to cellopentaose) have been compared with those of cellulose at various temperatures between 250 °C and that of liquid nitrogen.[36]

A study of the tautomerism of hexuloses in solution by ^{13}C n.m.r. spectroscopy is mentioned in Chapter 23.

Reactions

The chemistry of sucrose has been reviewed.[37] The Maillard reaction of reducing sugars with twenty amino-acids was faster for pentoses than for either hexoses or disaccharides.[38] The reaction was catalysed by organic acids at pH 5—6. Sodium poly(styrene sulphonate) at concentrations of 10^{-2}—10^{-3} mol l^{-1} inhibited

[27] G. V. Abagyan and A. S. Apresyan, *Studia Biophys.*, 1976, **53**, 95 (*Chem. Abs.*, 1976, **84**, 180 483a).
[28] A. Suggett and A. H. Clark, *J. Solution Chem.*, 1976, **5**, 1 (*Chem. Abs.*, 1976, **84**, 165 125s).
[29] A. Suggett, S. Ablett, and P. J. Lillford, *J. Solution Chem.*, 1976, **5**, 17 (*Chem. Abs.*, 1976, **84**, 165 126t).
[30] A. Suggett, *J. Solution Chem.*, 1976, **5**, 33 (*Chem. Abs.*, 1976, **84**, 165 127u).
[31] E. Lertes, A. Wiese, and E. Brauer, *Z. phys. Chem. (Frankfurt)*, 1976, **100**, 37 (*Chem. Abs.*, 1976, **85**, 124 246j).
[32] K. Arai, Y. Ogiwara, and K. Ebe, *Bull. Chem. Soc. Japan*, 1976, **49**, 1059.
[33] M. J. Blandamer, G. H. Beatham, C. H. Branch, and D. J. Reid, *J.C.S. Faraday I*, 1976, **72**, 2139.
[34] R. Huettenrauch and I. Keiner, *Pharmazie*, 1975, **30**, 804 (*Chem. Abs.*, 1976, **84**, 135 958y).
[35] H. Hatakeyama, H. Yoshida, and J. Nakano, *Carbohydrate Res.*, 1976, **47**, 203.
[36] H. Hatakeyama, C. Nagasaki, and T. Yurugi, *Carbohydrate Res.*, 1976, **48**, 149.
[37] R. Khan, *Adv. Carbohydrate Chem. Biochem.*, 1976, **33**, 236.
[38] W. Ciusa, P. Mazzaracchio, and G. Barbiroli, *Rass. Chim.*, 1975, **27**, 143 (*Chem. Abs.*, 1976, **84**, 136 024c).

the Maillard reaction and the decomposition of D-glucose to 5-hydroxymethylfurfural.[39] The mechanism of the reactions of hexuloses and partially methylated hexuloses with amino-acids has been studied.[40] For reactions in buffered methanol, the rate is greatest at pH 8.2 and there is a linear correlation between the proportion of the keto-form of the hexulose present at equilibrium and the reactivity. The products obtained when solutions of D-fructose and D-glucose at pH 4.5 are heated to high temperatures include four furans, seven substituted benzenes, an isobenzofuranone, a benzopyranone, and a benzofuran.[41]

Cellobiose was degraded in solutions of sodium hydroxide to give principally saccharinic and related acids, whereas D-glucose, D-mannose, D-fructose, and 3-deoxy-D-*glycero*-pentulose were the main products obtained when sodium hydrogen carbonate solution was used.[41a] In addition to the usual aliphatic acids, eleven phenols and two cyclopentenones were identified among the products obtained when D-xylose and D-glucose were heated with 0.63-M sodium hydroxide at 96 °C.[42] The degradation of D-gluco-oligosaccharides containing (1 → 3)- and (1 → 4)-linkages in saturated calcium hydroxide solution has been studied; the type of linkage present in the oligosaccharides is reflected in the rate of alkaline degradation.[43] However, the use of this technique in the sequence analysis of oligosaccharides of unknown structure requires relatively pure samples and a knowledge of whether or not the compound is branched. 3-Deoxyhexuloses were obtained in moderately good yield when isomeric 3-deoxyaldoses were treated with saturated solutions of calcium hydroxide.[44] Several reducing sugars have been treated with lead and tin hydroxides under the conditions used in the Danilov or Venus–Danilov reaction; 3-deoxy-D-*glycero*-tetronic acid was obtained from D-erythrose, whereas 3,5,6-tri-*O*-methyl(or acetyl)-D-glucofuranose and 3,4,6-tri-*O*-acetyl-D-glucopyranose afforded the corresponding 2-deoxy-D-*arabino*-hexonic acid derivatives.[45] The autocatalytic nature of the base-catalysed condensation of formose sugars can be eliminated by using an aldose or a ketose with an α-hydrogen atom as a co-catalyst.[46] An investigation of the condensation of formaldehyde to give sugars in the presence of lead catalysts has shown that the complexes formed between D-glucose and basic lead oxide have catalytic activity.[47]

The reversion products obtained when a solution of D-glucose was exposed to hydrogen chloride at 25 °C for several days included disaccharides of the trehalose type (6%), trisaccharides (4%), and oligo- and poly-saccharides (73%); the oligosaccharides are branched.[48] Oligosaccharides and low-molecular-weight

[39] A. Rubio, *Acta Cient. Venez.*, 1974, **25**, 209 (*Chem. Abs.*, 1976, **84**, 44 554x).
[40] K. Heyns and J. Heukeshoven, *Annalen*, 1976, 585.
[41] T. Popoff and O. Theander, *Acta Chem. Scand.* (*B*), 1976, **30**, 397.
[41a] L. Löwendahl and O. Samuelson, *Acta Chem. Scand.* (*B*), 1976, **30**, 691.
[42] I. Forsskahl, T. Popoff, and O. Theander, *Carbohydrate Res.*, 1976, **48**, 13.
[43] W. W. Luchsinger and B. A. Stone, *Carbohydrate Res.*, 1976, **46**, 1.
[44] Š. Kučár, *Coll. Czech. Chem. Comm.*, 1976, **41**, 2592.
[45] R. A. Gakhokidze, *Zhur. obshchei Khim.*, 1976, **46**, 1620 (*Chem. Abs.*, 1976, **85**, 192 996d).
[46] A. H. Weiss, *N.A.S.A. Contract Report*, 1975, NASA–CR–146639, *Sci. Tech. Aerosp. Report*, 1976, **14**, Abs. No. N 76–16177 (*Chem. Abs.*, 1976, **85**, 33 281a).
[47] Ya. B. Gorokhovatskii and N. P. Evmenenko, *Doklady Akad. Nauk S.S.S.R.*, 1976, **227**, 133 (*Chem. Abs.*, 1976, **85**, 5962e).
[48] I. S. Artem'eva, V. P. Levanova, and V. I. Sharkov, *Khim. Drev.*, 1975, 11 (*Chem. Abs.*, 1976, **84**, 59 890c).

polysaccharides containing residues of D-glucose and 3,6-anhydro-D-glucose in the ratios of 10—5 : 1 were formed on treatment of D-glucose with anhydrous hydrogen fluoride at 20 °C.[49] Twenty-one products and their respective *G*-values were reported in a study of the γ-radiolysis of aqueous solutions of cellobiose in the presence of nitrous oxide.[50] D-Xylose isomerases have been used to determine the anomeric configuration of D-xylose released on enzymic hydrolysis of β-D-xylopyranosides.[51]

The complexation of free sugars with metal ions (*e.g.* Ca^{2+} and La^{3+} ions) in aqueous solution [52] is referred to in Chapter 18, and the mechanism of oxidation of reducing sugars by molecular oxygen [53] is discussed in Chapter 22.

[49] U. Kraska and F. Micheel, *Carbohydrate Res.*, 1976, **49**, 195.
[50] C. von Sonntag, M. Dizdaroglu, and D. Schulte-Frohlinde, *Z. Naturforsch.*, 1976, **31b**, 857.
[51] H. Kersters-Hilderson, M. Claeyssens, E. van Doorslaer, and C. K. de Bruyne, *Carbohydrate Res.*, 1976, **47**, 269.
[52] R. E. Lenkinski and J. Reuben, *J. Amer. Chem. Soc.*, 1976, **98**, 3089.
[53] H. S. Isbell, *Carbohydrate Res.*, 1976, **49**, C1.

3
Glycosides

O-Glycosides

Synthesis.—Straightforward preparations of methyl β-L-rhamnopyranoside (in low yield) from L-rhamnose[54] and of methyl β-D-glucopyranoside from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[55] have been reported. A novel synthesis of simple alkyl glycosides has used dialkyl sulphates in DMF; for example, D-glucose afforded ethyl α-D-glucopyranoside in 82% yield when treated briefly with diethyl sulphate in DMF at 110 °C.[56] Methyl α- and β-D-[5-^{18}O]xylopyranosides have been prepared by methanolysis of 1,2-*O*-isopropylidene-α-D-[5-^{18}O]xylofuranose, which was obtained by way of oxygen exchange between [^{18}O]water and bis-(1,2-*O*-isopropylidene-α-D-xylopentodialdofuranose) 3,5′:5,5′-cyclic acetal.[56a] The glycosides were separated by fractional crystallization of the 2,4-benzeneboronates.

Several new methods for activating the anomeric centre of sugars for the synthesis of glycosides have been reported, including the use of trifluoromethanesulphonic anhydride[57] and a mixture of dichlorodiphenylsilane and a silver sulphonate,[58] although both methods yielded mixtures of α- and β-glycosides. 2,3,4-Tri-*O*-benzyl-6-*O*-(*N*-phenylcarbamoyl)-D-glucopyranosyl toluene-*p*-sulphonate has been used in stereoselective syntheses of a hexasaccharide derivative, methyl octadeca-*O*-benzyl-α-isomaltohexaoside, and methyl α-isomaltopentaoside.[59]

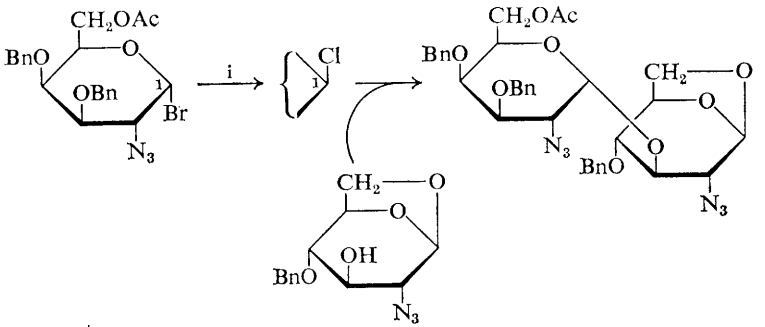
The nucleophilicity of an hydroxy-group to be linked to the anomeric centre of sugars can be enhanced by trialkylstannylation. Thus treatment of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with one equivalent each of tributylstannyl methoxide and stannic chloride in dichloromethane at 20 °C furnished an 86% yield of methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside.[60] Orthoesters were obtained in the reactions with tributylstannyl alkoxides when the glycosyl halide was epimerized (with tetraethylammonium bromide) *in situ* (see p. 40). t-Butyl ethers have also been used to enhance the nucleophilicity

[54] E. S. Evtushenko and Yu. S. Ovodov, *Khim. prirod. Soedinenii*, 1976, 87 (*Chem. Abs.*, 1976, **85**, 177 870q).
[55] H. Hönig and H. Weidmann, *Synthesis*, 1975, 804.
[56] A. Zamojski, H. Burzyńska, and M. Pietraszkiewicz, *Roczniki Chem.*, 1975, **49**, 2113.
[56a] W. D. Hitz, D. C. Wright, P. A. Seib, M. K. Hoffman, and R. M. Caprioli, *Carbohydrate Res.*, 1976, **46**, 195.
[57] J. Leroux and A. S. Perlin, *Carbohydrate Res.*, 1976, **47**, C8.
[58] S. Koto, N. Morishima, and S. Zen, *Chem. Letters*, 1976, 61.
[59] R. Eby and C. Schuerch, *Carbohydrate Res.*, 1976, **50**, 203.
[60] T. Ogawa and M. Matsui, *Carbohydrate Res.*, 1976, **51**, C13.

of hydroxy-groups in the synthesis of protected disaccharides (*e.g.* β-gentiobiose octa-acetate).[61]

The synthesis of 1,2-*cis*-glycosides from glycosyl halides continues to attract attention and the subject has been reviewed.[62] The effects of the substituent at O-2, the promoting salt, and the concentration of the alcohol on the stereoselectivity of the glycosidation of 2-*O*-acetyl(or methyl)-3,4,6-tri-*O*-methyl-α-D-glucopyranosyl bromide with cyclohexanol have been thoroughly examined.[63] Not unexpectedly, an acetoxy substituent at C-2 gave predominantly the β-glycoside, whereas the proportion (53—91%) of β-glycoside obtained with a methoxy substituent at C-2 depended both on the promoting salt and the concentration of alcohol used. The stereoselectivity of the reaction of 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranosyl bromide with cyclohexanol in the presence of mercuric cyanide increased as the concentration of the alcohol was increased, but decreased as the temperature was increased.[64] It was concluded that the stereochemical outcome depends on the relative rates of dissociation of the ion-pair (formed by heterolysis of the C—Br bond) and the reaction of the cation with the alcohol. Autocatalysis also plays a role in the reaction.

Glycosyl halides with a non-participating group at C-2 or halide-exchange processes have been used in the synthesis of 4-nitrophenyl 2-*O*-α-D-gluco-pyranosyl-β-D-galactopyranoside,[65] methyl and 4-isothiocyanatophenyl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-D-mannopyranoside,[65a] and α-(1 → 3)-linked disaccharides containing two hexosamine residues (see Scheme 3 and



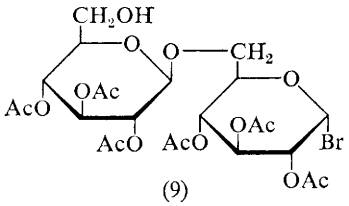
Reagent: i, $Et_4\overset{+}{N}Cl^-$–MeCN

Scheme 3

Chapter 8).[66] Other α-linked glycosides synthesized include (2-amino-2-deoxy- and 6-amino-6-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamine[67] and 4-nitrophenyl *N*-acetyl-α-neuraminide.[68]

[61] N. K. Kochetkov, E. M. Klimov, and V. I. Torgov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 2585.
[62] K. Eklind, *Chem. Comm. Univ. Stockholm*, 1975, 1 (*Chem. Abs.*, 1976, **84**, 122 158n).
[63] J. E. Wallace and L. R. Schroeder, *J.C.S. Perkin I*, 1976, 1938.
[64] J. E. Wallace and L. R. Schroeder, *J.C.S. Perkin II*, 1976, 1632.
[65] P. J. Garegg, I. J. Goldstein, and T. Iversen, *Acta Chem. Scand.* (*B*), 1976, **30**, 876.
[65a] K. Eklind, P. J. Garegg, and B. Gotthammer, *Acta Chem. Scand.* (*B*), 1976, **30**, 300, 305.
[66] H. Paulsen, C. Kolář, and W. Stenzel, *Angew. Chem. Internat. Edn.*, 1976, **15**, 440.
[67] S. Ogawa, Y. Funaki, K. Iwata, and T. Suami, *Bull. Chem. Soc. Japan*, 1976, **49**, 1975.
[68] A. S. Vzdykhan'ko, V. V. Glushenko, M. N. Mirzayanova, and A. Ya. Khorlin, *Invest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 699 (*Chem. Abs.*, 1976, **85**, 21 744r).

Condensations between 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide or 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl bromide and 1,2,4,6-tetra-*O*-acetyl-β-D-glucopyranose in the presence of mercuric salts were used in syntheses of laminaribiose and nigerose, respectively, and the stereoselectivity of these condensations was not affected when the aglycone was bound through C-1 to a polymeric support.[69] Derivatives of 6-*O*-β-D-glucopyranosyl-D-galactose and gentiobiose have been prepared by condensations involving 6-*O*-(t-butyl) ethers [*e.g.* 6-*O*-(t-butyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose],[61] and the disaccharide derivative (9) underwent self-condensation in acetonitrile in the



(9)

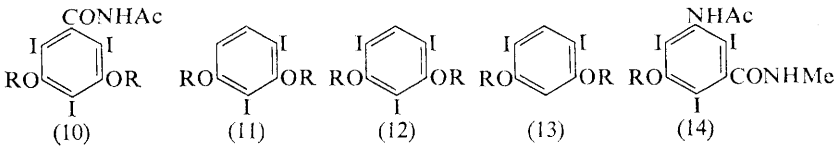
presence of mercuric salts to give a mixture of linear and cyclic oligosaccharides and a (1 → 6)-linked β-D-glucan having d.p. 9.[70] The anomeric configuration of the principal (1 → 6)-linked disaccharide obtained on condensation of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose with 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-glucopyranosyl bromide depended on the reaction conditions used; for example, the β-linked disaccharide is preponderant (*ca.* 85%) in benzene or dichloromethane in the presence of mercuric salts, whereas the α-linked disaccharide (75%) is obtained in acetonitrile.[70a] 2,3,4-Tri-*O*-acetyl-6-*O*-trichloroacetyl-α-D-glucopyranosyl bromide has been used as the glycosylating agent in a sequential synthesis of derivatives of gentio-triose and -tetraose, the trichloroacetyl group serving as a temporary protecting group.[71] Both α- and β-(1 → 2)-linked disaccharides were obtained when 2,3,4-tri-*O*-acetyl-6-deoxy-α-L-glucopyranosyl bromide was condensed with 1,3,4,6-tetra-*O*-acetyl-α-D-galacto- or -gluco-pyranose.[72] A synthesis of 3-*O*-α-L-rhamnopyranosyl-D-glucose has been reported.[73]

Monoglycosyl diglycerides have been prepared by way of glycosylation of 1,2-di-*O*-benzyl-*sn*-glycerol [74, 75] and β-D-glucopyranosides having a hydrophobic aglycone group terminated with a hydrophilic group have been synthesized by condensation of butane-1,4-diol or hexane-1,6-diol with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of mercuric cyanide and

[69] G. Excoffier, D. Y. Gagnaire, and M. R. Vignon, *Carbohydrate Res.*, 1976, **51**, 280.
[70] D. Y. Gagnaire and M. R. Vignon, *Carbohydrate Res.*, 1976, **51**, 140.
[70a] G. Excoffier, D. Y. Gagnaire, and M. R. Vignon, *Carbohydrate Res.*, 1976, **46**, 215.
[71] G. Excoffier, D. Y. Gagnaire, and M. R. Vignon, *Carbohydrate Res.*, 1976, **46**, 201.
[72] S. Kamiya, S. Esaki, and F. Konishi, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 273.
[73] F. Imperato, *J. Org. Chem.*, 1976, **41**, 3478.
[74] A. P. Kaplun, V. E. Kalugin, V. I. Shvets, and R. P. Evstigneeva, *Bioorg. Khim.*, 1975, **1**, 1675 (*Chem. Abs.*, 1976, **84**, 90 509j).
[75] S. G. Batrakov, E. F. Il'ina, and A. G. Panosyan, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 643 (*Chem. Abs.*, 1976, **85**, 143 396z).

deacetylation of the products.[76] Long-chain acylamidoalkyl β-glucopyranosides have been prepared by reaction of the appropriate acylamido alcohol with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide in nitromethane–benzene in the presence of mercuric cyanide, followed by catalytic deacetylation.[77]

The following glycosides have been obtained by way of conventional Koenigs–Knorr syntheses: the iodinated derivatives (10)—(14) (potential water-soluble

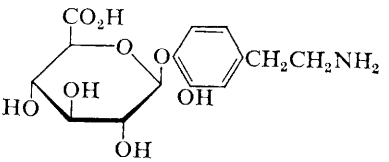


(10) (11) (12) (13) (14)

R = β-D-glucopyranosyl

contrast reagents for use in radiography),[78, 79] 4-methacryloylaminophenyl β-D-glucopyranoside, which could be polymerized by free-radical techniques,[80] 4-nitrophenyl 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α- and -β-D-galactopyranosides (for use, after reduction and linking to Sepharose, in the affinity chromatography of sialyltransferases),[81] *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-serine,[82] and β-D-glucopyranosyl derivatives of 3-*O*-methylmyricetin,[83] cirsilineol, cirsiliol,[84] and 6(7)-hydroxy-7(6)-methoxytetrahydroisoquinoline.[85]

A tumour substance, 4-*O*-(β-D-glucopyranosiduronic acid)-dopamine (15), has been synthesized by way of condensation of methyl (2,3,4-tri-*O*-acetyl-



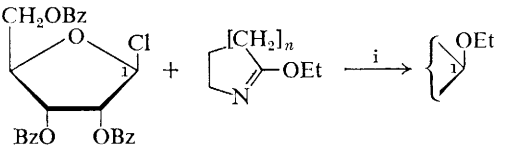
(15)

α-D-glucopyranosyl bromide)uronate with 3-benzyloxy-4-hydroxybenzaldehyde using an 'ion-pair' technique which involved extraction of the tetrabutylammonium phenolate into dichloromethane from an alkaline solution of the phenol and tetrabutylammonium hydrogen sulphate.[86] Koenigs–Knorr reactions

[76] B. R. Bhattacharyya, K. Ramaswamy, and R. K. Crane, *Carbohydrate Res.*, 1976, **47**, 167.
[77] H. M. Flowers, *Carbohydrate Res.*, 1976, **46**, 133.
[78] F. L. Weitl, M. Sovak, and M. Ohno, *J. Medicin. Chem.*, 1976, **19**, 353.
[79] F. L. Weitl, M. Sovak, T. M. Williams, and J. H. Lang, *J. Medicin. Chem.*, 1976, **19**, 1359.
[80] L. A. Carpino, H. Ringsdorf, and H. Ritter, *Makromol. Chem.*, 1976, **177**, 1631.
[81] K. L. Matta and J. J. Barlow, *Carbohydrate Res.*, 1976, **48**, 65.
[82] H. G. Garg and R. W. Jeanloz, *Carbohydrate Res.*, 1976, **49**, 482.
[83] S. C. Chhabra, S. R. Gupta, C. S. Sharma, and N. D. Sharma, *Indian J. Chem.*, 1976, **14B**, 384 (*Chem. Abs.*, 1976, **85**, 193 025y).
[84] L. Farkas and J. Strelisky, in 'Topics in Flavonoid Chemistry and Biochemistry', Proceedings of the 4th Hungarian Bioflavonoid Symposium, 1973 (Pub. 1975), p. 149 (*Chem. Abs.*, 1976, **85**, 63 294z).
[85] M. Barczaibeke and C. Szantay, *Acta Chim. Acad. Sci. Hung.*, 1974, **80**, 111 (*Chem. Abs.*, 1976, **84**, 90 500z).
[86] C. Hansson and E. Rosengren, *Acta Chem. Scand.* (*B*), 1976, **30**, 871.

have also been used to prepare the β-D-glucuronides of strophanthidine [87] and oestra-1,3,5(10)-triene-3,15α,16α,17β-tetraol.[88]

Glycosyl acetates have been used in the synthesis of β-D-glucuronides; for example, methyl D-glucopyranosyluronate β-tetra-acetate afforded higher yields of β-D-glucuronides than did the acetobromo-derivative in reactions with phenols and alcohols (including steroids) in 1,2-dichloroethane in the presence of stannic chloride.[89] Stannic chloride also catalysed the reaction between peracylated sugars and DMF dialkyl acetals to give high yields of 1,2-*trans*-glycosides, thereby avoiding the use of unstable glycosyl halides and of an excess of the aglycone.[90] Lactim ethers can also be used as a source of the aglycone under more vigorous conditions (Scheme 4). The α-glycoside formed when 1,2,3,4,6-penta-



n = 1, 2, or 3

Reagent: i, $SbCl_5$–CH_2Cl_2

Scheme 4

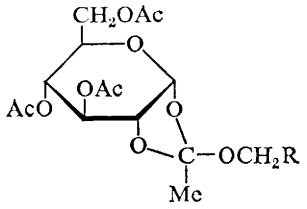
O-acetyl-β-D-glucopyranose reacts with phenol in the presence of stannic chloride does not appear to arise by anomerization of the β-glycoside.[91] An intermolecular process seems to be involved since stannic chloride did not anomerize the β-glycoside in the absence of phenol, whereas added 2-chlorophenol was incorporated into the α-glycoside produced. The process is apparently catalysed by diphenoxytin dichloride – which was isolated from the reaction mixture – and stannic chloride.

A practical preparation of 4-nitrophenyl β-D-mannopyranoside (7—8% yield) involved the fusion of 1,2,3,4,6-penta-*O*-acetyl-αβ-D-mannopyranose with 4-nitrophenol in the presence of zinc chloride (Helferich conditions), followed by deacetylation and chromatographic separation of the products on an ion-exchange resin,[92] although separation of the acetylated glycosides on silica gel has also been advocated.[93] A (1 → 6)-linked D-galactan and 6-*O*-β-D-galactopyranosyl-D-galactose have been recovered after deacetylation of the products resulting from the zinc chloride-catalysed polycondensation of 1,2,3,4-tetra-*O*-acetyl-β-D-galactopyranose.[94]

The synthesis of glycosides by the orthoester procedure continues to attract attention and the basicity of the alcohol used has been shown to influence the

[87] N. Sh. Pal'yants and N. K. Abubakirov, *Khim. prirod. Soedinenii*, 1975, **11**, 522 (*Chem. Abs.*, 1976, **84**, 59 921p).
[88] T. Nambara, K. Sudo, and M. Sudo, *Steroids*, 1976, **27**, 111 (*Chem. Abs.*, 1976, **84**, 150 907z).
[89] K. Honma, K. Nakazima, T. Uematsu, and A. Hamada, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 394.
[90] S. Hanessian and J. Banoub, *Tetrahedron Letters*, 1976, 657.
[91] T. D. Audichya, T. R. Ingle, and J. L. Bose, *Indian J. Chem.*, 1976, **14B**, 369 (*Chem. Abs.*, 1976, **85**, 193 024x).
[92] L. Rosenfeld and Y. C. Lee, *Carbohydrate Res.*, 1976, **46**, 155.
[93] K. Kawaguchi and N. Kashimura, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 241.
[94] E. E. Lee, G. Keaveney, A. Hartigan, and P. S. O'Colla, *Carbohydrate Res.*, 1976, **49**, 475.

ratio of α- and β-glycosides formed.[95] Thus, the ethyl derivative (16) gave only the β-glycoside, whereas the chloroethyl derivatives (17), (18), and (19) yielded



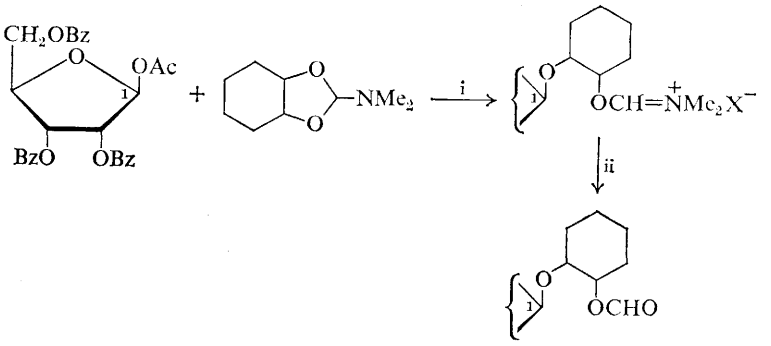
(16) R = Me
(17) R = CH_2Cl
(18) R = $CHCl_2$
(19) R = CCl_3

mixtures containing 16, 50, and 67%, respectively, of the corresponding α-glycoside. The orthoester procedure has been used in the D-glucosylation [74] and D-galactosylation [96] of 1,2-di-*O*-benzyl-*sn*-glycerol and in the preparation of the α-D-glucopyranoside from panaxadiol monoacetate [97] and of the cardenolide α-L-rhamnopyranosides convallatoxin, evomonoside, and periplorhamnoside [97a]

Oxidation (DMSO–P_2O_5–DMF) and then reduction ($NaBH_4$) of several aryl 3-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosides afforded the corresponding β-D-mannopyranosides in low yield [98] and standard transformations on 1,6-anhydro-2,3,3′-tri-*O*-benzoyl-4′,6′-*O*-benzylidene-β-lactose furnished 4-*O*-β-D-idopyranosyl-D-glucopyranose.[99] The synthesis of glycosides by the modification of unsaturated glyc-2-enopyranosides is dealt with in Chapter 14.

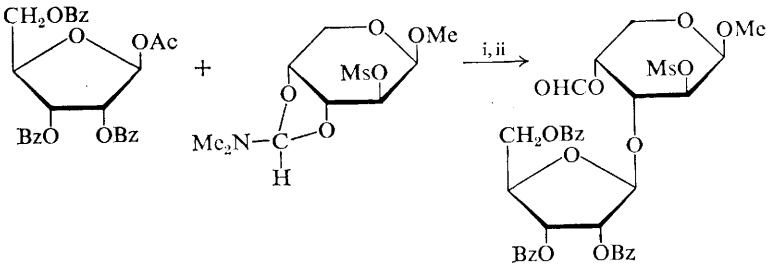
In addition to the approach outlined in Scheme 4, several other approaches to the synthesis of glycofuranosides have been reported during the past year. Hanessian and Banoub have used cyclic amide acetals derived from vicinal diols as the source of the aglycone in condensations with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose in the presence of stannic chloride (Scheme 5).[100] Disaccharide derivatives are obtained when the cyclic amide acetal is derived from carbohydrate vicinal diols (Scheme 6), and selective methanolysis of the formate ester exposed an hydroxy-group that can be subjected to further manipulation or glycosylation. 2,3,5-Tri-*O*-benzoyl-αβ-L-arabinofuranosyl bromide or chloride has been condensed with 4-nitrophenyl 2,3-di-*O*-acetyl-α-L-arabinofuranoside to yield, after deacylation, 4-nitrophenyl 5-*O*-α-L-arabinofuranosyl-α-L-arabinofuranoside,[101] with β-peltatin A [isolated from podophyllin (*Podophyllum peltatum*)] in glycosidation of the phenolic 8-OH group in an attempt to reduce

[95] P. J. Garegg and I. Kvarnström, *Acta Chem. Scand.* (*B*), 1976, **30**, 655.
[96] A. P. Kaplun, V. E. Kalugin, V. I. Shvets, and R. P. Evstigneeva, *Izvest. Vyssh. Uchebn. Zaved., Khim. khim. Tekhnol.*, 1975, **18**, 1502 (*Chem. Abs.*, 1976, **84**, 74 567w).
[97] N. I. Uvarova, G. I. Oshitok, A. K. Dzizenko, V. V. Isakov, and G. B. Elyakov, *Zhur. org. Khim.*, 1976, **12**, 984 (*Chem. Abs.*, 1976, **85**, 124 288z).
[97a] N. Sh. Pal'yants, A. F. Bochkov, and N. K. Abubakirov, *Khim. prirod. Soedinenii*, 1976, 58 (*Chem. Abs.*, 1976, **85**, 143 397n).
[98] N. Kashimura and K. Kawaguchi, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 1621.
[99] T. Chiba and S. Tejima, *Chem. and Pharm. Bull.* (*Japan*), 1976, **24**, 1684.
[100] S. Hanessian and J. Banoub, *Tetrahedron Letters*, 1976, 661.
[101] D. Arndt and A. Graffi, *Carbohydrate Res.*, 1976, **48**, 128.



Reagents: i, $SnCl_4$–CH_2Cl_2; ii, $NaHCO_3$

Scheme 5



Reagents: i, $SnCl_4$–CH_2Cl_2; ii, $NaHCO_3$

Scheme 6

its toxicity,[102] and with other cytotoxic phenols.[103, 104] The α- and β-anomers of 4-nitrophenyl 2,3,5-tri-*O*-benzyl-D-arabinofuranoside were obtained in the ratio of 7 : 1 when 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride was treated with 4-nitrophenol dissolved in dichloromethane in the presence of a molecular sieve as the acid acceptor.[105]

UDP-D-[^{14}C]glucuronic acid and the appropriate transferase have been used to prepare the β-D-glucuronides of 4-methylumbelliferone, 4-nitrophenol, and ethylmorphine.[106]

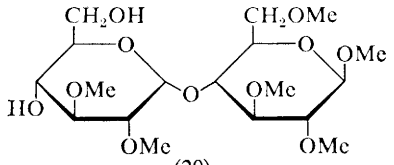
O-Glycosyldipeptides have been synthesized by lengthening the peptide chain at the CO_2H-terminus of *N*-(benzyloxycarbonyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-threonine 2-nitrophenyl ester [106a] and at the NH_2-terminus of the corresponding methyl ester after hydrogenolysis of the protecting *N*-substituent.[107]

[102] K. Schwabe, A. Graffi, and B. Tschiersch, *Carbohydrate Res.*, 1976, **48**, 277.
[103] D. Arndt, A. Graffi, and A. D. Teppke, *Pharmazie*, 1976, **31**, 287 (*Chem. Abs.*, 1976, **85**, 63 293y).
[104] D. Arndt and A. Graffi, *J. prakt. Chem.*, 1975, **317**, 752 (*Chem. Abs.*, 1976, **84**, 59 930r).
[105] E. Zissis and C. P. J. Glaudemans, *Carbohydrate Res.*, 1976, **50**, 292.
[106] E. Puhakainen, M. Lang, A. Ilvonen, and O. Hänninen, *Acta Chem. Scand.* (*B*), 1976, **30**, 685.
[106a] J. Martinez, A. Pavia, and F. Winternitz, *Carbohydrate Res.*, 1976, **50**, 15.
[107] J. Martinez, A. Pavia, and F. Winternitz, *Carbohydrate Res.*, 1976, **50**, 148.

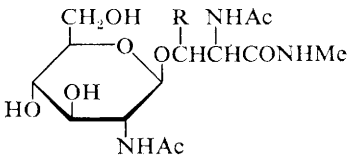
Hydrolysis and Related Reactions.—First-order rate coefficients, activation energies, and entropies of activation for the acid-catalysed hydrolysis of methyl β-D-glucopyranoside and seven methyl chlorodeoxyglycopyranosides have been determined from the amount of methanol produced.[107a] Increase in the number of chlorine substituents caused a decrease in the rate of hydrolysis, particularly when the C-2 hydroxy-group was replaced by chlorine. The kinetics of hydrolysis of β-cellobiose, methyl α-D-glucopyranoside, and sucrose have been examined over a wide range of concentrations of hydrochloric, sulphuric, and phosphoric acids.[108] Transition of the protonated forms from the chair to half-chair conformation (a cyclic carbonium ion being formed) was shown to be the rate-determining step of pyranoside hydrolysis – the interaction of a glycoside molecule with the medium, estimated from the proton chemical shifts ($\Delta\nu$), had a marked effect on this step. An equation relating the changes in rate constant to the Hammett acidity function (H_0) and the value $\Delta\nu$ was derived.

A linear free-energy relationship has been found between the rates of acetolysis of 2,4-dinitrophenyl glycopyranosides and the rates of acid-catalysed hydrolysis of the methyl glycopyranosides, except when participation by a neighbouring 2-acetamido-2-deoxy group intervenes.[109] The glycosyl acetates are formed mainly with inversion of configuration at the anomeric centre. A study of the methanolysis of the methyl glycosides of the disaccharides sophorose, cellobiose, gentiobiose, maltose, and isomaltose has been reported.[110] Hepta-*O*-acetylamygdalin was cleaved selectively with dichloromethyl methyl ether in the presence of zinc chloride to give 2,2′,3,3′,4,4′,6′-hepta-*O*-acetyl-α-gentiobiosyl chloride in good yield.[111]

The possibility of effecting selective cleavage of glycosidic linkages in partially methylated polysaccharides by oxidation of the unsubstituted hydroxy-groups and base-catalysed β-elimination from the oxidized product (see Vol. 6, pp. 116 and 157) has been tested with the methylated disaccharide (20).[112] The results



(20)



(21) R = H or Me

indicated that this degradation procedure should be applicable to bacterial polysaccharides containing hexopyranosyl residues that are acetalated at positions 4 and 6, since methylation, partial hydrolysis with acid, oxidation, and treatment with base should cleave the glycosidic bond of such residues. A procedure for the cleavage of *O*-glycosidic linkages between sugars and hydroxyamino-acids [*e.g.* in (21)] involved treatment with methylamine and sodium borohydride;

[107a] T. Ishii, A. Ishizu, and J. Nakano, *Carbohydrate Res.*, 1976, **48**, 33.
[108] Y. V. Moiseev, N. A. Khalturinskii, and G. E. Zaikov, *Carbohydrate Res.*, 1976, **51**, 23.
[109] D. Cocker and M. L. Sinnott, *J.C.S. Perkin II*, 1976, 618.
[110] B. Piekarska, *Roczniki Chem.*, 1975, **49**, 1919.
[111] I. Farkas, I. F. Szabó, and R. Bognár, *Annalen*, 1976, 440.
[112] P.-K. Jansson, L. Kenne, and S. Svensson, *Acta Chem. Scand.* (*B*), 1976, **30**, 61.

this procedure afforded stable derivatives both of the amino-acid and sugar residues.[113]

Additional physical and analytical data for the u.v.(253.7 nm)-photolysis of phenyl β-D-glucopyranosides in aqueous solution have been provided.[114] The data permit a number of experimental observations to be explained, although it is still not possible to propose a valid mechanism for D-glucosidic bond scission.

Protection of the anomeric centre of sugars with the trichloroethyl group, which can be removed with zinc and dilute acid, is mentioned in Chapter 20.

Other Features of Glycosides.—Consideration of the electron density at the anomeric centre of glycosides has provided a rationale for the fact that equatorially oriented aglycones, which have a lower electron density at C-1 and therefore at H-1, interact more strongly than axially oriented aglycones with DMSO.[115] That the mobilities of β-glycoside derivatives on DMSO-impregnated chromatography paper are lower than those of the corresponding α-glycosides has been known for some time. Measurement of the ^{1}H n.m.r. spectra of acetylated anomers of methyl D-gluco-, D-galacto-, and D-manno-pyranosides in [^{2}H]chloroform containing increasing concentrations of [$^2H_6$]DMSO indicated that there is a specific interaction between H-1 of the β-glycopyranosides and the partially negative oxygen atom of DMSO. Another study, on non-carbohydrate derivatives, is relevant to differences in the reactivities of axial and equatorial hydrogens at the anomeric centre of pyranosides; thus u.v.-irradiation of *cis*- and *trans*-2-methoxy-4-methyltetrahydropyran in benzene containing benzophenone gave 3-methyl-δ-valerolactone and methyl 3-methyl-5-phenylvalerate by way of abstraction of the hydrogen atom at C-2 (Scheme 7).[116] Since the



Reagent: i, *hν*–PhCOPh–PhH

Scheme 7

cis-isomer reacted more rapidly than the *trans*-isomer, abstraction of the axial hydrogen atom is more easily achieved (*cf.* Vol. 6, p. 24).

The configuration at the benzylic centre of naturally occurring and synthetic glycosides of mandelonitrile can be assigned by ^{1}H n.m.r. spectroscopy, since the chemical shifts of the methine and anomeric protons depend on the configuration at the benzylic centre.[117]

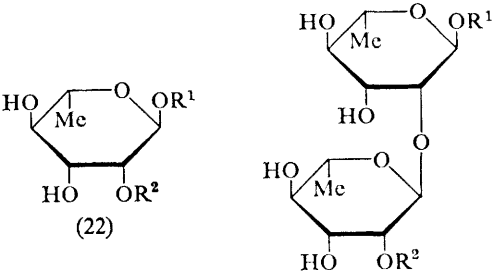
The α- and β-anomers of ethyl 2-amino-2-deoxy-D-glucopyranoside have been separated on cationic resins prior to *N*-acylation with a series of fatty-acid anhydrides.[118]

[113] Z. I. Lebedeva, L. A. Baratova, S. M. Avaeva, I. V. Medvedeva, M. N. Mirzayanova, and A. Ya. Khorlin, *Bioorg. Khim.*, 1975, **1**, 923 (*Chem. Abs.*, 1976, **84**, 105 986p).
[114] W. G. Filby, G. O. Phillips, and M. G. Webber, *Carbohydrate Res.*, 1976, **51**, 269.
[115] P. J. Garegg and T. Iversen, *Acta Chem. Scand.* (*B*), 1976, **30**, 185.
[116] K. Hayday and R. D. McKelvey, *J. Org. Chem.*, 1976, **41**, 2222.
[117] U. Schwarzmaier, *Chem. Ber.*, 1976, **109**, 3250.
[118] S. Hirano, M. Ishigami, and Y. Ohe, *J. Org. Chem.*, 1976, **41**, 4038.

Kinetic methods have been used to investigate the binding of 4-nitrophenyl α-D-mannopyranoside with concanavalin A.[119]

Natural Products.—A review of flavone and flavonol glycosides has appeared.[120]

A bitter-tasting component produced by the defence mechanism of an Australian insect (*Acripeza reticulata* Guér) has been identified as (−)-1-methylpropyl β-D-glucopyranoside.[121] 3-*O*-β-D-Glucopyranosyl-2-*O*-isovaleryl-D-glucose is the carbohydrate component of a glycoside isolated from coffee.[122] Phenols were glycosylated to give 4-*O*-methyl-β-D-glucopyranosyl derivatives when incubated with *Sporotrichum sulfurescens*,[123] while growth of a *Pseudomonas* in the presence of n-alkanes as a source of carbon produced the L-rhamnopyranoside esters (22) and (23).[124]



R^1 = $Me[CH_2]_6CHCH_2CO_2CH[CH_2]_6Me$ with CH_2CO_2H

R^2 = $\cdot OCCH{=}CH[CH_2]_6Me$

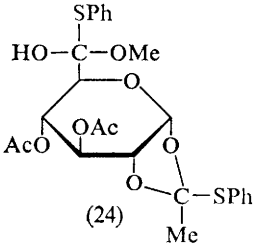
O-α-L-Arabinofuranosylhydroxyproline has been isolated following alkaline degradation of rice-bran proteoglycans[125] and the urine of ABO secretors has been shown to contain α-L-fucopyranosyl-*myo*-inositol.[126]

S-Glycosides

Treatment of alkyl 1-thio-β-D-glucopyranoside tetra-acetates with boron trifluoride in dichloromethane at room temperature afforded mixtures of the α- and β-anomers, in the ratio of *ca.* 7 : 3, which were readily separated by chromatography on silica gel.[127] Boron trifluoride also catalysed the condensation of equimolar proportions of 1,2-*trans*-related monosaccharide peresters and alkyl, alkenyl, and some aryl thiols to provide a convenient synthesis of esters

[119] S. D. Lewis, J. A. Shafer, and I. J. Goldstein, *Arch. Biochem. Biophys.*, 1976, **172**, 689.
[120] J. B. Harborne and C. A. Williams, *Flavonoids*, 1975, **1**, 376 (*Chem. Abs.*, 1976, **85**, 160426b).
[121] J. Cable and H. Nocke, *Austral. J. Chem.*, 1975, **28**, 2737.
[122] H. Obermann and G. Spiteller, *Chem. Ber.*, 1976, **109**, 3450.
[123] K. Kieslich, H.-J. Vidic, K. Petzoldt, and G.-A. Hoyer, *Chem. Ber.*, 1976, **109**, 2259.
[124] M. Yamaguchi, A. Sato, and A. Yukuyama, *Chem. and Ind.*, 1976, 741.
[125] T. Yamagishi, K. Matsuda, and T. Watanabe, *Carbohydrate Res.*, 1976, **50**, 63.
[126] G. Lennartson, A. Lundblad, B. Lindberg, and J. Lönngren, *Biochem. Biophys. Res. Comm.*, 1976, **69**, 920.
[127] B. Erbing and B. Lindberg, *Acta Chem. Scand.* (*B*), 1976, **30**, 611.

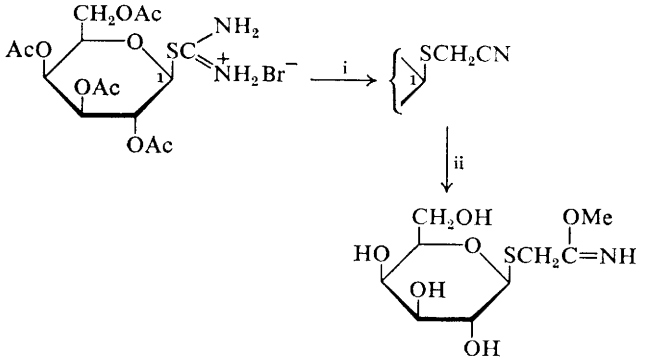
of 1,2-*trans*-thioglycosides.[127a] Stannic chloride has been used to catalyse the formation of 1,2-*trans*-thioglycosides from peracetylated glycopyranoses and thiophenol and of the unusual thio-orthoester (24) from methyl (1,2,3,4-tetra-



(24)

O-acetyl-α-D-glucopyranosyl)uronate.[128] 1,2-*trans*-Thioglycosides have also been obtained by thiolysis of 1,2-(t-butyl orthoacetates).[129]

Peracetylated glycopyranosyl 2-pseudothioureas have been converted into 2-imino-2-methoxyethyl 1-thioglycopyranosides by the reactions shown in Scheme 8.[130] The iminoethers reacted with amines, amino-acids, and proteins



Reagents: i, $ClCH_2CN$–K_2CO_3–$NaHSO_3$; ii, NaOMe

Scheme 8

under mild alkaline conditions to form amidines, and this provides a means of attaching sugars (*e.g.* β-D-glucopyranose, α-D-mannopyranose, and 2-acetamido-2-deoxy-β-D-glucopyranose) to proteins.

Acetobromo-sugars have been used to glycosylate 4-methyl-3-phenyl-1,2,4-triazoline-5-thione, 2-phenyl-1,3,4-thiadiazoline-5-thione,[131] and 4-ethylamino-6-isopropylamine-2-mercapto-*s*-triazine,[132] yielding the *S*-glycosides preferentially.

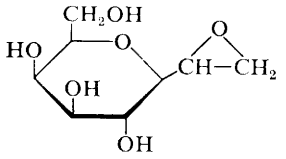
[127a] R. J. Ferrier and R. H. Furneaux, *Carbohydrate Res.*, 1976, **52**, 63.
[128] K. Honma and A. Hamada, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 818.
[129] K. Honma and A. Hamada, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 1165.
[130] Y. C. Lee, C. P. Stowell, and M. J. Krantz, *Biochemistry*, 1976, **15**, 3956.
[131] G. Wagner, B. Dietzsch, and U. Krake, *Pharmazie*, 1975, **30**, 694 (*Chem. Abs.*, 1976, **84**, 90 504d).
[132] M. Poje, B. Mihanovic, and P. Mildner, *Bull. Sci., Cons. Acad. Sci. Arts R.S.F. Yougosl., Sect. A*, 1975, **20**, 273 (*Chem. Abs.*, 1976, **84**, 59 928w).

The S—aryl bond of 2,4-dinitrophenyl 1-thio-β-D-glucopyranoside was cleaved on treatment with benzyl bromide, the benzyl carbonium ion presumably acting as a sulphur-specific Lewis acid.[133]

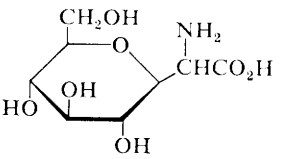
C-Glycosides

Details of the synthesis and reactions of glycofuranosylethynes, prepared *via* the reaction of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose with ethynyl magnesium bromide, have been published (see Vol. 8, p. 27).[134] It is noteworthy that the glycofuranosylethynes do not obey Hudson's rules of isorotation.

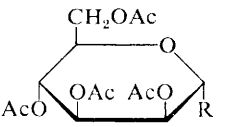
Diastereoisomeric β-D-galactopyranosyl epoxyethanes (25) resulted when 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide was treated with vinylmagnesium bromide, followed by epoxidation and deacetylation.[135] 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide reacted with the 2-phenyloxazolone



(25)

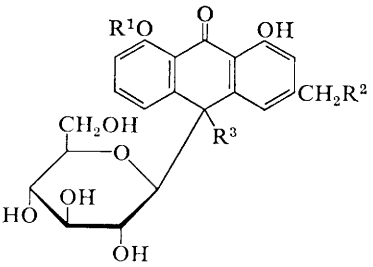


(26)



(27) R = $CONH_2$
(28) R = CMe_2OH

anion to give 2-*C*-β-D-glucopyranosyl-(*RS*)glycine (26) following methanolysis and deacetylation.[136] Photochemical addition of formamide to 2,3,4,6-tetra-*O*-acetyl-2-hydroxy-D-glucal afforded the α-D-*manno* adduct (27) (7%), the



	R^1	R^2	R^3
(29)	H	H	OH
(30)	β-D-glucopyranosyl	OH	H
(31)	β-D-glucopyranosyl	H	H

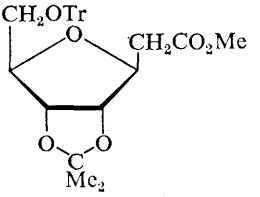
isomeric α-D-*gluco* adduct (55%), and the tertiary alcohol (28) (23%) arising from solvent (t-butyl alcohol) participation.[137]

The 9-anthrone derivatives (29) (from the heartwood of *Cassia garrettiana* Craib),[138] (30), and (31) (from the bark of *Rhamnus kurshianus*)[139] and several

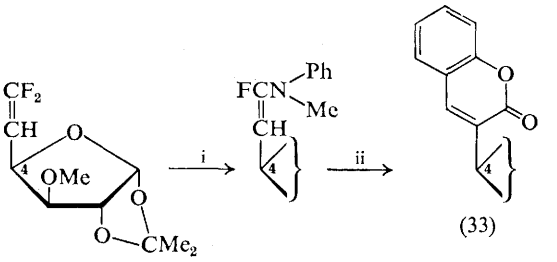
[133] N. G. Morozova, L. V. Volkova, E. G. Gutsalenko, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1976, **12**, 960 (*Chem. Abs.*, 1976, **85**, 94 647w).
[134] J. G. Buchanan, A. D. Dunn, and A. R. Edgar, *J.C.S. Perkin I*, 1976, 68.
[135] S. D. Shiyan, O. E. Lakhtina, M. L. Shul'man, and A. Ya. Khorlin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 197 (*Chem. Abs.*, 1976, **84**, 150 852c).
[136] A. Rosenthal and A. J. Brink, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 343.
[137] A. Rosenthal and M. Ratcliffe, *Canad. J. Chem.*, 1976, **54**, 91.
[138] K. Hata, M. Kozawa, and K. Baba, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 1688.
[139] H. Wagner and G. Demuth, *Z. Naturforsch.*, 1976, **31b**, 267.

flavonoid *C*-glycosides (from *Swertia perennis* L. and *Gentiana pyrenaica* L., *etc.*) [140–143] have been characterized.

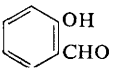
A Wittig reaction has been used in an improved large-scale synthesis of the *C*-glycofuranoside derivative (32),[144] and the *C*-glycoside analogue (33) has been



(32)



(33)

Reagents: i, $PhN^{-}MeLi^{+}$; ii, 

Scheme 9

synthesized by the reactions shown in Scheme 9 [145] (see also the section on *C*-nucleosides in Chapter 21).

Glycofuranosyl cyanides are dealt with in Chapter 7.

[140] K. Hostettmann and A. Jacot-Guillarmod, *Helv. Chim. Acta*, 1976, **59**, 1584.
[141] J. Krause, *Z. Pflanzenphysiol.*, 1976, **79**, 465 (*Chem. Abs.*, 1976, **85**, 106 667t).
[142] A. Marston, K. Hostettmann, and A. Jacot-Guillarmod, *Helv. Chim. Acta*, 1976, **59**, 2596.
[143] R. Tschesche and K. Struckmeyer, *Chem. Ber.*, 1976, **109**, 2901.
[144] T. J. Cousineau and J. A. Secrist, tert., *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 185.
[145] J. M. J. Tronchet, B. Baehler, and A. Bonenfant, *Helv. Chim. Acta*, 1976, **59**, 941.

4

Ethers and Anhydro-sugars

Ethers

Phase-transfer catalysts (*e.g.* benzyltriethylammonium chloride and tetrabutylammonium bromide) have been used in the preparation of *O*-methyl and *O*-benzyl ethers and acetals of formaldehyde (*e.g.* methyl 4,6-*O*-benzylidene-2,3-*O*-methylene-α-D-mannopyranoside).[146] A new cyclic ether, methyl 4,6-*O*-benzylidene-2,3-*O*-ethylene-α-D-glucopyranoside, resulted when methyl 4,6-*O*-benzylidene-α-D-glucopyranoside reacted with 1,2-dichloroethane under basic aqueous conditions in the presence of a phase-transfer catalyst. Partially protected sugars (*e.g.* 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose) have also been methylated and benzylated by reaction with methyl and benzyl trifluoromethanesulphonate (triflate), respectively, using 2,6-di-t-butylpyridine as base.[147] The reactions with benzyl triflate, prepared *in situ* from benzyl alcohol and trifluoromethanesulphonic anhydride, occurred readily at *ca.* −70 °C, whereas those with methyl triflate were performed in refluxing dichloromethane.

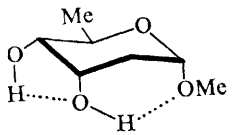
Methyl Ethers.—6-*O*-Methyl-L-sorbose, 5-*O*-methyl-D-fructose and -L-sorbose, and 5,6-di-*O*-methyl-L-sorbose have been synthesized.[148] U.v. spectroscopy was used to determine the proportion of the acyclic form of these and other ketoses present in methanol solution; the extinction coefficient (ε = 34.7) for the carbonyl group was determined using 5,6-di-*O*-methyl-L-sorbose, which cannot cyclize. Of the other ketoses examined, 6-*O*-methyl-L-sorbose was found to contain the highest proportion (7.4%) of the acyclic form at equilibrium.

A new route for preparing methyl 4,6-di-*O*-methyl-α-D-mannopyranoside *via* methyl 2,3-di-*O*-toluene-*p*-sulphonyl-α-D-mannopyranoside has been described.[149] Mass-spectral fragmentation pathways proposed for 5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-, 2,5-di-*O*-acetyl-3,4,6-tri-*O*-methyl-, and 3,5-di-*O*-acetyl-2,4,6-tri-*O*-methyl-D-mannononitrile were also confirmed.

Studies of the partial methylation of methyl 2,6-dideoxy-α-D-*ribo*-hexopyranoside have shown that the 3- and 4-*O*-methyl ethers are formed at comparable rates, although the 4-*O*-methyl ether reacted more rapidly than the 3-*O*-methyl ether in forming the disubstituted derivative.[150] Hydrogen-bonding between O-1 and the hydroxy-group at C-3 increases the nucleophilicity of O-3

[146] P. Di Cesare and B. Gross, *Carbohydrate Res.*, 1976, **48**, 271.
[147] J. M. Berry and L. D. Hall, *Carbohydrate Res.*, 1976, **47**, 307.
[148] K. Heyns and J. Heukeshoven, *Annalen*, 1976, 269.
[149] F. R. Seymour, M. E. Slodki, R. D. Plattner, and L. W. Tjarks, *Carbohydrate Res.*, 1976, **46**, 189.
[150] M. Marek, K. Kefurt, J. Staněk, jun., and J. Jarý, *Coll. Czech. Chem. Comm.*, 1976, **41**, 2596.

in the 4-*O*-methyl derivative, whereas the hydroxy-groups of the parent diol (34) are equally reactive since both are involved in hydrogen-bonding. Related studies on the partial methylation and benzylation of methyl 4,6-*O*-benzylidene-α-



(34)

and -β-D-glucopyranoside have demonstrated that HO-2 is either as reactive as, or more reactive than, HO-3 in the α-series, but that it is relatively less reactive in the β-series.[151] A report on the products obtained on partial methylation of methyl α-D-xylopyranoside and methyl L-rhamnopyranosides has also appeared.[152]

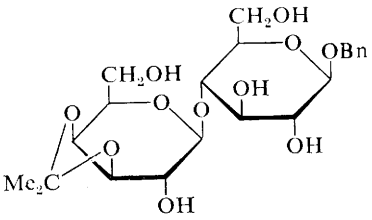
4-*O*-Methyl-D-galactose [153, 153a] and 4-*O*-methyl-D-glucose [153] have been identified as components of extracellular polysaccharides produced by certain *Rhizobium* species.

Benzyl and Other Substituted Alkyl Ethers.—Phase-transfer catalysts have been used in the benzylation of such protected sugar derivatives as methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside [154] and in the monobenzylation of monosaccharide diols (*e.g.* methyl 4,6-*O*-benzylidene-D-glucopyranosides), where the total yield of monobenzylated products may be substantially improved.[155]

The separation of fifteen *O*-benzyl ethers of methyl α-D-glucopyranoside by g.l.c. of their TMS derivatives has been examined; although neither the 2,3- and 2,4-di- nor the 2,3,6- and 2,4,6-tri-*O*-benzyl ethers could be separated by g.l.c., the proportion of each of these components in a mixture could be determined by mass spectrometry.[156] 2,3,6-Tri-*O*-benzyl-D-glucose has been obtained by benzylation of phenyl 2,2′,3,3′,4′,6,6′-hepta-*O*-acetyl-α-lactoside with benzyl chloride and potassium hydroxide, followed by acidic hydrolysis of the benzylated lactoside and chromatographic separation of the benzylated monosaccharides.[157] Selective benzylation of the β-lactoside derivative (35) gave the 2,2′,3,6,6′-penta- and 2′,3,6,6′- and 2,2′,6,6′-tetra-*O*-benzyl ethers in 38, 7, and 30% yield, respectively, suggesting that the high nucleophilicity of HO-2′ may arise from intramolecular hydrogen-bonding.[158]

Benzyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside and benzyl 6-*O*-allyl-2,4-di-*O*-benzyl-β-D-galactopyranoside, which were required as intermediates in the

[151] Y. Kondo, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 1879.
[152] E. V. Evtushenko and Yu. S. Ovodov, *Khim. prirod. Soedinenii*, 1975, **11**, 682 (*Chem. Abs.*, 1976, **84**, 150 895u).
[153] L. D. Kennedy and R. W. Bailey, *Carbohydrate Res.*, 1976, **49**, 451.
[153a] W. F. Dudman, *Carbohydrate Res.*, 1976, **46**, 97.
[154] S. Czernecki, C. Georgoulis, and C. Provelenghiou, *Tetrahedron Letters*, 1976, 3535.
[155] P. J. Garegg, T. Iversen, and S. Oscarson, *Carbohydrate Res.*, 1976, **50**, C12.
[156] J. M. Küster, H. Luftmann, and I. Dyong, *Chem. Ber.*, 1976, **109**, 2223.
[157] O. E. Brodde, *Carbohydrate Res.*, 1976, **48**, 299.
[158] J. M. Küster, I. Dyong, and D. Schmeer, *Chem. Ber.*, 1976, **109**, 1253.



(35)

synthesis of oligosaccharides, have been prepared by straightforward routes.[159] Improved procedures for preparing 2,3,4,6-tetra-*O*-benzyl-D-manno-, -gluco-, and -galacto-pyranoses have been reported.[160]

Benzyl ethers have been used as temporary blocking groups in the synthesis of a blood-group H-specific trisaccharide, 2-acetamido-2-deoxy-4-*O*-(2-*O*-α-L-fucopyranosyl-β-D-galactopyranosyl)-D-glucopyranose.[161]

Methyl α-D-fucopyranoside reacted with trityl chloride in pyridine to give the 2- and 3-*O*-trityl derivatives (ratio 3 : 2) in good yield.[162] A report describing the use of insoluble polymers containing pendant trityl chloride residues to block one of the primary hydroxy-groups of polyhydric alcohols (*e.g.* butane-1,2,4-triol) is of interest.[163]

The structures of 3-*O*-[(*R*)-1-carboxyethyl]-L-rhamnose, a constituent of the *O*-antigenic lipopolysaccharide of *Sh. dysenteriae* type 5,[164, 165] 4-*O*-[(*S*)-1-carboxyethyl]-D-glucose, a constituent of the extracellular polysaccharide material from *Aerococcus viridans* var. *homari*,[165, 166] and 4-*O*-(1-carboxyethyl)-D-mannose, a constituent of the extracellular polysaccharide of *Mycobacterium lacticolum*,[167] have been determined by chemical and physicochemical methods and, in one instance, by synthesis.[164]

The 2-, 3-, and 6-*O*-(2-hydroxyethyl) ethers of D-glucose have been obtained by way of treatment of appropriately protected derivatives (*e.g.* 1,2:3,5-di-*O*-methylene-α-D-glucofuranose) with sodium and then with 2-bromoethanol.[168]

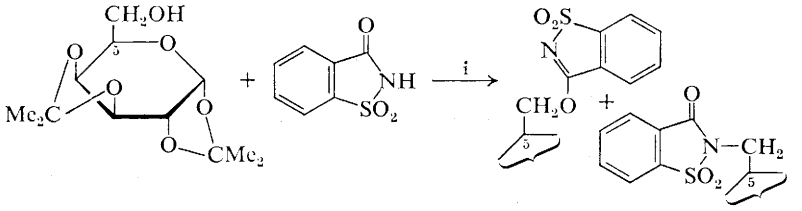
Allyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside has been used as the starting material in preparations of 2-*O*-allyl-3,4,6-tri-*O*-benzyl-D-glucopyranose and 2-*O*-allyl-3,4-di-*O*-benzyl-D-glucopyranose, the 1-(4-nitrobenzoates) of which are potential intermediates for the synthesis of glycolipids that occur in the cytoplasmic membranes of Streptococci.[169] Allyl ethers can be cleaved on

[159] A. Lubineau, A. Thieffry, and A. Veyrières, *Carbohydrate Res.*, 1976, **46**, 143.
[160] S. Koto, N. Morishima, Y. Miyata, and S. Zen, *Bull. Chem. Soc. Japan*, 1976, **49**, 2639.
[161] J.-C. Jacquinet and P. Sinaÿ, *Tetrahedron*, 1976, **32**, 1693.
[162] T. Otake and T. Sonobe, *Bull. Chem. Soc. Japan*, 1976, **49**, 1050.
[163] J. M. Fréchet and L. J. Nuyens, *Canad. J. Chem.*, 1976, **54**, 926.
[164] N. K. Kochetkov, B. A. Dmitriev, and L. V. Backinowsky, *Carbohydrate Res.*, 1976, **51**, 229.
[165] N. K. Kochetkov, B. A. Dmitriev, L. V. Backinowsky, and V. L. L'vov, *Bioorg. Khim.*, 1975, **1**, 1238 (*Chem. Abs.*, 1976, **84**, 105 922q).
[166] L. Kenne, B. Lindberg, B. Lindquist, J. Lönngren, B. Arie, R. G. Brown, and J. E. Stewart, *Carbohydrate Res.*, 1976, **51**, 287.
[167] N. K. Kochetkov, O. S. Chizhov, A. F. Sviridov, and Kh. A. Arifkhodzhaev, *Bioorg. Khim.*, 1976, **2**, 1140 (*Chem. Abs.*, 1976, **85**, 138 900e).
[168] B. H. Thewlis, *Stärke*, 1975, **27**, 336 (*Chem. Abs.*, 1976, **84**, 17 625q).
[169] P. A. Gent and R. Gigg, *Carbohydrate Res.*, 1976, **49**, 325.

heating in methanol with palladized charcoal in the presence of a trace of acid; under these conditions the allyl ether isomerizes to the prop-1-enyl ether, which is hydrolysed by the acid present.[170]

O-Tetrahydrofuran-2-yl ethers were obtained when a mixture of sulphuryl chloride and THF reacted with a solution of an alcohol (or a thiol) in THF in the presence of triethylamine.[171] The tetrahydrofuranyl group can be quantitatively removed by hydrolysis under very mild acidic conditions.

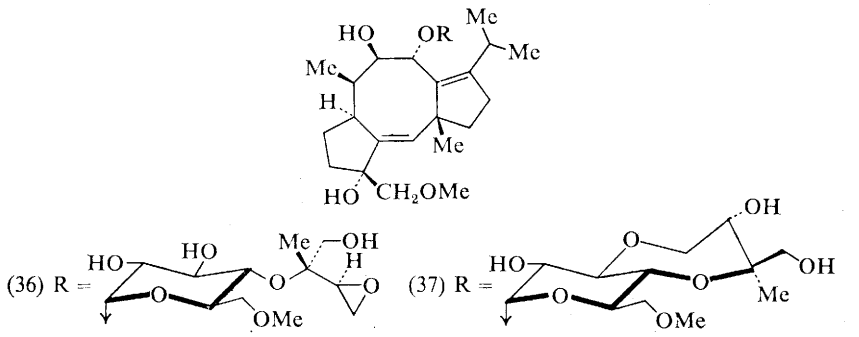
Novel carbohydrate–saccharin derivatives have been prepared by the procedure shown in Scheme 10.[172]



Reagent: i, $EtO_2CN{=}NCO_2Et$–PPh_3

Scheme 10

The structures of the unusual ether derivatives (36) and (37) (cotylenins F and G) and those of related plant glycosides have been reported.[173, 173a]

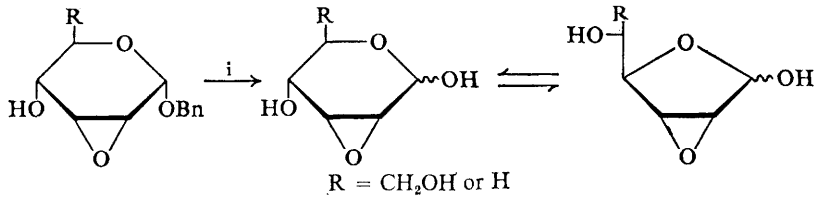


Intramolecular Ethers (Anhydro-sugars)

Oxirans.—All the stereoisomeric methyl 2,3-anhydro-6-deoxy-DL-hexopyranosides or their 4-*O*-acetyl derivatives or both have been prepared by epoxidation (hydrogen peroxide–benzonitrile) of methyl 2,3,6-trideoxy-DL-hex-2-enopyranosides or their 4-acetates.[174]

[170] R. Boss and R. Scheffold, *Angew. Chem. Internat. Edn.*, 1976, **15**, 558.
[171] C. G. Kruse, N. L. J. M. Broekhof, and A. van der Gen, *Tetrahedron Letters*, 1976, 1725.
[172] W. A. Szarek, C. Depew, and J. K. N. Jones, *J. Heterocyclic Chem.*, 1976, **13**, 1131.
[173] T. Sassa, M. Togashi, and T. Kitaguchi, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 1735.
[173a] T. Sassa and A. Takahama, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 2213.
[174] O. Achmatowicz, jun., and B. Szechner, *Carbohydrate Res.*, 1976, **50**, 23.

2,3-Anhydro-D-allose and 2,3-anhydro-D-ribose, potential hexokinase inhibitors, have been prepared as shown in Scheme 11.[175] Crystalline 2,3-anhydro-α-D-allo

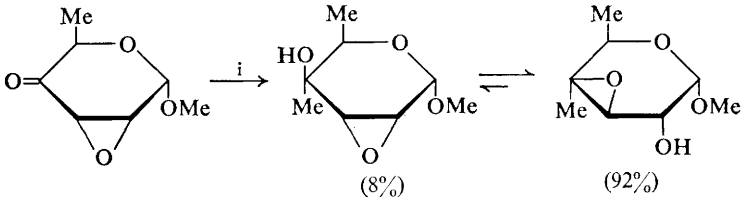


Reagent: i, H_2–Pd–C–THF (or EtOAc)

Scheme 11

pyranose underwent rapid mutarotation when dissolved in water to give an equilibrium mixture containing mainly the α-pyranose (41%) and β-furanose (42%) forms, together with the β-pyranose and α-furanose forms (5 and 12%, not individually assigned).

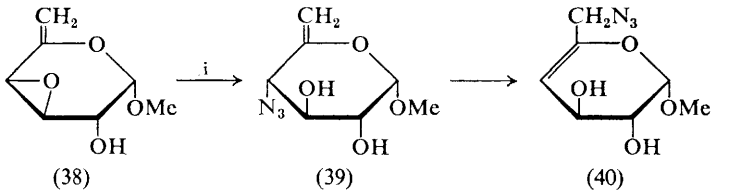
A careful study of the equilibration of α-hydroxyoxirans in the propane and pyranoside series (Scheme 12) has shown that the more highly substituted oxiran is favoured.[176]



Reagent: i, MeLi

Scheme 12

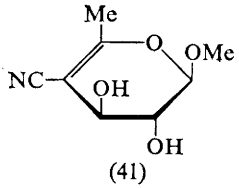
Compounds containing both oxiran and vinyl ether groups [*e.g.* (38)] have been shown to be useful intermediates in the regio- and stereo-selective synthesis of rare sugars.[177] Opening of the oxiran ring with ammonia provided a route to derivatives of methyl 4-acetamido-4,6-dideoxy-D-glucopyranoside, while the 4-azido-derivative (39), obtained with sodium azide, rapidly rearranged to give the 6-azido-derivative (40) (Scheme 13). The β-anomer of (38) afforded the



Reagent: i, NaN_3–H_2O

Scheme 13

[175] J. G. Buchanan, D. M. Clode, and N. Vethaviyasar, *J.C.S. Perkin I*, 1976, 1449.
[176] H. Paulsen and K. Eberstein, *Chem. Ber.*, 1976, **109**, 3891.
[177] M. Brockhaus, W. Gorath, and J. Lehmann, *Annalen* 1976, 89.



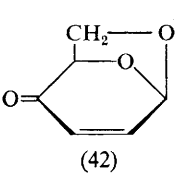
(41)

unsaturated 6-deoxy-derivative (41) on treatment with sodium cyanide, presumably *via* the 4-cyanohex-5-enoside.

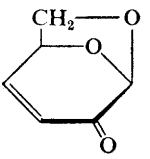
Methylation of methyl 3,4-anhydro-α-DL-talopyranoside, followed by acid-catalysed opening of the oxiran ring, has been used in a synthesis of methyl 2,6-di-*O*-methyl-α-DL-mannopyranoside (methyl DL-curamicoside).[178] Ring-opening of methyl 3,4-anhydro-6-*O*-trityl-DL-allopyranoside with ammonia *etc.*, afforded derivatives of DL-kanosamine (3-amino-3-deoxy-DL-glucose).

Oxirans are also mentioned in Chapters 14, 15, 16, and 21.

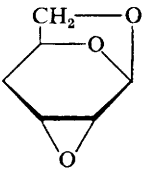
Other Anhydrides.—The enones (42) and (43) have been prepared by oxidation of the corresponding allylic alcohols with manganese dioxide.[179] Whereas the oxiran derivative (44) afforded the allylic alcohol (45) on treatment with butyl-lithium, the *lyxo*-isomer (46) gave the 2-substituted glycal (47). Enols with equatorially oriented hydroxy-groups were obtained when the enones (42) and (43) were reduced with sodium borohydride.



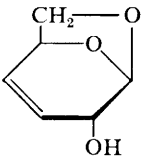
(42)



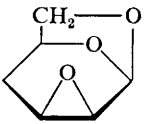
(43)



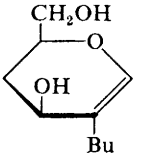
(44)



(45)



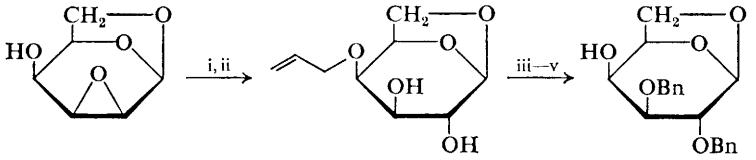
(46)



(47)

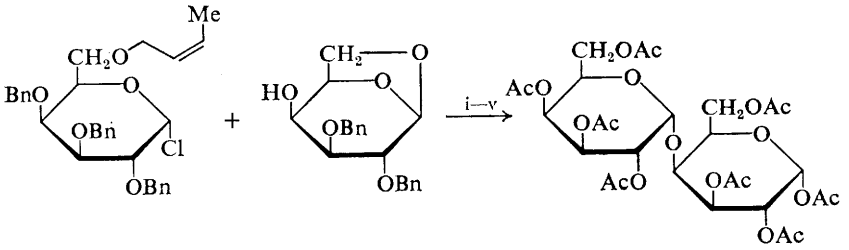
Several routes have been used to prepare 1,6-anhydro-2,3-di-*O*-benzyl-β-D-galactopyranose (*e.g.* see Scheme 14), which was condensed with 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranosyl chloride to give a disaccharide derivative that was converted into 1,2,3,6-tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-α-D-galactopyranose (Scheme 15).[180]

[178] A. Banaszek, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1975, **23**, 633 (*Chem. Abs.*, 1976, **84**, 59 933u).
[179] P. Köll, T. Schultek, and R.-W. Rennecke, *Chem. Ber.*, 1976, **109**, 337.
[180] P. A. Gent, R. Gigg, and A. A. E. Penglis, *J.C.S. Perkin I*, 1976, 1395.



Reagents: i, $CH_2{=}CHCH_2Br$–NaH–DMF; ii, HO^-; iii, BnCl–NaH–DMF; iv, Bu^tOK–DMSO; v, H_3O^+

Scheme 14



Reagents: i, $Et_4\overset{+}{N}Cl^-$–Et_3N–$ClCH_2CH_2Cl$; ii, Bu^tOK–DMSO; iii, H_2–Pd–C; iv, Ac_2O–py; v, Ac_2O–AcOH–H_2SO_4

Scheme 15

It has been shown that 1,6-anhydro-L-*glycero*-D-*manno*-heptopyranose is one of the products formed on acidic hydrolysis or methanolysis of an L-*glycero*-D-*manno*-heptose-containing polysaccharide from *Bordetella pertussis* endotoxin.[181] Alkaline treatment of 2-chlorophenyl β-maltotrioside deca-acetate afforded 1,6-anhydro-β-maltotrioside, which was isolated as the crystalline nona-acetate.[182]

Other 1,6-anhydro-sugars are referred to in Chapters 6, 7, 11, and 23.

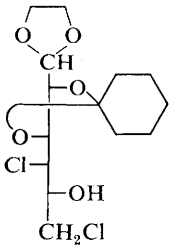
[181] R. Chaby and P. Szabó, *Carbohydrate Res.*, 1976, **49**, 489.
[182] K. Takeo, K. Mine, and T. Kuge, *Carbohydrate Res.*, 1976, **48**, 197.

5
Acetals

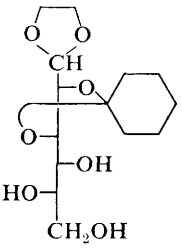
Acetals Derived from Carbohydrate Carbonyl Groups

The formation and chemistry of dithioacetals of sugars have been reviewed.[183]

4,6-Dichloro-4,6-dideoxy-D-galactose reacted with cyclohexanone ethylene acetal in the presence of an acid catalyst to give the acyclic derivative (48), which was converted into the corresponding 5-ulose.[184] Treatment with base converted (48) into the 5,6-anhydro-derivative, which was hydrolysed to give the L-idose diacetal (49), presumably by way of a 4,5-anhydro-D-glucose derivative.



(48)



(49)

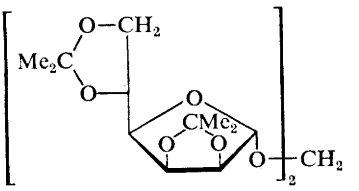
Acetals Derived from Carbohydrate Hydroxy-groups

From Single Hydroxy-groups.—The formation and hydrolysis of *O*-tetrahydrofuran-2-yl derivatives have been mentioned in Chapter 4. The β-methoxyethoxymethyl group has also been recommended for the protection of hydroxy-groups; it may be introduced using aprotic-basic or -neutral conditions and its removal can be accomplished under aprotic conditions under the influence of a mild Lewis acid (*e.g.* zinc bromide or titanium tetrachloride).[185] Pojer and Angyal have shown that suitably protected monosaccharides are transformed into methylthiomethyl ethers, with little or no competing oxidation, when treated with a mixture of acetic anhydride and DMSO containing acetic acid.[186] The methylthiomethyl protecting group can be conveniently removed with methyl iodide in moist acetone, with the addition of solid sodium hydrogen carbonate for compounds that are sensitive to acid. Methylthiomethyl groups are stable to basic or weakly acidic conditions and are reduced by Raney nickel to the

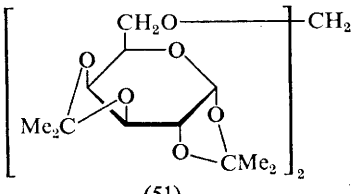
[183] J. D. Wander and D. Horton, *Adv. Carbohydrate Chem. Biochem.*, 1976, **32**, 15.
[184] H. Paulsen, H. Salzburg, and H. Redlich, *Chem. Ber.*, 1976, **109**, 3598.
[185] E. J. Corey, J.-L. Gras, and P. Ulrich, *Tetrahedron Letters*, 1976, 809.
[186] P. M. Pojer and S. J. Angyal, *Tetrahedron Letters*, 1976, 3067.

corresponding methyl ethers, thereby providing a novel preparation of methyl ethers under mildly acidic or neutral conditions.

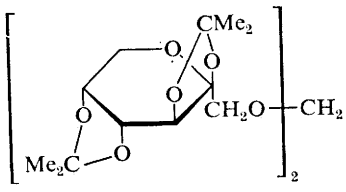
Such methylene derivatives as (50)—(52) were obtained when suitably protected monosaccharides reacted with dichloromethane in a two-phase system in the presence of a phase-transfer catalyst.[146]



(50)



(51)



(52)

From Diol Groups on Cyclic Carbohydrates.—Acid-catalysed acetonation of D-*glycero*-D-*galacto*-heptose yielded only 1,2:3,4:6,7-tri-*O*-isopropylidene-α-D-*glycero*-D-*galacto*-heptopyranose, whereas D-*glycero*-L-*gluco*- and D-*glycero*-L-*manno*-heptose reacted in the furanose form to give 1,2:5,6- (major) and 1,2:6,7-di-*O*-isopropylidene-β-D-*glycero*-L-*gluco*-heptofuranose (minor), and 2,3:5,6- (major) and 2,3:6,7-di-*O*-isopropylidene-β-D-*glycero*-L-*manno*-heptofuranose (minor), respectively.[187] Unsaturated acetal derivatives have been prepared by heating methyl D-hexopyranosides with acrolein, crotonaldehyde, and cinnamaldehyde, *etc.*, in the presence of toluene-*p*-sulphonic acid; methyl α- and β-D-glucopyranoside and methyl α-D-galactopyranoside afforded 4,6-acetals, whereas methyl α-D-mannopyranoside gave 2,3:4,6-diacetals.[188] Methyl 4,6-*O*-benzylidene-2,3-*O*-methylene-α-D-mannopyranoside was formed when the parent *cis*-2,3-diol was treated with dichloromethane in a two-phase system in the presence of a phase-transfer catalyst.[146]

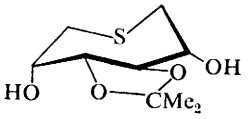
Acetalation of 1,6-anhydro-1(6)-thio-D-glucitol with acetone, formaldehyde, or benzaldehyde furnished 2,3:4,5-diacetals whose structures, after desulphurization, were established by m.s.[189] On partial hydrolysis of the 2,3:4,5-di-*O*-isopropylidene derivative, one of the isopropylidene groups was removed and the other migrated to O-3 and -4 to give the monoacetal (53).

Methyl 2,3-*O*-benzylidene-4-*O*-methyl-α-L-rhamnopyranoside (54) was converted by butyl-lithium at −30 °C into methyl 2,6-dideoxy-4-*O*-methyl-

[187] B. A. Dmitriev, A. Ya. Chernyak, O. S. Chizhov, and N. K. Kochetkov, *Carbohydrate Res.*, 1976, **47**, 25.

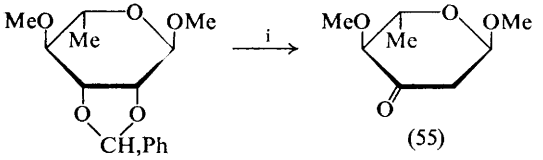
[188] Z. Jedlinski, J. Maslinska-Solich, and A. Dworak, *Uniw. Adama Michiewicza Poznaniu, Mat., Fiz., Chem.*, 1975, **18**, 227 (*Chem. Abs.*, 1976, **84**, 135 995h).

[189] J. Kuszmann, P. Sohár, and G. Horváth, *Carbohydrate Res.*, 1976, **50**, 45.



(53)

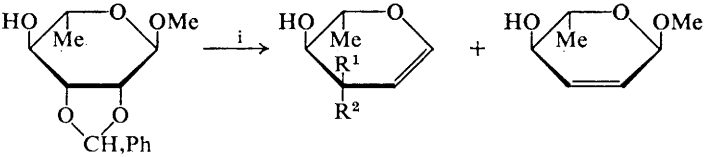
α-L-*erythro*-hexopyranosid-3-ulose (55) (Scheme 16), whereas methyl 2,3-*O*-benzylidene-α-L-rhamnopyranoside reacted only at 0 °C to give a mixture of 1,5-anhydro-3-*C*-butyl-1,2,6-trideoxy-L-*ribo*- and -L-*arabino*-hex-1-enitol and methyl 2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranoside (Scheme 17).[190] It appears



(54) (55)

Reagent: i, BuLi–C_6H_{14}

Scheme 16



R^1 = OH, R^2 = Bu
R^1 = Bu, R^2 = OH

Reagent: i, BuLi–C_6H_{14}

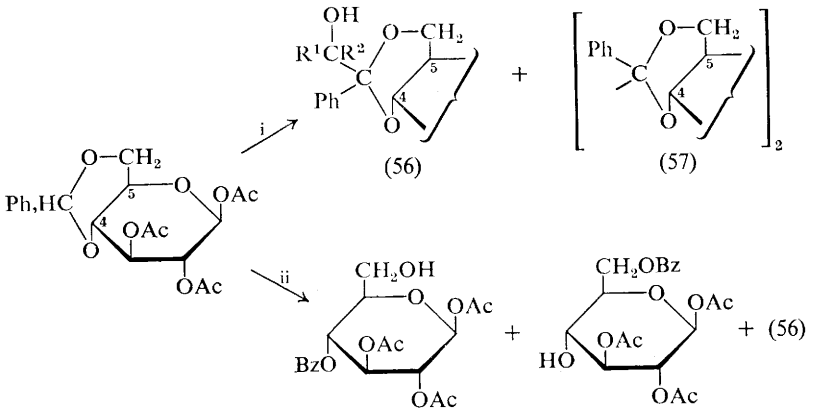
Scheme 17

that the oxyanion formed at C-4 on reaction with the reagent impedes abstraction of a second proton, necessitating more vigorous reaction conditions which lead to elimination products.

The photoreactions of 1,2,3-tri-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranose in the presence of benzophenone, acetophenone, or acetone have been studied in deoxygenated and in aerated benzene solutions at room temperature (Scheme 18).[191] Photolysis under deoxygenated conditions gave the cross-adducts (56) and the dimer (57), whereas 1,2,3-tri-*O*-acetyl-4- and -6-*O*-benzoyl-β-D-glucopyranose and (56) were obtained in the presence of oxygen.

The direction of hydrogenolytic ($LiAlH_4$–$AlCl_3$) ring-cleavage of 2,3-*O*-benzylidene acetals of hexopyranosides has been shown to depend on the configuration at the acetal carbon atom.[192] For the *exo*-isomers, the reagent attacks

[190] D. M. Clode, D. Horton, and W. Weckerle, *Carbohydrate Res.*, 1976, **49**, 305.
[191] M. Suzuki, T. Inai, and R. Matsushima, *Bull. Chem. Soc. Japan*, 1976, **49**, 1585.
[192] A. Lipták, P. Fügedi, and P. Nánási, *Carbohydrate Res.*, 1976, **51**, C19.



Reagents: i, R^1R^2CO-$h\nu$-N_2; ii, R^1R^2CO-$h\nu$-O_2

Scheme 18

mainly the axial oxygen atom of the dioxolan ring, yielding a derivative containing axial hydroxyl and equatorial *O*-benzyl groups, whereas the reagent attacks mainly the equatorial oxygen atom of the dioxolane ring of the *endo*-isomers, giving a derivative containing equatorial hydroxyl and axial *O*-benzyl groups. For example, benzyl *exo*- and *endo*-2,3-*O*-benzylidene-α-L-rhamnopyranoside gave benzyl 3- and 2-*O*-benzyl-α-L-rhamnopyranoside in the ratio of 98 : 2 and 2 : 98, respectively, on hydrogenolytic ring-cleavage. Comparable results were obtained in the hydrogenolytic ring-cleavage reactions of 3,4-*O*-benzylidenepyranosides.[192a]

1,2-*O*-Isopropylidene- and 1,2-*O*-cyclohexylidene-α-D-glucofuranose have been used as reagents in the asymmetric reduction of prochiral enones (*e.g.* 3,3,5-trimethylcyclohex-2-enone) catalysed by ruthenium complexes [$RuCl_2(PPh_3$ and $RuH_2(PPh_3)_4$].[193]

6,6′-Dichloro-6,6′-dideoxysucrose reacted with a mixture of 2,2-dimethoxypropane, DMF, and toluene-*p*-sulphonic acid to give, after acetylation, the corresponding 1′,2:3,4-di- (39%) and 1′,2-*O*-isopropylidene (37%) derivatives.[194] A similar reaction of methyl 6-chloro-6-deoxy-α-D-glucopyranoside with this reagent furnished the corresponding 2,3- and 3,4-*O*-isopropylidene derivatives in yields of 29 and 9%, respectively, after benzoylation.

From Diol Groups on Acyclic Carbohydrates.—The known tri-*O*-benzylidene derivative of D-glucitol has been assigned a 1,3(*R*):2,4(*S*):5,6(*S*)-structure, while a new diastereoisomer has been shown to differ only in the configuration at the benzylic centre [5,6(*R*)] of the dioxolan ring.[195] In addition to further work on the known 1,3(*R*):4,6(*R*)-di- and 1,3(*R*):2,5:4,6(*R*)-tri-*O*-benzylidene derivatives of D-mannitol, five new di-*O*-benzylidene derivatives have been isolated and characterized following acid-catalysed benzylidenation of D-mannitol in DMSO.[196]

[192a] A. Lipták, *Tetrahedron Letters*, 1976, 3551.
[193] G. Descotes and D. Sinou, *Tetrahedron Letters*, 1976, 4083.
[194] R. Khan, M. R. Jenner, and H. F. Jones, *Carbohydrate Res.*, 1976, **49**, 259.
[195] D. J. Brecknell, R. M. Carman, J. J. Kibby, and L. T. Nicholas, *Austral. J. Chem.*, 1976, **29**, 1859.
[196] D. J. Brecknell, R. M. Carman, and J. J. Kibby, *Austral. J. Chem.*, 1976, **29**, 1749.

Acid-catalysed dibutylidenation of 1-deoxy- and 3-*O*-methyl-D-glucitol under conditions of thermodynamic control yielded the 2,4:5,6-diacetals as the principal products, whereas 2-deoxy-D-*arabino*-hexitol (*i.e.* 2-deoxy-D-glucitol) gave mainly the 1,3:4,6-diacetal.[197]

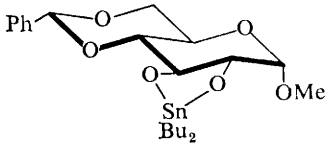
[197] T. G. Bonner, D. Lewis, and L. Yüceer, *Carbohydrate Res.*, 1976, **49**, 119.

6
Esters

Carboxylic Esters

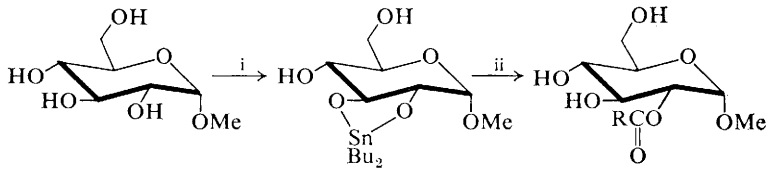
A review has appeared covering the selective esterification of hydroxy-groups in carbohydrates.[6]

A convenient method for preparing 2-esters of some methyl 4,6-*O*-benzylidene-α-D-hexopyranosides involved reaction of the 2,3-*O*-dibutylstannylene derivatives with an acid chloride.[198] For example, methyl 4,6-*O*-benzylidene-2,3-*O*-dibutylstannylene-α-D-glucopyranoside (58) reacted with benzoyl, myristoyl, lauroyl,



(58)

and toluene-*p*-sulphonyl chlorides in *p*-dioxan to give the corresponding 2-esters in 70—90% yield. Methyl 4,6-*O*-benzylidene-2,3-*O*-dibutylstannylene-α-D-galacto- and -allo-pyranosides were also selectively esterified at O-2, whereas the 2- and 3-esters were both obtained with methyl 4,6-*O*-benzylidene-2,3-*O*-dibutylstannylene-α-D-manno- and -β-D-gluco-pyranosides. The success of this procedure appears to depend on selective reactivity at O-2 when the tin atom of the 2,3-*O*-dibutylstannylene derivative is capable of co-ordination to the α-methoxy-group. 2-Esters (60) could also be prepared in high yield by reaction of methyl α-D-glucopyranoside with an equimolar proportion of dibutyltin oxide in methanol, to yield the 2,3-*O*-dibutylstannylene derivative (59), and then with an acid chloride in the presence of triethylamine (Scheme 19). Other examples of



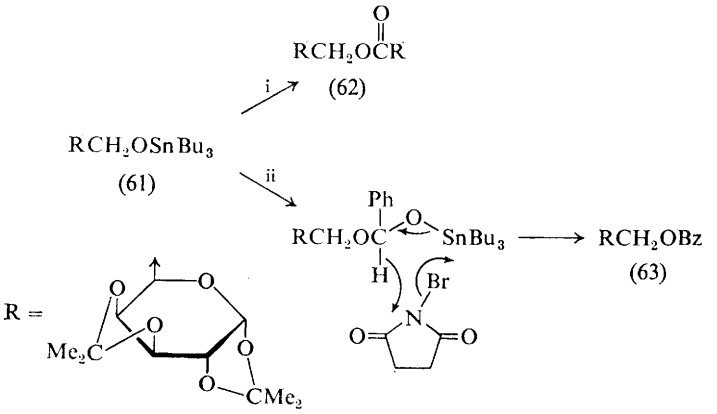
(59) (60)

Reagents: i, Bu_2SnO–MeOH; ii, RCOCl–Et_3N

Scheme 19

[198] R. M. Munavu and H. H. Szmant, *J. Org. Chem.*, 1976, **41**, 1832.

the formation and reactions of *O*-dibutylstannylene derivatives are referred to in Chapter 18.[199–201] Tributylstannyl ethers such as (61) reacted with NBS in dry carbon tetrachloride to give the dimeric ester (62), or with NBS in the presence of benzaldehyde to give the 6-benzoate (63) (Scheme 20).[202] Similarly, the



Reagents: i, NBS–CCl_4; ii, PhCHO–NBS–CCl_4

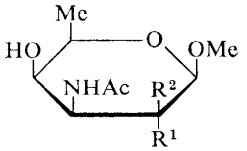
Scheme 20

reaction of 1,2:5,6-di-*O*-isopropylidene-3-*O*-tributylstannyl-α-D-glucofuranose with NBS and n-hexanal furnished the corresponding 3-hexanoate in high yield.

The products obtained on selective benzoylation of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside with either *N*-benzoylimidazole or benzoyl chloride depend on the solvent used – the principal products in chloroform are the 2- and 3-benzoates, whereas in either pyridine or acetone a significant proportion of the 2,3-dibenzoate is also formed.[203] It is suggested that the observed solvent effects might be related to the hydrogen-bonding ability of the solvent. Selective dimolar benzoylation of methyl β-L-arabinopyranoside with benzoyl cyanide in DMF gave mainly the 2,4-dibenzoate.[204]

Partial acetylation of methyl 3-acetamido-3,6-dideoxy-β-D-galactopyranoside (64) in pyridine with either acetic anhydride or acetyl chloride gave mixtures of the 2-, 4-, and 2,4-di-acetates, and that of the isomeric D-talopyranoside (65) gave the corresponding 2,4-diacetate and predominantly, or exclusively, the 2-acetate.[205] The relative reactivities of the hydroxy-groups of (64), (65), and the corresponding 2- and 4-acetates were calculated and the ratios obtained showed that the relative reactivity of an hydroxy-group in the galactopyranoside (64) alters when the other hydroxy-group is acetylated. For example, HO-4 of (64) is more reactive than HO-2 towards acetylation with acetyl chloride in pyridine,

[199] C. Augé, S. David, and A. Veyrières, *J.C.S. Chem. Comm.*, 1976, 375.
[200] M. A. Nashed and L. Anderson, *Tetrahedron Letters*, 1976, 3503.
[201] A. J. Crowe and P. J. Smith, *J. Organometallic Chem.*, 1976, **110**, C57.
[202] T. Ogawa and M. Matsui, *J. Amer. Chem. Soc.*, 1976, **98**, 1629.
[203] J. Staněk, jun. and J. Jarý, *Annalen*, 1976, 163.
[204] S. A. Abbas, A. H. Haines, and A. G. Wells, *J.C.S. Perkin I*, 1976, 1351.
[205] J. Staněk, jun., K. Čapek, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1975, **40**, 3698.



(64) R^1 = OH; R^2 = H
(65) R^1 = H; R^2 = OH

whereas the relative reactivities of HO-4 and -2 in the 2- and 4-acetates, respectively, are reversed.

Ammonolysis of 1,2,2′,3′,4′,6,6′-hepta-*O*-benzoyl-β-cellobiose afforded both cellobiose (*ca.* 40%) and 6-*O*-benzoylcellobiose (59%);[206] similar results have previously been observed with the corresponding β-maltose and β-lactose heptabenzoates, signifying that a benzoyl group at O-3 is required for the migration reaction that leads to formation of nitrogen-containing disaccharide derivatives. The 2′-benzoate (48%) was obtained as the principal product when 1,6-anhydro-4′,6′-*O*-benzylidene-β-maltose reacted with two molar equivalents of benzoyl chloride in pyridine,[207] and the 2,4,5-trichlorophenoxyacetylation of sucrose has been shown to occur preferentially at positions 1′, 6, and 6′.[207a] In a re-investigation of the conversion of β-maltose octa-acetate into 1,2′,3,3′,4′,6,6′-hepta-*O*-acetyl-β-maltose, *via* the corresponding 2-trichloroacetyl β-chloride derivative, the intermediates have been isolated in crystalline form and fully characterized.[208]

Insoluble polymers containing pendant trityl chloride groups have been used to block one of the primary hydroxy-groups of symmetrical αω-diols [163, 209] and simple polyhydroxy-compounds.[163] Acetylation or benzoylation followed by acid cleavage from the polymer yielded monoesterified products from the diols, although acyl migration occurred on similar treatment of some polyhydroxy-compounds.

Acyl migration and intramolecular aminolysis have been observed on hydrogenolysis of protecting groups in the synthesis of D-glucopyranosyl esters of aspartic [210] and glutamic acids.[211]

The regioselective 2′-*O*-debenzoylation of fully benzoylated purine and pyrimidine ribonucleosides using hydrazine hydrate is mentioned in Chapter 21.[212]

Benzoyl cyanide in the presence of triethylamine has been used for the rapid (*ca.* 30 min) perbenzoylation of aldoses,[213] and benzoyltetrazole has been shown to be an excellent benzoylating reagent for the hydroxy-groups of most common nucleosides;[214] some selectivity between primary and secondary hydroxy-groups could be achieved. The allylic hydroxy-group of several unsaturated pyranosides

[206] I. M. Vazquez, I. M. E. Thiel, and J. O. Deferrari, *Carbohydrate Res.*, 1976, **47**, 241.
[207] M. Mori, M. Haga, and S. Tejima, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 1173.
[207a] J. Arct and Z. Eckstein, *Roczniki Chem.*, 1976, **50**, 1883.
[208] K. Takeo, *Carbohydrate Res.*, 1976, **48**, 290.
[209] T. M. Fyles and C. C. Leznoff, *Canad. J. Chem.*, 1976, **54**, 935.
[210] S. Valenteković and D. Keglević, *Carbohydrate Res.*, 1976, **47**, 35.
[211] D. Keglević, J. Horvat, and F. Plavšić, *Carbohydrate Res.*, 1976, **47**, 49.
[212] Y. Ishido, N. Nakazaki, and N. Sakairi, *J.C.S. Chem. Comm.*, 1976, 832.
[213] A. Holy, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 251.
[214] J. Stawinski, T. Hozumi, and S. A. Narang, *J.C.S. Chem. Comm.*, 1976, 243.

(*e.g.* methyl 2,3-dideoxy-α-DL-*glycero*-pent-2-enopyranoside) reacted with benzoic acid in the presence of diethylazodicarboxylate and triphenylphosphine to give the benzoate of inverted configuration, but without allylic rearrangement.[215]

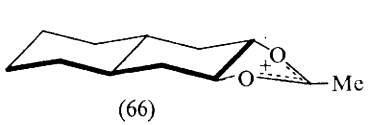
The trichloroacetyl group has been used as a temporary protecting group in the sequential synthesis of derivatives of gentio-triose and -tetraose.[71] Metalation of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose using n-butyl-lithium in THF at *ca.* −30 °C, followed by acylation with hexadecanoyl chloride, furnished a mixture of the α- and β-esters in a ratio of 9 : 1, whereas after metalation in benzene at 60 °C the ratio of the α- and β-esters obtained was 1 : 8.[216] This sequence therefore provides a single pathway to 1-*O*-acyl-α- and -β-D-glucopyranoses. 2,5-Di-*O*-benzyl-3-deoxy-D-*threo*-pentofuranose reacted with 4-nitrobenzoyl chloride in pyridine to give a 64% yield of the α-(4-nitrobenzoate).[217]

The conversion of primary alcohols into chlorofluoroacetic esters is referred to in Chapter 7, while the formation of the unusual L-rhamnopyranosyl esters (22) and (23)[124] is dealt with in Chapter 3. The advantages of using trifluoroacetyl derivatives in the m.s. of methyl hexopyranosides is referred to in Chapter 24.[218]

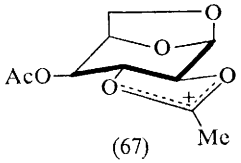
Acyloxonium Ions and Orthoesters

Several cyclic alkyl orthoacetates derived from methyl pentopyranosides have been prepared by orthoester exchange and the *endo*-*C*-methyl isomer shown to preponderate.[219] Treatment of α-acetoxyoxirans (*e.g.* methyl 4-*O*-acetyl-2,3-anhydro-β-L-lyxopyranoside) and orthoacetates with boron trifluoride and then with either lithium borohydride or diborane yielded ethylidene acetals in which the *C*-methyl group is *endo*. The reaction of cyclic orthoacetates of methyl β-L-arabinopyranoside and some derivatives with dry acetic acid proceeded *via* an acyclic acetoxonium ion to yield only products having the L-*arabino* configuration. Paulsen and his co-workers have reported examples of five-membered acetoxonium ions fused *trans* to a six-membered ring.[220] For example, the acetoxonium ion (66) was obtained from the corresponding vicinal *trans* (*eq*,*eq*)-diacetate on treatment with trifluoromethanesulphonic acid. 2,3,4-Tri-*O*-acetyl-1,6-anhydro-β-D-idopyranose similarly gave the *trans*-fused acetoxonium ions (67) and (68), whose structures were deduced from the products obtained on hydrolysis. *cis*-Fused acetoxonium ions having the 2,3-D-*gulo*, 3,4-D-*altro*, and 2,3-D-*talo* configurations, which must have arisen by way of boat conformations, were also identified. An *X*-ray analysis of the dioxolane salt (69) has shown that the ring is planar, while electron spectroscopy and ^{13}C n.m.r. measurements on the hexachloroantimonate salt of the acetoxonium ion (70) have provided information on the charge distribution, which indicated that the carbocation (71) contributes to the structure.[221] The reactions of acetylated pentopyranosyl bromides and related compounds with dibromomethyl methyl ether and zinc

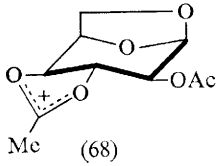
[215] G. Grynkiewicz and H. Burzyńska, *Tetrahedron*, 1976, **32**, 2109.
[216] P. E. Pfeffer, E. S. Rothman, and G. G. Moore, *J. Org. Chem.*, 1976, **41**, 2925.
[217] T. D. Audichya, *Indian J. Chem.*, 1976, **14B**, 111 (*Chem. Abs.*, 1976, **85**, 177 809b).
[218] S. Ando, T. Ariga, and T. Yamakawa, *Bull. Chem. Soc. Japan*, 1976, **49**, 1335.
[219] J. G. Buchanan and A. R. Edgar, *Carbohydrate Res.*, 1976, **49**, 289.
[220] H. Paulsen, H. Höhne, and P. L. Durette, *Chem. Ber.*, 1976, **109**, 597.
[221] H. Paulsen and R. Dammeyer, *Chem. Ber.*, 1976, **109**, 1837.



(66)



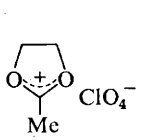
(67)



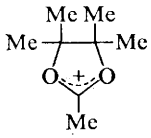
(68)

bromide, which involve the formation of intermediate acetoxonium ions, are discussed in Chapter 7.[222]

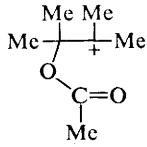
Steroidal orthoesters (72) have been prepared in good yield from the 1,2-(t-butyl orthoester) (73),[223] which also reacted with carboxylic acids in



(69)



(70)



(71)

chlorobenzene to give 1-*O*-acyl-β-D-glucopyranose tetra-acetates.[129] The reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with one equivalent of tributylstannyl methoxide in the presence of tetraethylammonium bromide afforded the *endo*-*C*-methyl 1,2-orthoacetate in high yield – and the procedure was also used to prepare 1,2-orthoesters from more complex tributylstannyl alkoxides – which can be transformed subsequently into 1,2-*trans*-glycosides.[60]

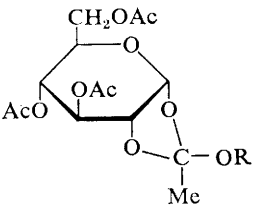
The conversion of adenosine into a 2′,3′-*O*-alkoxymethylene derivative is noted in Chapter 21,[224] and the formation of the thio-orthoester (24) from methyl (1,2,3,4-tetra-*O*-acetyl-α-D-glucopyranosyl)uronate is mentioned in Chapter 3.[128]

Phosphates

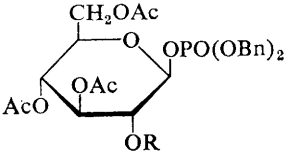
Phosphates and phosphonates of biochemical interest [225] and the forms adopted by ketose phosphates in aqueous solution [226] have been discussed. The pK_a values of each of the six phosphoric monoester groups of *myo*-inositol hexaphosphate (phytic acid) have been determined by the use of ^{31}P n.m.r. spectroscopy.[227] The conformation adopted by *myo*-inositol hexaphosphate in basic and neutral solutions was also discussed.

[222] K. Bock, C. Pedersen, and P. Rasmussen, *Acta Chem. Scand.* (*B*), 1976, **30**, 172.
[223] N. I. Uvarova, N. F. Samoshina, G. I. Oshitok, and G. B. Elyakov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1907 (*Chem. Abs.*, 1976, **84**, 59 906n).
[224] D. E. Gibbs and J. G. Verkade, *Synthetic Comm.*, 1976, **6**, 103.
[225] D. W. Hutchinson, *Organophosphorus Chem.*, 1976, **7**, 131 (*Chem. Abs.*, 1976, **85**, 143 365p).
[226] G. R. Gray, *Accounts Chem. Res.*, 1976, **9**, 418.
[227] A. J. R. Costello, T. Glonek, and T. C. Myers, *Carbohydrate Res.*, 1976, **46**, 159.

Procedures for the preparation of α-D-glucopyranose 1-phosphate from potato starch [227a] and for the synthesis of 4,6-dideoxy-α- and -β-L-*lyxo*-hexopyranose 1-phosphates [228] and 2,6-dideoxy-α-L-*arabino*-hexopyranose 1-phosphate [229] have been described. The orthoesters (73) and (74) were stereospecifically



(72) R = cholesteryl, *etc.*
(73) R = CMe_3
(74) R = Me, Et, Pr^i, or Ph



(75) R = H or Ac

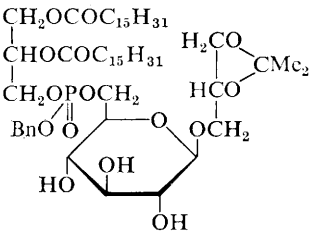
phosphorylated using dibenzyl phosphate to give the β-ester (75); analogous 1-(diphenyl phosphates) were similarly prepared.[230] Catalytic hydrogenolysis of these esters and deacetylation gave β-D-glucopyranose 1-phosphate. An identical route was used to prepare β-D-galactopyranose and α-D-mannopyranose 1-phosphates.[231] Some 1-(phthalimidoalkyl phosphate) derivatives of D-glucopyranose were obtained when the 1,2-(t-butyl orthoester) (73) was treated with an appropriate phthalimidophenyl phosphate.[232]

The dimerization of D-erythrose 4-phosphate in aqueous solution to form a cyclic bis-hemiacetal has been discussed.[233] 1,2-*O*-Cyclohexylidene- and 1,2-*O*-isopropylidene-α-D-glucofuranose reacted with hexa-alkylphosphortriamides to give the corresponding 3,5,6-phosphites, which afforded 1,2-substituted 6-deoxy-6-halogeno-α-D-glucofuranose 3,5-phosphorohalogenidates on treatment with either chlorine or bromine.[234] Replacement of halogen at phosphorus by hydroxy- and amino-groups, and the isomerization of phosphorohalogenidates in DMF were also investigated. The relative stabilities toward hydrolysis of the cyclic phosphate groups in ribonucleoside 3′,5′-phosphates and methyl α-D-glucopyranoside 4,6-phosphate have been discussed in the introduction to a paper which reports the crystal structure of the cyclohexylammonium salt of the pyranoside cyclic phosphate.[235]

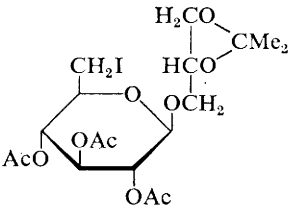
[227a] G. V. Silonova and N. O. Lisovskaya, *Metody. Sovrem. Biokhim.*, 1975, 67 (*Chem. Abs.*, 1976, **84**, 31 321u).
[228] V. N. Shibaev, Yu. Yu. Kusov, V. A. Petrenko, and N. K. Kochetkov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 887 (*Chem. Abs.*, 1976, **85**, 108 900n).
[229] V. N. Shibaev, Yu. Yu. Kusov, and V. A. Petrenko, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1843 (*Chem. Abs.*, 1976, **84**, 31 319z).
[230] L. L. Danilov, L. V. Volkova, and R. P. Evstigneeva, *Zhur. obshchei Khim.*, 1975, **45**, 2307 (*Chem. Abs.*, 1976, **84**, 105 919n).
[231] L. L. Danilov, L. V. Volkova, V. A. Bondarenko, and R. P. Evstigneeva, *Bioorg. Khim.*, 1975, **1**, 905 (*Chem. Abs.*, 1976, **84**, 105 921p).
[232] M. A. Grum-Grzhimailo, L. V. Volkova, and R. P. Evstigneeva, *Zhur. obshchei Khim.*, 1976, **46**, 1386 (*Chem. Abs.*, 1976, **85**, 143 372p).
[233] P. F. Blackmore, J. F. Williams, and J. K. Macleod, *F.E.B.S. Letters*, 1976, **64**, 222.
[234] N. K. Kochetkov, E. E. Nifant'ev, M. P. Koroteev, Z. K. Zhane, and A. A. Borisenko, *Carbohydrate Res.*, 1976, **47**, 221.
[235] C. L. Coulter, *J. Amer. Chem. Soc.*, 1976, **98**, 4997.

The preparation of carbohydrate enol phosphates, using the Perkow reaction,[236] and of uridine 2′,3′-(adamant-1-yl)phosphonates [237] are dealt with in Chapters 18 and 21, respectively.

The phosphoglycolipid (76) has been synthesized by reaction of the silver salt of 1,2-di-*O*-palmitoyl-*sn*-glycerol 3-(benzyl phosphate) with the 6-deoxy-6-iodo derivative (77), followed by deacetylation using buffered (pH 9.2) hydrazine.[238]



(76)

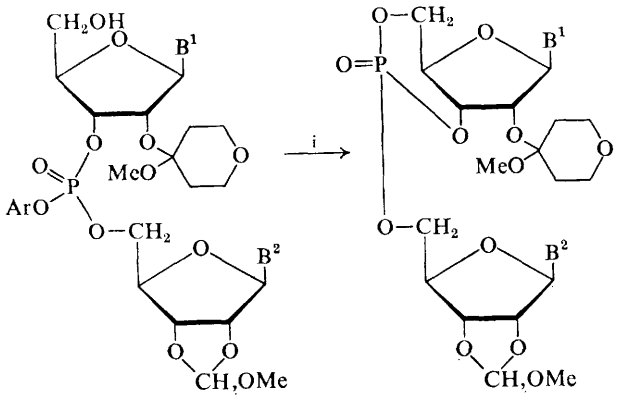


(77)

Hydrolysis with alkali has been used to establish the anomeric configuration of β-D-mannopyranosyl retinyl phosphate synthesized by rat-liver microsomes.[239] The principal phosphoglycolipids synthesized by *Nocardia asteroides* have been identified as dimannosylphosphoinositides.[240] Heavy-metal (preferably Zn^{2+}) ions catalysed the hydrolysis of the sugar-phosphate bond of mono- and oligosaccharyl dolichyl phosphates at 100 or 65 °C; the Zn^{2+}-catalysed reaction followed first-order kinetics.[241] A new procedure for the synthesis of the pyrophosphate linkage – which was used in the preparation of nucleoside dipyrophosphates from nucleoside 3′,5′-diphosphates – is mentioned in Chapter 21.[242]

The importance of protecting the terminal 3′- and 5′-hydroxy-groups of nucleotide intermediates with a base-stable (*e.g.* tetrahydropyranyl) group in the synthesis of oligoribonucleotides by the phosphotriester approach has been underlined.[243] Mild alkaline hydrolysis of 5′-ester protecting groups lead to cyclization of the intermediate 5′-hydroxy derivatives with the formation of dinucleoside 3′,5′-cyclic phosphates (Scheme 21). More drastic alkaline hydrolysis of phosphotriester intermediates with free vicinal 3′- or 5′-hydroxy-groups gave products with 3′ → 3′- and 5′ → 5′-internucleotide linkages, respectively, in addition to 3′ → 5′-internucleotide linkages. Tetrabutylammonium fluoride in THF can be used for the selective removal of the phosphate-protecting group from a phosphotriester intermediate without affecting the internucleotide linkage.[244] Cyanoethyl, 2,2,2-trichloroethyl, and phenyl groups, which are

[236] J. Thiem, D. Rasch, and H. Paulsen, *Chem. Ber.*, 1976, **109**, 3588.
[237] S. Ya. Melnik, T. P. Nedorezova, and M. N. Preobrazhenskaya, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 129.
[238] L. V. Volkova, N. G. Morozova, V. P. Sokolov, T. V. Lipatova, and R. P. Evstigneeva, *Bioorg. Khim.*, 1975, **1**, 765 (*Chem. Abs.*, 1976, **84**, 31 359n).
[239] G. C. Rosso, S. Masushige, C. D. Warren, T. C. Kiorpes, and G. Wolf, *J. Biol. Chem.*, 1976, **251**, 6465.
[240] G. K. Khuller, *Experientia*, 1976, **32**, 1371.
[241] P. Zatta, *Experientia*, 1976, **32**, 693.
[242] G. N. Bennett, G. R. Gough, and P. T. Gilham, *Biochemistry*, 1976, **15**, 4623.
[243] J. H. van Boom, P. M. J. Burgers, P. H. van Deursen, J. F. M. de Rooy, and C. B. Reese, *J.C.S. Chem. Comm.*, 1976, 167.
[244] K. K. Ogilvie, S. L. Beaucage, and D. W. Entwistle, *Tetrahedron Letters*, 1976, 1255.

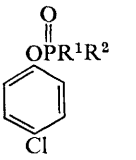


$B^1 = B^2$ = uracil-1-yl; Ar = C_6H_4Cl-*o*

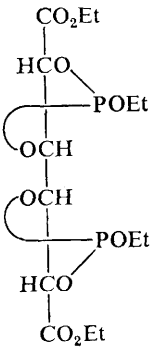
Reagent: i, 0.1M-NaOH in aqueous dioxan

Scheme 21

commonly used in nucleotide phosphotriester syntheses, were rapidly removed at room temperature, and tetrabutylammonium fluoride in THF containing glacial acetic acid could be used to remove an *O'*-alkylsilyl protecting group in the presence of 2,2,2-trichloroethyl or phenyl groups. 2,2,2-Trichloroethyl 2-chlorophenyl phosphorochloridate [245] and (78) [246] have been recommended as phosphorylating reagents for the synthesis of internucleotide linkages in the phosphotriester approach to oligonucleotides. The synthesis and hydrolysis of P^1-(nucleoside 5'-) P^1-amino-triphosphates [247] and the reaction of the bis-(cyclic phosphite) (79) with sulphur, to give the corresponding bis(thiono-phosphate),[248] have been described.



(78) $R^1 = R^2$ = triazolyl;
R^1 = triazolyl; R^2 = *N*-methylimidazolyl

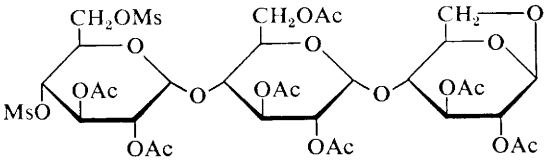


(79)

[245] J. H. van Boom, P. M. J. Burgers, and P. H. van Deursen, *Tetrahedron Letters*, 1976, 869.
[246] P. Cashion, K. Porter, T. Cadger, G. Sathe, T. Tranquilla, H. Notman, and E. Jay, *Tetrahedron Letters*, 1976, 3769.
[247] A. Simoncsits and J. Tomasz, *Tetrahedron Letters*, 1976, 3995.
[248] L. I. Gurarii and E. T. Mukmenev, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1646 (*Chem. Abs.*, 1976, **84**, 5295u).

Sulphonates

Lactose has been converted into its 3-epimer, namely 4-*O*-β-D-galactopyranosyl-D-allopyranose, by a route that involved a benzoate displacement on methyl 3-*O*-methanesulphonyl-β-lactoside hexabenzoate, which was obtained by selective hexa-*O*-benzoylation of methyl β-lactoside followed by methanesulphonylation.[12] Benzoate displacements on both sulphonate groups of 4,6-di-*O*-methanesulphonyl-αα-trehalose hexabenzoate gave α-D-glucopyranosyl α-D-galactopyranoside octabenzoate, which furnished α-D-glucopyranosyl α-D-galactopyranoside on debenzoylation,[11] while a related displacement on 2,2′,2″,3,3′,3″,6′-hepta-*O*-acetyl-1,6-anhydro-4″,6″-di-*O*-methanesulphonyl-β-maltotriose (80) was used to invert the configuration at C-4″ in a synthesis



(80)

of *O*-α-D-galactopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 4)-D-glucopyranose.[249] Details have appeared on the preparation and properties of primary and secondary 2,2,2-trifluoroethane-, pentafluorobenzene-, and trifluoromethanesulphonic esters of sugars (see Vol. 9, p. 50); trifluoromethanesulphonates (triflates) in particular were highly reactive (*cf.* toluene-*p*-sulphonates) in S_N2 displacements with, for example, pyridine and iodide ion.[250]

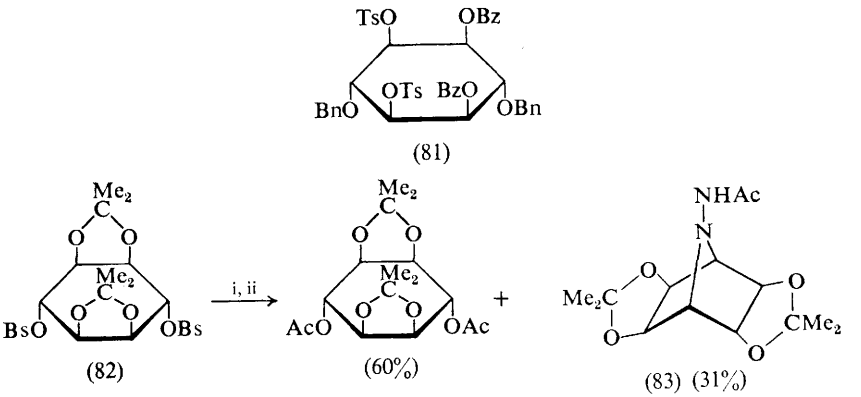
The HO-4 group of methyl 2,6-di-*O*-methanesulphonyl-α-D-glucopyranoside has been shown to be more reactive than the HO-3 group towards sulphonyl chlorides in pyridine.[251] Benzoylation of either the isolated 2,4,6-trimethanesulphonate or, more conveniently, the mixture from monomethanesulphonylation of the disulphonate gave crystalline methyl 3-*O*-benzoyl-2,4,6-tri-*O*-methanesulphonyl-α-D-glucopyranoside, although displacement of the 6-*O*-methanesulphonyloxy-group with chloride ion proved to be a troublesome side-reaction during benzoylation of the triester. Selective toluene-*p*-sulphonylation of methyl β-maltotrioside and acetylation afforded methyl 2,2′,2″,3,3′,3″,4″-hepta-*O*-acetyl-6,6′,6″-tri-*O*-toluene-*p*-sulphonyl-β-maltotrioside, which underwent nucleophilic displacements with iodide, bromide, thioacetate, chloride, and azide ions to give the 6,6′,6″-tri-iodo, -tribromo, -tri-*S*-acetyl, -trichloro, and -triazido derivatives, respectively.[252] Selective toluene-*p*-sulphonylation of the tritosylate was also investigated. An improved synthesis of 6,6′-disubstituted derivatives of sucrose has used 1′,6,6′-tris-*O*-tri-isopropylbenzenesulphonyl-(tripsyl)-sucrose as the starting material, the 6- and 6′-tripsyloxy-groups being selectively displaced.[253] Subsequent reaction of the 1′-tripsyloxy-group with sodium benzoate, under forcing conditions, effected sulphonate displacement

[249] K. Takeo, T. Matsunami, and T. Kuge, *Carbohydrate Res.*, 1976, **51**, 73.
[250] L. D. Hall and D. C. Miller, *Carbohydrate Res.*, 1976, **47**, 299.
[251] H. B. Sinclair, *Carbohydrate Res.*, 1976, **50**, 247.
[252] K. Takeo, *Carbohydrate Res.*, 1976, **51**, 85.
[253] R. G. Almquist and E. J. Reist, *Carbohydrate Res.*, 1976, **46**, 33.

and gave, after debenzoylation, sucrose derivatives substituted at C-6 and C-6′. This route was used to obtain 6,6′-diazido-6,6′-dideoxysucrose in 17% overall yield from sucrose. The preparation of 6-amino-6-deoxysucrose from 1′,6,6′-tri-*O*-tripsylsucrose is referred to in Chapter 8.[254]

Prolonged treatment of methyl β-lactoside with methanesulphonyl chloride in DMF produced mixtures of the 3,3′,4′,6,6′-pentachloride, the 3,3′,6,6′- and 3,4′,6,6′-tetrachlorides, and the 3,6,6′- and 3′,6,6′-trichlorides, chlorination at a secondary position always occurring with inversion of configuration.[255] The unexpected displacement that occurred at C-3′ of the lactoside, despite the *vic*-axial group at C-4′, was attributed to an abnormally high concentration of the 3′-*O*-formyliminium ($ROCH{=}\overset{+}{N}Me_2$) ion, which undergoes displacement with chloride ion.

The sulphonyloxy-groups in the *muco*-inositol derivative (81) resisted nucleophilic displacement (*e.g.* with sodium azide in DMF, DMSO, or HMPT), presumably due to the *vic*-axial benzyloxy group at C-3, and the reaction of the 3,6-di-(4-bromobenzenesulphonate) (82) with anhydrous hydrazine proceeded in part by S—O bond cleavage and in part by displacement of both sulphonyloxy-groups by the same nitrogen atom to yield, after acetylation, the *N*-acetamido-epimine derivative (83) (Scheme 22).[256]

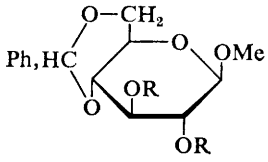


Reagents: i, N_2H_4 at 90 °C; ii, Ac_2O–py

Scheme 22

The β-D-glucopyranoside 2,3-disulphonates (84) have been reported to undergo rapid elimination to give methyl 4,6-*O*-benzylidene-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranoside in high yield by the Tipson–Cohen reaction, in contrast to a previous assertion.[257] In a general study of the reaction of sulphonic esters with magnesium halides, it was pointed out that the presence of several hydroxy-groups in a carbohydrate derivative (*e.g.* methyl 6-*O*-toluene-*p*-sulphonyl-α-D-galactopyranoside) hinders the displacement reaction and that the yield of

[254] R. G. Almquist and E. J. Reist, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 261.
[255] R. S. Bhatt, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1976, **49**, 103.
[256] K. E. Espelie and L. Anderson, *Carbohydrate Res.*, 1976, **46**, 53.
[257] T. Yamazaki, H. Sugiyama, N. Yamaoka, K. Matsuda, and S. Seto, *Carbohydrate Res.*, 1976, **50**, 279.



(84) R = Ms or Ts

the halogeno compound could be improved by prior acetylation of the hydroxy-groups.[258]

2-*O*-Methanesulphonyl-α-D-mannopyranose has been prepared and shown to give 1,6-anhydro-β-D-glucopyranose, presumably through 1,2-anhydro-α-D-glucopyranose as an intermediate, on treatment with alkali.[175]

Other Esters

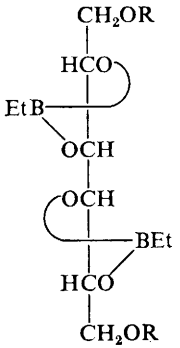
The isomeric L-fucose 2-, 3-, and 4-sulphates have been separated by chromatography on DEAE-cellulose (by elution with borate buffer) following sulphation (pyridine–sulphur trioxide) of L-fucose under optimal conditions for monosulphate formation.[259] Methyl α-L-fucopyranoside 2-(barium sulphate) was readily hydrolysed on heating with 1% acetic acid to give methyl α-L-fucopyranoside (60%) and L-fucose 2-sulphate (40%), and this class of compound appears to be more susceptible to acid hydrolysis than glycoside 3-, 4-, or 6-sulphates. Sulphation of 2-(benzyloxycarbonyl)amino-2-deoxy-D-glucose with chlorosulphonic acid gave the 4,6-disulphate, and the enzymic desulphation of this and other mono- and di-sulphated derivatives of 2-amino-2-deoxy-D-glucose and -galactose was examined.[259a] (D-Fructose 6-sulphate) 1-phosphate has been prepared by sulphation (sulphur trioxide–pyridine in DMF) of D-fructose and enzymic phosphorylation of the isolated 6-sulphate with phosphofructokinase.[260] ^{13}C N.m.r. spectroscopy has been used to determine the position of the sulphate group in each of three isomeric methyl 3-*O*-methyl-α-D-mannopyranoside sulphates.[261] The preparation of L-ascorbic acid 2-[^{35}S]sulphate is referred to in Chapter 17.[262]

Several new carbonate derivatives of methyl α- and β-D-glucopyranoside, including methyl 2- and 3-*O*-methoxycarbonyl-α- and -β-D-glucopyranosides and methyl α- and β-D-glucopyranoside 2,3-carbonates, have been obtained by standard procedures.[263] Inhibition of yeast α-D-glucosidase by methyl 2- and 3-*O*-methoxycarbonyl-α-D-glucopyranoside indicated that such compounds may be valuable as active-site-directed, irreversible inhibitors for glycosidases.

The synthesis of deoxy-sugars from thiocarbonate derivatives is noted in Chapter 13.[264]

[258] P. Place, M.-L. Roumestant, and J. Gore, *Bull. Soc. chim. France*, 1976, 169.
[259] P. F. Forrester, P. F. Lloyd, and C. H. Stuart, *Carbohydrate Res.*, 1976, **49**, 175.
[259a] A. A. Farooqui, *Experientia*, 1976, **32**, 1242.
[260] T. M. Martensen and T. E. Mansour, *Biochem. Biophys. Res. Comm.*, 1976, **69**, 844.
[261] A. I. Usov, S. V. Yarotskii, and L. K. Vasyanina, *Bioorg. Khim.*, 1975, **1**, 1583 (*Chem. Abs.*, 1976, **84**, 90 508h).
[262] R. R. Muccino, R. Markezich, G. G. Vernice, C. W. Perry, and A. A. Liebman, *Carbohydrate Res.*, 1976, **47**, 172.
[263] C. J. Gray, K. Al-Dulaimi, and S. A. Barker, *Carbohydrate Res.*, 1976, **47**, 321.
[264] D. H. R. Barton and R. Subramanian, *J.C.S. Chem. Comm.*, 1976, 867.

1,2,3,4,5,6-Hexakis-*O*-diethylborylgalactitol (obtained by reaction of galactitol with activated triethylborane) was converted into (85) on pyrolysis at 150 °C and into (86) on pyrolysis at 230 °C.[265] Selective deborylation of either (85) or (86) with methanol afforded the diol (87), which was used to prepare



(85) R = BEt_2
(86) R, R = BEt
(87) R = H

1,6-disubstituted galactitol derivatives. Similar deboronylation of D-mannitol 1,2:3,4:5,6-tris-ethylboronate gave the 3,4-ethylboronate, from which 1,2,5,6-tetrasubstituted D-mannitol derivatives were obtained.[266] Acylation of methyl β-D-xylopyranoside 2,4-ethylboronate and methyl β-L-arabinopyranoside 3,4-ethylboronate, *etc.*, followed by deboronylation, has also been used to prepare partially esterified glycosides.[267] The acetonation of monosaccharide borate or phenylboronate complexes (prepared *in situ*) has resulted in improved preparations of 2,3-*O*-isopropylidene-D-mannofuranose and 3,4-*O*-isopropylidene-L-arabinopyranose and in the synthesis of the new monoacetal 1,2-*O*-isopropylidene-β-L-arabinopyranose.[268]

The mass spectrometry of acetylated alkylboronate derivatives of monosaccharides is referred to in Chapter 24.[269]

Several pairs of *cis*- and *trans*-diols have been separated on a poly(styrylboronic acid) resin,[270] and the procedure may be of use in carbohydrate chemistry.

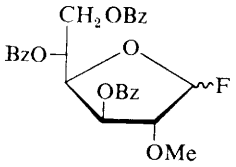
Xylitol reacted with boric acid under reduced pressure to give mixtures of boric esters and polymeric xylitol-borate complexes,[271] and a cryoscopic study of the borate complexes formed by pentaerythritol has been reported.[271a] N.m.r. spectroscopic studies have confirmed that borax complexes with HO-1 and -2 and with HO-3, -5, and -6 of D-glucose in the α-furanose form to give bidentate and terdentate complexes, respectively.[272] Only those D-glucobioses that are (1 → 3)- or (1 → 6)-linked formed complexes with borax.

[265] W. V. Dahlhoff and R. Köster, *J. Org. Chem.*, 1976, **41**, 2316.
[266] W. V. Dahlhoff, W. Schüssler, and R. Köster, *Annalen*, 1976, 387.
[267] R. Köster and W. V. Dahlhoff, *Annalen*, 1976, 1925.
[268] B. E. Stacey and B. Tierney, *Carbohydrate Res.*, 1976, **49**, 129.
[269] J. Wiecko and W. R. Sherman, *J. Amer. Chem. Soc.*, 1976, **98**, 7631.
[270] E. Seymour and J. M. J. Fréchet, *Tetrahedron Letters*, 1976, 3669.
[271] V. Grundsteins, E. Svarcs, and A. Ievins, *Latv. P.S.R. Zinat. Akad. Vestis, kim. Ser.*, 1975, 387 (*Chem. Abs.*, 1976, **84**, 59 901g).
[271a] L. A. Slashcheva, E. Svarcs, and R. G. Belousova, *Latv. P.S.R. Zinat. Akad. Vestis, kim. Ser.*, 1975, 458 (*Chem. Abs.*, 1976, **84**, 31 332y).
[272] A. de Bruyn and M. Anteunis, *Acta Cienc. Indica*, 1976, **2**, 1 (*Chem. Abs.*, 1976, **85**, 177 882v).

7
Halogenated Sugars

Glycosyl Halides

Prolonged treatment of 1,2,3,5-tetra-*O*-benzoyl-β-D-xylofuranose with anhydrous hydrogen fluoride gave, after aqueous work-up and benzoylation, principally 2,3,5-tri-*O*-benzoyl-α-D-lyxofuranosyl fluoride, whereas similar treatment of D-xylo-furanosyl and -pyranosyl and D-lyxo-furanosyl and -pyranosyl tetra-acetates yielded complex mixtures.[273] The reaction of 1,3,4,6-tetra-*O*-benzoyl-2-*O*-methyl-β-D-glucopyranose with anhydrous hydrogen fluoride yielded initially the α-glycopyranosyl fluoride, which reacted further to give, after aqueous work-up and benzoylation, the anomeric furanosyl fluorides (88).[274]



(88)

3,5,6-Tri-*O*-acetyl-2-*O*-methyl-β-D-mannofuranosyl fluoride (40%) and the corresponding α-anomer (11%) were obtained following prolonged treatment of 1,3,4,6-tetra-*O*-acetyl-2-*O*-methyl-β-D-mannopyranose with anhydrous hydrogen fluoride, aqueous work-up, and acetylation. Similar ring-contractions were observed with 1,3,4,6-tetra-*O*-acetyl-2-bromo(chloro)-2-deoxy-β-D-glucopyranose. A mixture of 1,2,3,4,6-penta-*O*-acetyl-α- and -β-D-fructofuranose gave the 1,3,4,6-tetra-*O*-acetyl-α- and -β-D-fructofuranosyl fluorides when treated briefly with anhydrous hydrogen fluoride.[275] The acetylated α- and β-D-fructofuranosyl fluorides were separated by chromatography on silica gel and appear to adopt E_2 or $^3T_2$ and ^2E or $^2T_0$ conformations, respectively, in chloroform-*d*.

α-D-Glucopyranosyl fluoride has been shown to serve as the D-glucopyranosyl donor for the glycosyltransferase from *Streptococcus mutans*, yielding a D-glucan of high molecular weight and fluoride ions.[276]

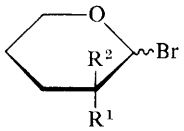
Boron trifluoride etherate catalysed the reaction of 1,2-*trans*-glycopyranose acetates (*e.g.* 1,2,3,4-tetra-*O*-acetyl-β-D-xylopyranose) with dichloromethyl

[273] K. Bock and C. Pedersen, *Acta Chem. Scand.* (*B*), 1976, **30**, 727.
[274] K. Bock and C. Pedersen, *Acta Chem. Scand.* (*B*), 1976, **30**, 777.
[275] B. Erbing and B. Lindberg, *Acta Chem. Scand.* (*B*), 1976, **30**, 12.
[276] W. R. Figures and J. R. Edwards, *Carbohydrate Res.*, 1976, **48**, 245.

methyl ether to give the corresponding 1,2-*trans*-glycopyranosyl chloride, which is formed *via* a 1,2-acetoxonium ion intermediate.[277] However, hepta-*O*-acetylamygdalin and methyl 2,2′,3,3′,4,4′,6′-hepta-*O*-acetyl-β-gentiobioside were each cleaved to give 2,2′,3,3′,4,4′,6′-hepta-*O*-acetyl-α-gentiobiosyl chloride when treated with dichloromethyl ether in the presence of zinc chloride.[111] Solutions of *O*-acetyl-α-glycosyl bromide derivatives of D-glucose, cellobiose, and lactose in HMPT were converted into the corresponding β-chlorides at room temperature by the action of lithium chloride.[278] Hydrolysis of the acetylated β-cellobiosyl and β-lactosyl chlorides gave 2-hydroxyglycosyl acetates in yields that, depending on the conditions, varied from 16 to 60%.

Reaction of β-maltotriose hendeca-acetate with phosphorus pentachloride afforded 2′,2″,3,3′,3″,4″,6,6′,6″-nona-*O*-acetyl-2-*O*-trichloroacetyl-β-maltotriosyl chloride (42%), which could be isomerized ($TiCl_4$ in chloroform) to the corresponding α-chloride.[182] Selective ammonolysis of the trichloroacetyl group in the α- and β-chlorides gave the 2-hydroxy derivatives.

Methyl (methyl 2,3,4-tri-*O*-acetyl(or methyl)-α-D-glucopyranosid)uronate reacted with dichloro(or dibromo)methyl methyl ether and zinc chloride to give the 1,2-*cis*-glycopyranosyl halides, whereas the less stable 1,2-*trans*-glycopyranosyl halides were obtained when methyl (1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosyl)-uronate was treated with dihalogenomethyl methyl ethers in the presence of boron trifluoride etherate.[279] Both *cis*- and *trans*-1,2-diacetoxycyclohexane reacted with dibromomethyl methyl ether and zinc bromide to give *trans*-2-bromocyclohexyl acetate, which is presumably formed *via* an acetoxonium ion.[222] Further reaction with the reagent yielded *trans*-1,2-dibromocyclohexane. Similar treatment of 3-acetoxy-2-bromotetrahydropyrans (89) gave the dibromo derivatives (90). These results suggest that 2,3,4-tri-*O*-acetylpentopyranosyl

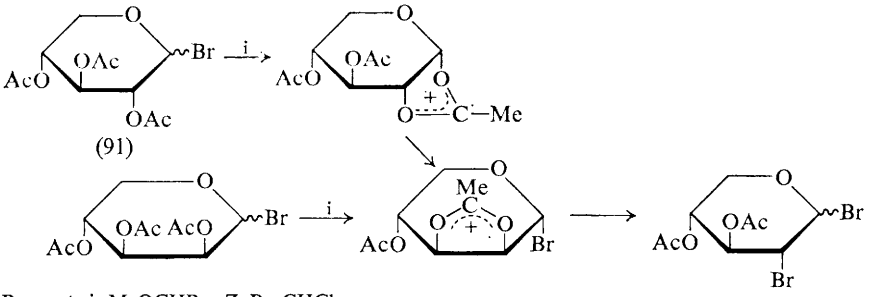


(89) R^1 = OAc; R^2 = H
(90) R^1 = H; R^2 = Br

bromides [*e.g.* (91)] react with dibromomethyl methyl ether and zinc bromide by the type of mechanism illustrated in Scheme 23.

Pedersen's group has also examined the reactions of partially benzoylated sugars with hydrogen bromide in glacial acetic acid.[280] Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside, for example, reacted with hydrogen bromide in glacial acetic acid to give 2,3,5-tri-*O*-benzoyl-6-bromo-6-deoxy-β-D-galactofuranosyl bromide, which was converted, *via* reduction of the derived 1,2,3,5-tetra-*O*-benzoyl-6-bromo-6-deoxy-β-D-galactofuranose with either palladized charcoal or zinc in acetic acid, into 1,2,3,5-tetra-*O*-benzoyl-β-D-fucofuranose and

[277] I. Farkas, I. F. Szabó, R. Bognár, and D. Anderle, *Carbohydrate Res.*, 1976, **48**, 136.
[278] W. E. Dick and D. Weisleder, *Carbohydrate Res.*, 1976, **46**, 173.
[279] P. Kováč, I. Farkas, V. Mihálov, R. Palovčík, and R. Bognár, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 57.
[280] A. Fogh, I. Lundt, and C. Pedersen, *Acta Chem. Scand.* (*B*), 1976, **30**, 624.



Reagent: i, $MeOCHBr_2$–$ZnBr$–$CHCl_3$

Scheme 23

1,2,3-tri-*O*-benzoyl-5,6-dideoxy-α-L-*arabino*-hex-5-enofuranose, respectively. Treatment of methyl 2,3,5-tri-*O*-(4-nitrobenzoyl)-β-D-ribofuranoside with hydrogen bromide in glacial acetic acid was used to prepare the corresponding β-bromide, which could be converted into 2,5-anhydro-3,4,6-tri-*O*-(4-nitrobenzoyl)-D-allononitrile with mercuric cyanide or hydrolysed to 2,3,5-tri-*O*-(4-nitrobenzoyl)-D-ribofuranose.[281] A four-step synthesis of 2,3,5-tri-*O*-(4-nitrobenzoyl)-αβ-D-xylofuranosyl bromide from methyl 3,5-*O*-isopropylidene-β-D-xylofuranoside was also described.

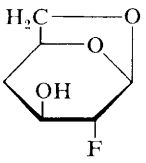
Per-*O*-benzyl-α-D-galacto- and -α-L-fuco-pyranosyl bromides have been synthesized by benzylation of the appropriate allyl glycopyranosides, which were then treated in turn with potassium t-butoxide in DMSO, mercuric oxide, 4-nitrobenzoyl chloride, and hydrogen bromide in dichloromethane.[282]

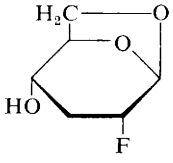
The synthesis and hydrogenolysis of glycosyl halides are also referred to in Chapters 13 and 14, respectively.

Other Halogenated Derivatives

Carbohydrate derivatives containing a $-CH_2F$ function have been obtained by the reaction of primary sulphonates (*e.g.* 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose) with potassium fluoride in ethylene glycol, although the yields were uniformly poor.[283] The reaction of the corresponding primary alcohols with the fluoramine reagent [*N*-(2-chloro-1,1,2-trifluoroethyl)-*NN*-diethylamine] generally yielded the *O*-chlorofluoroacetyl derivatives; however, 1,2:3,5-di-*O*-methylene-α-D-glucofuranose was rapidly converted into the 6-deoxy-6-fluoro derivative in 66% yield. Syntheses of methyl 2,5-dideoxy-5-fluoro-β-D-*erythro*-pentofuranoside (*via* a fluoride-ion displacement on an appropriate sulphonate) [283a] and of 1,6-anhydro-2,4-dideoxy-2-fluoro-β-D-*xylo*-hexopyranose (92) and 1,6-anhydro-2,3-dideoxy-2-fluoro-β-D-*ribo*-hexopyranose (93) (by standard transformations on 1,6-anhydro-4-*O*-benzyl-2-deoxy-2-fluoro-β-D-glucopyranose) [284] have been reported. Acetolysis of (92) and (93),

[281] H. S. El. Khadem, T. D. Audichya, D. A. Niemeyer, and J. Kloss, *Carbohydrate Res.*, 1976, **47**, 233.
[282] K. James and R. V. Stick, *Austral. J. Chem.*, 1976, **29**, 1159.
[283] L. Evelyn and L. D. Hall, *Carbohydrate Res.*, 1976, **47**, 285.
[283a] M. von Janta-Lipinski, G. Kowollik, K. Gaertner, and P. Langen, *Nucleic Acids Res., Spec. Publ.*, 1975, 1 (3rd Symp. Chem. Nucleic Acids Components, 1975, S 45).
[284] J. Doležalová, M. Černý, T. Trnka, and J. Pacák, *Coll. Czech. Chem. Comm.*, 1976, **41**, 1944.

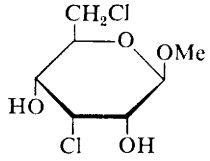

(92)

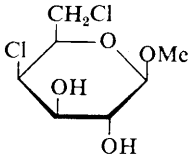

(93)

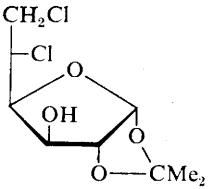
followed by deacetylation, furnished the free dideoxyfluoro-sugars. Chapters 14, 16, 21, and 23 also contain references to fluorinated sugars, while the synthesis and reactions of 1,2-substituted 6-deoxy-6-halogeno-α-D-glucofuranose 3,5-phosphorohalogenidates are noted in Chapter 6.[234]

vic-Diol groups of sugars can be transformed into chlorodeoxy acetyl groupings on reaction with acetylsalicyloyl chloride.[285] Thus the action of acetylsalicyloyl chloride on 3-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose in *p*-dioxan gave principally 3,5-di-*O*-acetyl-6-chloro-6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose. Some 1,3-diols (*e.g.* 1,2-*O*-cyclohexylidene-α-D-xylofuranose) also reacted with acetylsalicyloyl chloride to give terminal chlorodeoxy-sugars.

The regioselectivity of the reductive dechlorination of chlorodeoxy-sugars, by hydrogenation over Raney nickel in the presence of triethylamine, has been investigated by Jones's group.[286] Reductive dechlorination occurred at C-3 only of methyl 3,6-dichloro-3,6-dideoxy-β-D-allopyranoside (94), whereas methyl 4,6-dichloro-4,6-dideoxy-β-D-galactopyranoside (95) gave a mixture containing mainly methyl 4-chloro-4,6-dideoxy-β-D-galactopyranoside and some methyl 6-chloro-4,6-dideoxy-β-D-*xylo*-hexopyranoside. There was no discrimination between the primary and secondary chloro-groups of 5,6-dichloro-5,6-dideoxy-1,2-*O*-isopropylidene-β-L-idofuranose (96), which was converted into the fully


(94)


(95)

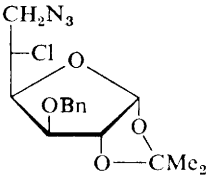

(96)

reduced derivative on reductive dechlorination. ^{13}C N.m.r. spectroscopy was used in assigning structures to various new chlorodeoxy-sugars and their reduced products.

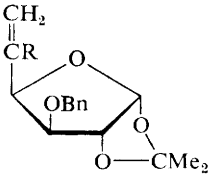
The thermal degradation of derivatives of 6-chloro-6-deoxy-D-glucopyranose (*e.g.* methyl or phenyl 6-chloro-6-deoxy-α- and -β-D-glucopyranosides) proceeded more readily than that of the parent sugars, since the hydrogen chloride liberated catalysed additional degradation.[287]

[285] A. A. Akhrem, G. V. Zaitseva, and I. A. Mikhailopulo, *Carbohydrate Res.*, 1976, **50**, 143.
[286] W. A. Szarek, A. Zamojski, A. R. Gibson, D. M. Vyas, and J. K. N. Jones, *Canad. J. Chem.*, 1976, **54**, 3783.
[287] F. Shafizadeh, Y. Z. Lai, and C. R. Nelson, *J. Appl. Polymer Sci.*, 1976, **20**, 139 (*Chem. Abs.*, 1976, **84**, 59 893f).

3-*O*-Benzyl-1,2-*O*-isopropylidene-6-*O*-toluene-*p*-sulphonyl-α-D-glucofuranose reacted with sulphuryl chloride to give the 5-chloro-5-deoxy-β-L-idofuranose derivative, which could be converted into (97) with sodium azide in DMF, or (98) with sodium iodide in acetone, or (99) with sodium methoxide.[288] An



(97)



(98) R = H
(99) R = Cl

improved chlorosulphation procedure using sulphuryl chloride has afforded 4,6-dichloro-4,6-dideoxy-α-D-galactopyranosyl 6-chloro-6-deoxy-β-D-fructofuranoside 1′,2,3,3′,4′-pentachlorosulphate from sucrose.[289]

Acetonation of 6,6′-dichloro-6,6′-dideoxysucrose with 2,2-dimethoxypropane, DMF, and toluene-*p*-sulphonic acid gave the 1′,2:3,4-di- and the 1′,2-monoacetals, which were isolated following acetylation.[194] Similar treatment of methyl 6-chloro-6-deoxy-α-D-glucopyranoside gave the corresponding 2,3- and 3,4-acetal derivatives in yields of 28% and 9%, respectively.

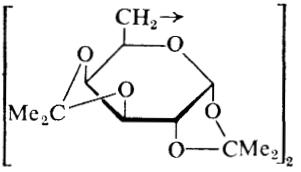
The acid-catalysed hydrolysis of methyl chlorodeoxyglycopyranosides is referred to in Chapter 3,[107a] and other references to chlorodeoxy-sugars occur in Chapters 6, 16, and 21.

Methyl 2,3-*O*-benzylidene-4-*O*-methyl-α-L-rhamnopyranoside reacted with NBS in carbon tetrachloride to give methyl 2-*O*-benzoyl-3-bromo-3,6-dideoxy-4-*O*-methyl-α-L-altropyranoside.[290] This and the reactions of other benzylidene acetals with NBS, which were used in the synthesis of rare deoxy-sugars, are covered in more detail in Chapter 13.

N-Methyldicyclohexylcarbodi-imidium iodide (prepared from DCC and methyl iodide) in refluxing THF has been used to prepare deoxyiodo-sugar derivatives (*e.g.* 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose and 5′-deoxy-5′-iodo-2′,3′-*O*-isopropylideneuridine) from the corresponding primary alcohols.[291] An attempt has been made to prepare Grignard reagents from 6-deoxy-6-iodo derivatives of sugars; however, 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose reacted with magnesium in refluxing THF to give a mixture of 1,2:3,4-di-*O*-isopropylidene-α-D-fucopyranose and the dimer (100), whereas other 6-deoxy-6-iodo derivatives afforded complex mixtures.[292]

The unusual transformation that occurs when 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose is treated with iodine and thallium(I)

[288] E. B. Rathbone and G. R. Woolard, *Carbohydrate Res.*, 1976, **48**, 143.
[289] H. Parolis, *Carbohydrate Res.*, 1976, **48**, 132.
[290] C. Monneret, J. Florent, N. Gladieux, and Q. Khuong-Huu, *Carbohydrate Res.*, 1976, **50**, 35.
[291] A. R. Gibson, D. M. Vyas, and W. A. Szarek, *Chem. and Ind.*, 1976, 67.
[292] E. I. Stout, W. M. Doane, and V. C. Trinkus, *Carbohydrate Res.*, 1976, **50**, 282.



(100)

salts is discussed in Chapter 16,[293] while reference to iodinated derivatives of nucleosides is made in Chapter 21.

[293] A. A. Akhrem, N. B. Khripach, and I. A. Mikhailopulo, *Carbohydrate Res.*, 1976, **50**, C6.

8
Amino-sugars

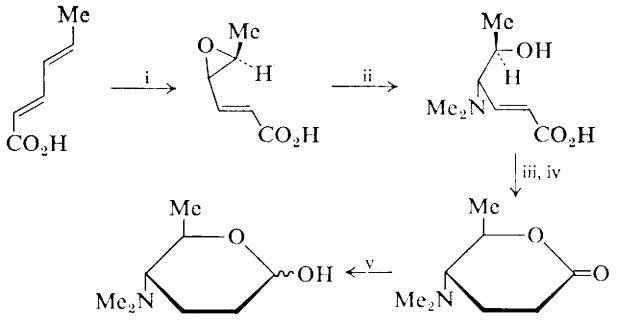
Natural Products

The D-*gluco* configuration tentatively assigned to a 2,3-diamino-2,3-dideoxyhexose isolated from the lipid A moiety of lipopolysaccharides from *Rhodopseudomonas viridis* has now been established by comparison of the c.d. spectrum of the di-*N*-acetyl derivative with that of synthetic 2,3-diacetamido-2,3-dideoxy-D-glucose.[294]

Amino-sugars found in antibiotics or introduced into nucleosides are dealt with in Chapters 20 and 21, respectively.

Synthesis

Ring-opening of oxirans has been used in the synthesis of a number of amino-sugars, including a new synthesis of D-forosamine (4-dimethylamino-2,3,4,6-tetradeoxy-D-*erythro*-hexose) (101) from sorbic acid (Scheme 24);[295] the initial



(101)

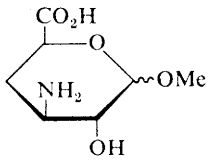
Reagents: i, $MeCO_3H$; ii, Me_2NH; iii, H_2–cat.; iv, Ac_2O; v, Bu^i_2AlH

Scheme 24

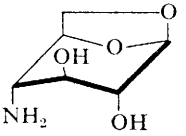
racemic oxiran was resolved prior to regioselective cleavage with dimethylamine. Jarý and his co-workers have reported syntheses, *via* oxiran intermediates, of methyl 3-acetamido-3,6-dideoxy-β-D-allo-, -altro-, and -gulo-pyranosides, thereby completing the synthesis of all the isomeric methyl 3-acetamido-3,6-dideoxy-β-D-hexopyranosides.[296] Both anomers of racemic (methyl 3-amino-3,4-dideoxy-

[294] G. Keilich, J. Roppel, and H. Mayer, *Carbohydrate Res.*, 1976, **51**, 129.
[295] I. Dyong, R. Knollmann, and N. Jersch, *Angew. Chem. Internat. Edn.*, 1976, **15**, 302.
[296] K. Čapek, J. Staněk, jun., J. Čapková, and J. Jarý, *Coll. Czech. Chem. Comm.*, 1975, **40**, 3887.

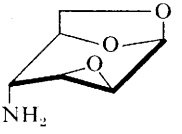
xylo-hexopyranosid)uronic acid (102) have been obtained *via* ring-opening of t-butyl (methyl 2,3-anhydro-4-deoxy-α-or -β-D-*ribo*-hexopyranosid)uronate with ammonia [297] – the D-sugar is a derivative of ezoaminuroic acid, a component of ezomycin A_2. Derivatives of racemic kanosamine (3-amino-3-deoxy-DL-glucose) have been synthesized by a route that involved ring-opening of methyl 3,4-anhydro-6-*O*-trityl-α-DL-allopyranoside with ammonia.[178] In continuing their studies on derivatives of 1,6-anhydro-sugars, Černý and Pacák have described a synthesis of 4-amino-1,6-anhydro-4-deoxy-β-D-glucopyranose (103), by way of opening of the oxiran ring of 1,6:3,4-dianhydro-2-*O*-benzyl-β-D-galactopyranose with ammonia, and have established the ease of isomerization of these *trans*-amino-oxirans [*e.g.* (104)] into *trans*-hydroxy-epimines [*e.g.* (105)] as *altro* < *gulo* < *galacto* < *manno* (104) [298] (see also Vol. 8, p. 63).



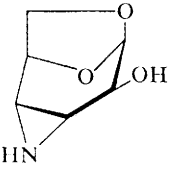
DL-(102)



(103)

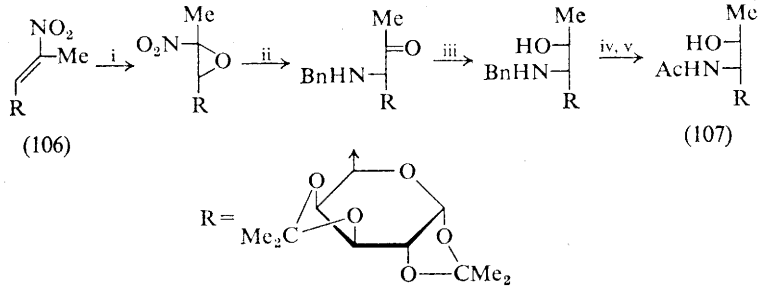


(104)



(105)

The lincosamine derivative (107) has been prepared from the unsaturated nitro-sugar (106) by the sequence of reactions shown in Scheme 25.[299] The



(106)

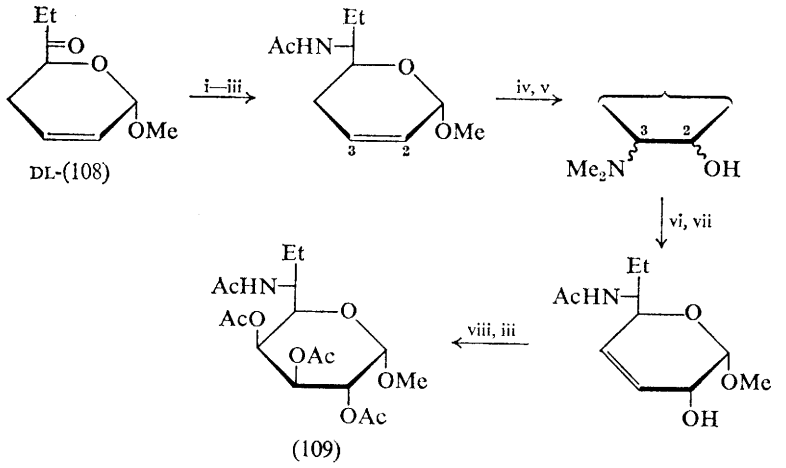
(107)

Reagents: i, H_2O_2–HO^-; ii, $BnNH_2$–DMF; iii, $NaBH_4$; iv, H_2–Pd; v, Ac_2O–MeOH

Scheme 25

various reactions proceeded with high regio- or stereo-selectivity, but an analogous sequence using azide ion, instead of benzylamine, to introduce the amino-group was less stereoselective. Total syntheses of the racemic 7-deoxy-lincosamine derivative (109) and related ring stereoisomers from the 2,6-disubstituted dihydropyran (108) are outlined in Scheme 26, although mixtures of stereoisomers were obtained at several stages of the syntheses.[300] Syntheses of lincosamine and one of its epimers are also mentioned in Chapter 16.

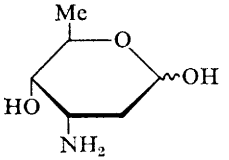
[297] J. Mieczkowski and A. Zamojski, *Bull. Acad. polon. Sci., Sér. Sci. Chim.*, 1975, **23**, 581 (*Chem. Abs.*, 1976, **84**, 31 341a).
[298] M. Černý, I. Černý, and J. Pacák, *Coll. Czech. Chem. Comm.*, 1976, **41**, 2942.
[299] G. R. Woolard, E. B. Rathbone, W. A. Szarek, and J. K. N. Jones, *J.C.S. Perkin I*, 1976, 950.
[300] A. Konowal, O. Achmatowicz, jun., and A. Zamojski, *Roczniki Chem.*, 1976, **50**, 879.



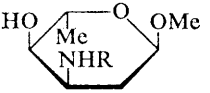
Reagents: i, NH_2OH; ii, $LiAlH_4$; iii, Ac_2O; iv, *m*-$ClC_6H_4CO_3H$; v, Me_2NH; vi, H_2O_2; vii, Δ; viii, OsO_4–H_2O_2

Scheme 26

Following their recent synthesis of daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose) from methyl α-D-mannopyranoside (see Vol. 9, p. 62), Horton and Weckerle have described a related and high-yielding route to 3-amino-2,3,6-trideoxy-D-*ribo*-hexose (110), the 5-epimer of daunosamine and the enantiomer of the naturally occurring amino-sugar ristosamine.[301] A closely related synthesis has afforded the ristosamine derivative (111), which is a key

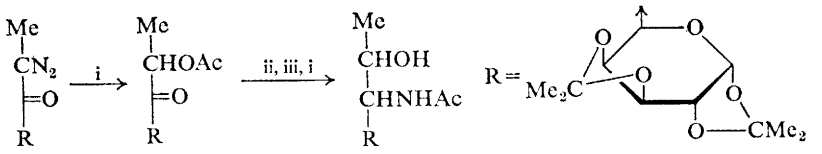


(110)



(111) R = $COCF_3$

intermediate in the synthesis of new analogues of the antitumour anthracycline antibiotics.[302] Ketoacetates have also been used in syntheses of the lincosamine derivative (107) and its three diastereoisomers at C-6 and C-7 (Scheme 27).[302a]



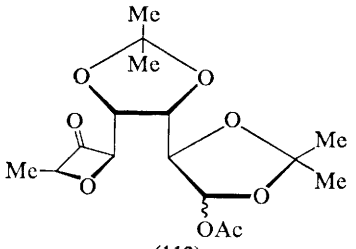
Reagents: i, Ac_2O; ii, NH_2OH; iii, $LiAlH_4$

Scheme 27

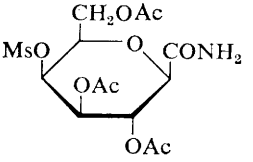
[301] D. Horton and W. Weckerle, *Carbohydrate Res.*, 1976, **46**, 227.
[302] F. Arcamone, A. Bargiotti, G. Cassinelli, S. Penco, and S. Hanessian, *Carbohydrate Res.*, 1976, **46**, C3.
[302a] S. M. David and J.-C. Fischer, *Carbohydrate Res.*, 1976, **50**, 239.

As well as yielding a mixture of epimeric ketoacetates, in which the epimer required for the synthesis of lincosamine derivatives is a minor component, acetolysis of the diazoketone also gave the 3-oxetanone (112) (19% yield), a product of pyranose-ring opening.

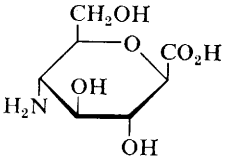
A standard displacement with azide ion, *etc.*, has been used in the conversion of 3,4,7-tri-*O*-acetyl-2,6-anhydro-5-*O*-methanesulphonyl-D-*glycero*-L-*manno*-heptonamide (113) into 5-amino-2,6-anhydro-5-deoxy-D-*glycero*-D-*gulo*-heptonic acid (114), which, after formation of the methyl ester hydrochloride, underwent polycondensation to give oligomeric or polymeric amides on treatment with methanolic sodium methoxide.[303] Both 3-acetamido-2,3,6-trideoxy-L-hexosides (116) and (117) have been synthesized by introduction of an amino-group *via* azide-displacement of the allylic acetoxy-group in the glycal derivative (115); (116) and (117) are derivatives of L-acosamine and L-ristosamine, respectively.[304]



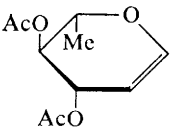
(112)



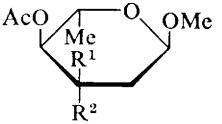
(113)



(114)



(115)



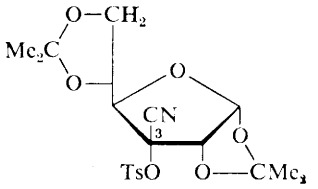
(116) R^1 = H; R^2 = NHAc
(117) R^1 = NHAc; R^2 = H

2-Amino-2,6-dideoxy-D-allose and -altrose hydrochlorides have been prepared by application of the Kuhn modification of the cyanohydrin synthesis to 5-deoxy-D-ribose, the overall yields of the two amino-sugars being roughly the same as those obtained using the nitromethane synthesis.[305]

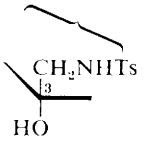
Reduction of the 3-*C*-cyano 3-sulphonate derivative (118) with lithium aluminium hydride furnished the branched-chain sugar (119) and the *spiro*-epimine (120), which, on further reduction and *N*-acetylation, gave 3-acetamido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-3-*C*-methyl-α-D-glucofuranose.[306] The latter branched-chain amino-sugar was also converted into related D-xylofuranose derivatives by a conventional chain-shortening procedure, following removal of the 5,6-*O*-isopropylidene group.

2-Amino-2,3-dideoxy-L-*ribo*-hexose (121) has been synthesized from 2-amino-2-deoxy-L-glucose by a route that did not require protection of the anomeric

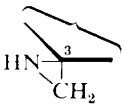
[303] E.-F. Fuchs and J. Lehmann, *Carbohydrate Res.*, 1976, **49**, 267.
[304] K. Heyns, M. Lim, and J. I. Park, *Tetrahedron Letters*, 1976, 1477.
[305] D. Horton and A. Liav, *Carbohydrate Res.*, 1976, **47**, 326.
[306] J.-M. Bourgeois, *Helv. Chim. Acta*, 1976, **59**, 2114.



(118)

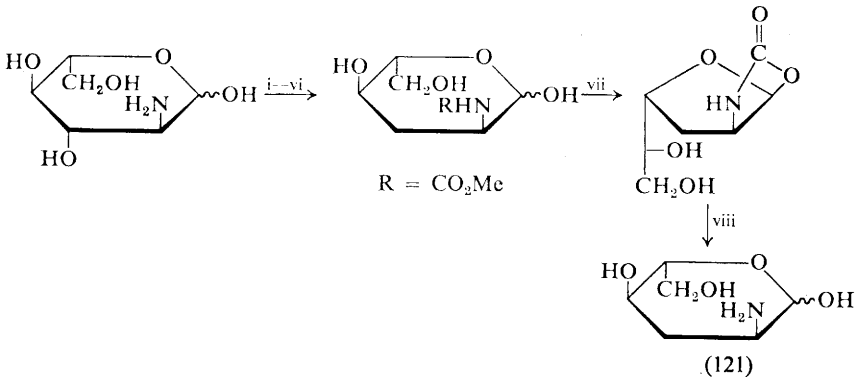


(119)



(120)

hydroxy-group (Scheme 28).[307] Solvolysis of methyl 3-acetamido-3,6-dideoxy-2-*O*-methanesulphonyl-β-D-galactopyranoside in 95% 2-methoxyethanol containing sodium acetate yielded mainly methyl 3-acetamido-3,6-dideoxy-β-D-talopyranoside, *via* an oxazoline intermediate, and a small proportion of methyl 2-acetamido-2,6-dideoxy-β-D-idopyranoside, *via* an aziridine intermediate.[205]



(121)

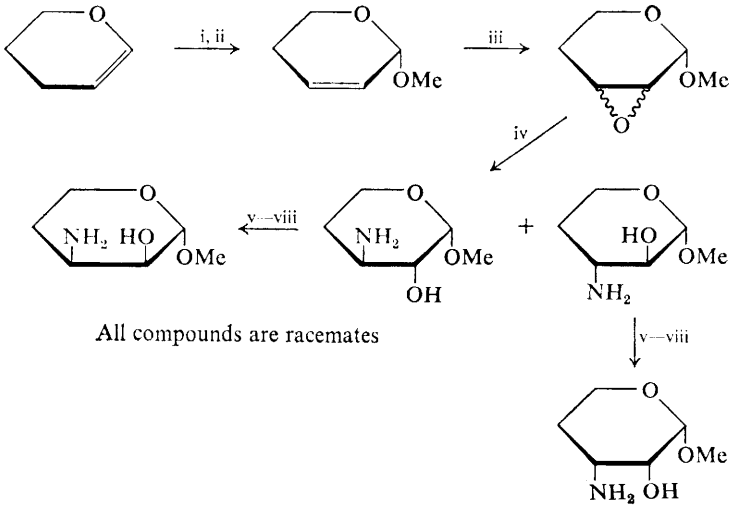
Reagents: i, $ClCO_2Me$; ii, $PhCHO-ZnCl_2$; iii, MsCl–py; iv, NaI–DMF; v, H_2–Ni; vi, $AcOH-H_2O$; vii, $Ba(OH)_2$; viii, H_3O^+

Scheme 28

Several derivatives of racemic amino-sugars have been synthesized from heterocyclic precursors. Thus, the four isomeric methyl 3-amino-3,4-dideoxy-DL-pentopyranosides have been derived from dihydro-(4*H*)-pyran, as indicated in Scheme 29,[308] furfural has been converted into derivatives of 6-acetamido-6-deoxy-DL-mannopyranose,[309] and 3-cyanopyridine has been transformed into the racemic nojirimycin (5-amino-5-deoxy-DL-glucopyranose) derivative (122) by the sequence of reactions outlined in Scheme 30.[310]

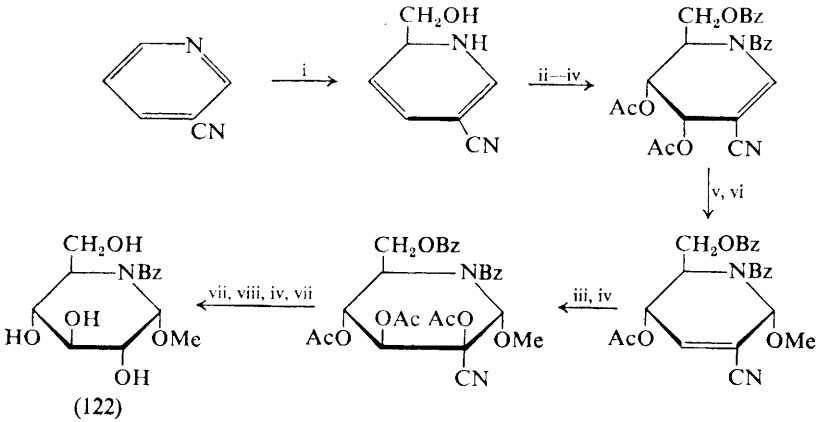
3-(β-D-Ribofuranosyl)-DL-alanine (123), an analogue of the sugar moiety of the polyoxins and a useful intermediate in the synthesis of *C*-nucleosides, has been synthesized from 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allose by modification of the classical Erlenmeyer azlactone synthesis,[311] and a number of biologically

[307] H. Sano, T. Tsuchiya, Y. Ban, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1976, **49**, 313.
[308] D. Descours, D. Anker, and H. Pacheco, *Compt. rend.*, 1976, **283**, *C*, 691.
[309] O. Achmatowicz, jun. and G. Grynkiewicz, *Roczniki Chem.*, 1976, **50**, 719.
[310] M. Natsume and M. Wada, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2567.
[311] A. Rosenthal and A. J. Brink, *Carbohydrate Res.*, 1976, **47**, 332.



Reagents: i, NBS–MeOH; ii, MeONa–MeOH; iii, H_2O_2; iv, NH_3; v, Ac_2O–MeOH; vi, MsCl–py; vii, NaOAc–H_2O; viii, NaOH

Scheme 29



(122)

Reagents: i, *hν*–MeOH; ii, BzCl–NaH–DMF; iii, OsO_4 or $KMnO_4$; iv, Ac_2O–py; v, NBS–MeOH; vi, $Bu^t_4N^+OAc^-$; vii, KOH; viii, $NaBH_4$

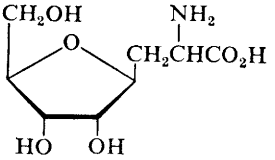
Scheme 30

active 1-amino-1-deoxy-D-fructopyranose derivatives were obtained when D-glucose reacted with amino-acids in an Amadori rearrangement.[312] 5-Methoxytryptamine was used to displace the sulphonyloxy-group of methyl 6-*O*-toluene-*p*-sulphonyl-α-D-glucopyranoside in a synthesis of methyl 6-deoxy-6-(5-methoxytryptamino)-α-D-glucopyranoside, which was isolated as its tetra-acetate.[313]

[312] B. N. Stepanenko and N. N. Borodina, *Prikl. Biokhim. i Mikrobiol.*, 1976, **12**, 5 (*Chem. Abs.*, 1976, **85**, 143 425h).

[313] O. V. Lukin, M. M. Vigdorchik, and N. N. Suvorov, *Zhur. Vsesoyuz. Khim.*, 1975, **20**, 479.

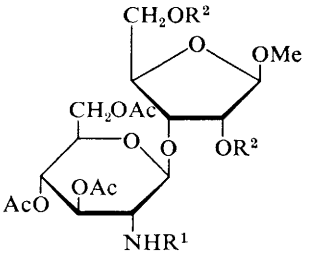
References to the synthesis of other amino-sugar derivatives occur in Chapters 3, 7, 14, and 15.



(123)

Disaccharides containing Amino-sugars

Interest in the synthesis of disaccharides containing one or two amino-sugar residues has continued during the past year. Derivatives of neobiosamine C and its β-(1 → 3)-linked isomer (124) have been prepared from the appropriate



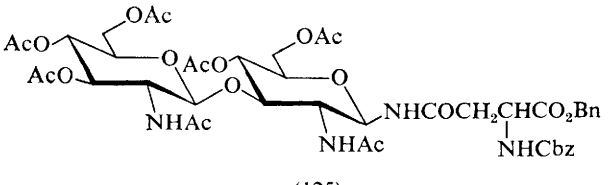
(124) R^1 = ·$C_6H_3(NO_2)_2$; R^2 = $COC_6H_4NO_2$-*p*

monosaccharide derivatives using the Koenigs–Knorr procedure.[314] Selective displacement of the 6-tripsyloxy-group in 1′,6,6′-tri-*O*-tripsylsucrose (see p. 60) with sodium azide in HMPT, *etc.*, has been used to prepare 6-amino-6-deoxysucrose.[254] α-Linked disaccharides containing residues of 2-amino-2-deoxy- and various diaminodideoxy-D-glucopyranoses have been synthesized by Koenigs–Knorr condensation of the corresponding mono- or di-azidoglycosyl β-chloride (*e.g.* 6-*O*-acetyl-2,4-diazido-3-*O*-benzyl-2,4-dideoxy-β-D-glucopyranosyl chloride) with, for example, 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose, benzyl 2,4-diazido-3-*O*-benzyl-2,4-dideoxy-β-D-glucopyranoside, and 1,6-anhydro-2,4-diazido-2,4-dideoxy-β-D-glucopyranose[315] (see also Chapter 3). The derivative (125) of 2-acetamido-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-L-aspartoyl-2-deoxy-β-D-glucopyranosylamine has been prepared by way of the oxazoline (126), since an attempt to synthesize it by a conventional Koenigs–Knorr condensation, using 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl azide, proved to be unsuccessful.[316] A high-yielding synthesis of *N*-acetyllactosamine (2-acetamido-2-deoxy-4-*O*-β-D-galactopyranosyl-α-D-glucopyranose) was based on the reaction of 3,4,6-tri-*O*-acetyl-α-D-galactopyranose 1,2-(t-butyl orthoacetate) with benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyrano-

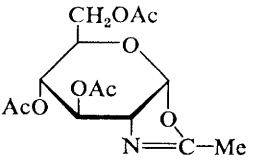
[314] H. Fukami, S. Ikeda, H. Kohno, and M. Nakajima, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 2383.

[315] H. Paulsen, O. Lockhoff, B. Schröder, B. Sumfleth, and W. Stenzel, *Tetrahedron Letters*, 1976, 2301.

[316] E. Walker-Nasir and R. W. Jeanloz, *Annalen*, 1976, 1262.



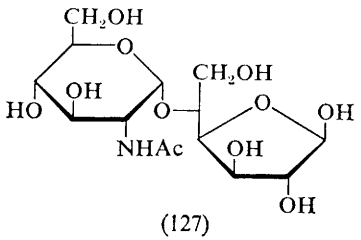
(125)



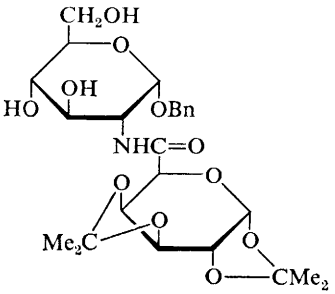
(126)

side, followed by removal of the protecting groups by deacetylation and hydrogenolysis.[317] 5-*O*-(2-Acetamido-2-deoxy-α-D-glucopyranosyl)-β-D-glucofuranose (127) has been obtained by condensation of dimeric 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-D-glucopyranosyl chloride with 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone, followed by reduction (borane) of the derived 2-benzoyloxyimino derivative, *N*-acetylation, and removal of the isopropylidene acetal.[318]

In addition to oligomers obtained from the amino-acid (114), two other reports have described the synthesis of disaccharides linked through amide bonds. Hexosaminides (*e.g.* benzyl 2-amino-2-deoxy-α-D-glucopyranoside) have been condensed directly, or in the presence of DCC, with hexuronic acid derivatives (*e.g.* 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranosyluronic acid) to give the corresponding *N*-acylamino derivatives [*e.g.* (128)],[319] and a similar



(127)



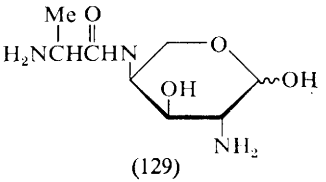
(128)

procedure was used to prepare amide-linked disaccharides [*e.g.* 2-(2-amino-2-deoxy-D-glucopyranuronyl)amino-2-deoxy-D-glucopyranose] containing an hexosaminuronic acid residue.[320]

Di- and Poly-amino-sugars

Derivatives of 2-amino-2-deoxy-D-glucose have been used as the starting materials in a new synthesis of prumycin (129) [4-(D-alanylamino)-2-amino-2,4-dideoxy-

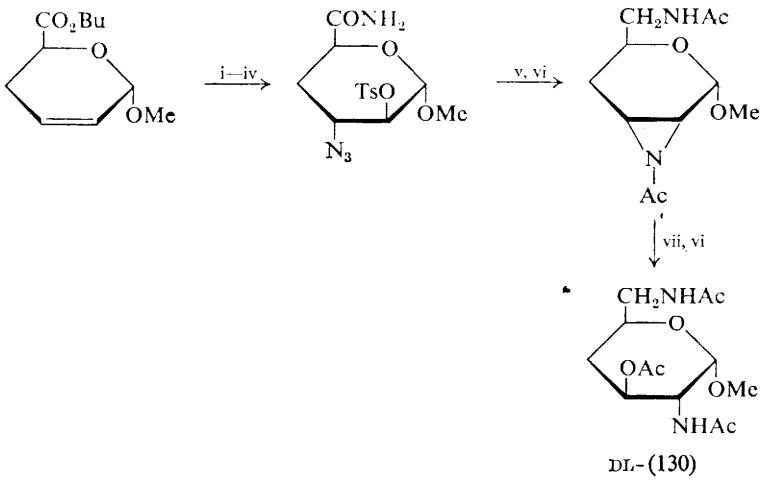
[317] J.-C. Jacquinet and P. Sinaÿ, *Carbohydrate Res.*, 1976, **46**, 138.
[318] W. A. R. van Heeswijk, P. de Haan, and J. F. G. Vliegenthart, *Carbohydrate Res.*, 1976, **48**, 187.
[319] J. Yoshimura, Y. Motoki, M. Ikeda, N. Oda, and H. Hashimoto, *Nippon Kagaku Kaishi*, 1975, 1958 (*Chem. Abs.*, 1976, **84**, 17 633r).
[320] J. Yoshimura, H. Ando, T. Sato, S. Tsuchida, and H. Hashimoto, *Bull. Chem. Soc. Japan*, 1976, **49**, 2511.



(129)

L-arabinopyranose][321] and in the synthesis of new derivatives of 2,4-diamino-2,4-dideoxy-D-glucose and -galactose.[322] Another synthesis of prumycin is reported in Chapter 20.

Epimine intermediates have been used in the synthesis of the racemic 4-deoxyneosamine C derivative (130) (Scheme 31)[323] and of the 2,3-diamino-2,3,4-trideoxy-D-*arabino*-hexopyranose derivative (131) (Scheme 32).[324] A



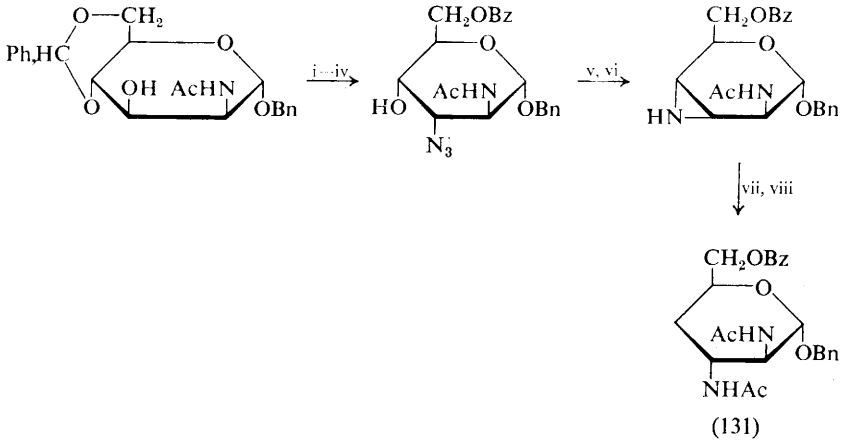
DL-(130)

Reagents: i, NH_3; ii, *m*-$ClC_6H_4CO_3H$; iii, NaN_3–$EtOCH_2CH_2OH$; iv, TsCl–py; v, $LiAlH_4$; vi, Ac_2O–py; vii, 80% AcOH

Scheme 31

reinvestigation of the apparently anomalous diequatorial ring-opening of methyl 2,3-benzoylepimino-4,6-*O*-benzylidene-2,3-dideoxy-α-D-allopyranoside (132) by sodium azide in refluxing DMF has shown that methyl 2-azido-3-benzamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-altropyranoside (133) (*trans*-diaxial ring-opening) is the principal product, although some methyl 3-azido-2-benzamido-4,6-*O*-benzylidene-2,3-dideoxy-α-D-glucopyranoside (134) (*trans*-diequatorial ring-opening) is also formed (Scheme 33).[325] The azidolysis of methyl 2,3-benzoylepimino-4,6-*O*-benzylidene-2,3-dideoxy-α-D-mannopyranoside in the presence of ammonium chloride afforded only methyl 3-azido-2-benzamido-4,6-*O*-

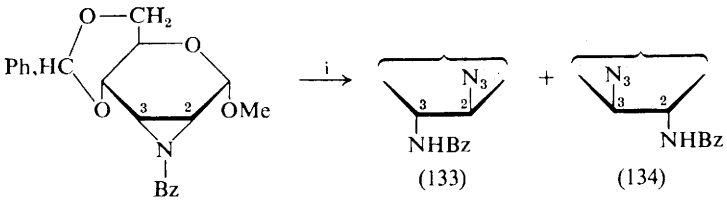
[321] A. Hasegawa, N. Aritake, and M. Kiso, *Carbohydrate Res.*, 1976, **51**, C10.
[322] A. Hasegawa and S. Kosuge, *Gifu Daigaku Nogakubu Kenkyu Hokoku*, 1975, **38**, 185 (*Chem. Abs.*, 1976, **84**, 180 509p).
[323] A. Banaszek and A. Zamojski, *Carbohydrate Res.*, 1976, **51**, 276.
[324] J. Yoshimura, M. Iwakawa, and Y. Ogura, *Bull. Chem. Soc. Japan*, 1976, **49**, 2506.
[325] R. D. Guthrie and G. J. Williams, *J.C.S. Perkin I*, 1976, 801.



(131)

Reagents: i, MsCl–py; ii, LiN_3–HMPT; iii, AcOH; iv, BzCl(1 mol.)–py; v, SO_2Cl_2; vi, Bu_3SnH; vii, H_2–Ni; viii, Ac_2O–py

Scheme 32



(132) (133) (134)

Reagent: i, NaN_3–DMF

Scheme 33

benzylidene-2,3-dideoxy-α-D-altropyranoside, resulting from *trans*-diaxial ring-opening. The conditions necessary for formation of the oxazoline on azidolysis of D-*manno*-epimine derivatives were also investigated.

Methyl 3-deoxy-3-nitro-β-D-xylofuranoside has been used as the starting material in the synthesis of the stereoisomeric methyl 2,3,5-triacetamido-2,3,5-trideoxy-β-D-pentofuranosides, a key intermediate being a 2,3-unsaturated nitro-sugar.[326] 3,4-Di-*O*-acetyl-6-azido-2,6-dideoxy-2-(2,4-dinitroanilino)-α-D-glucopyranosyl chloride, a useful intermediate in the synthesis of α-glycosides of 2,6-diamino-sugars, has been prepared from 2-deoxy-2-(4-methoxybenzylidene)amino-D-glucopyranose by a series of conventional transformations.[327]

Syntheses of derivatives of methyl 2,4-diamino-2,4,6-trideoxy-α-D-idopyranoside, methyl 2,4-diamino-2,4-dideoxy-β-L-xylopyranoside,[328] and 1,6-anhydro-2,4-diamino-2,4-dideoxy-β-D-glucopyranose[329] have been reported; conformational studies on these and related derivatives are referred to in Chapter 23.

[326] T. Takamoto, H. Tanaka, T. Kawahara, and R. Sudoh, *Chem. Letters*, 1976, 137.
[327] S. Ogawa, H. Fujimori, and T. Suami, *Bull. Chem. Soc. Japan*, 1976, **49**, 2585.
[328] H. Paulsen and H. Koebernick, *Chem. Ber.*, 1976, **109**, 90.
[329] H. Paulsen and H. Koebernick, *Chem. Ber.*, 1976, **109**, 104.

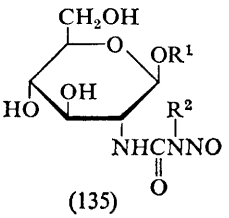
The conversion of 2,6-diamino-2,6-dideoxy-D-glucopyranose derivatives into 3- and 4-deoxy-D-*ribo*-hexopyranose analogues is mentioned in Chapter 13.

Reactions and Other Features

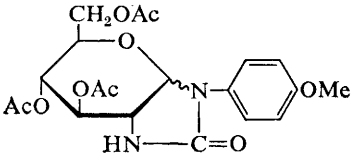
Deamination of 2-amino-1,5-anhydro-2-deoxy-D-mannitol with nitrous acid gave principally 1,5-anhydro-2-deoxy-D-*erythro*-hex-3-ulose (68%), by migration of H-3, and some 2-deoxy-D-*arabino*-hexose (8%) and 1,5-anhydro-D-glucitol (6%).[330] It was established that 2-deoxy-D-*arabino*-hexose is formed *via* a hydride-shift mechanism, rather than by acid-catalysed hydration of D-glucal produced by an elimination pathway.

Indole-3-acetic acid has been condensed with 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose to give the *N*-acyl derivative,[330a] and a series of *N*-alkyl-*N*-nitrosourea derivatives (135; R^1 or R^2 = Me, Et, Pr^n, Bu^n) of 2-amino-2-deoxy-D-glucopyranose, which are analogues of streptozotocin (135; R^1 = H, R^2 = Me), have been prepared; these analogues, particularly (135; R^1 = R^2 = Me), showed high antitumour activity.[331, 332] 1,3,4,6-Tetra-*O*-acetyl-2-amino-2-deoxy-α- and -β-D-glucopyranoses gave urea derivatives on reaction with a series of substituted-phenylisocyanates, whereas 2-amino-2-deoxy-D-glucose reacted with 4-methoxyphenylisocyanate to give, after acetylation, the heterocyclic derivative (136).[333]

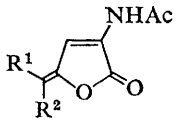
Reinvestigation of the reaction of 2-amino-2-deoxy-D-gluconic acid with hot acetic anhydride has shown that the principal products are the (*E*)- and (*Z*)-furanone derivatives (137) and the pyranone (138).[334] The crystalline



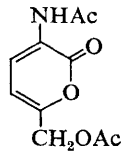
(135)



(136)



(137) R^1 = H; R^2 = CH_2OAc
R^1 = CH_2OAc; R^2 = H



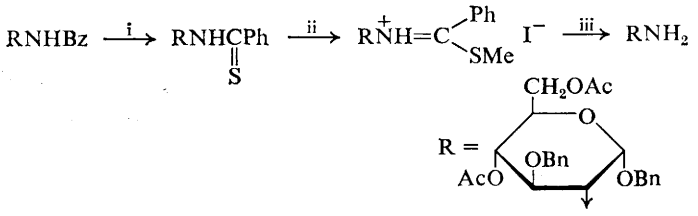
(138)

[330] J. A. Ballantine, G. Hutchinson, and J. M. Williams, *Carbohydrate Res.*, 1976, **50**, C9.
[330a] O. V. Lukin, M. M. Vigdorchik, K. F. Turchin, and N. N. Suvorov, *Zhur. org. Khim.*, 1976, **12**, 565 (*Chem. Abs.*, 1976, **85**, 33 312m).
[331] T. Machinami, K. Kobayashi, Y. Hayakawa, and T. Suami, *Bull. Chem. Soc. Japan*, 1975, **48**, 3761.
[332] M. Iwasaki, M. Ueno, K. Ninomiya, J. Sekine, Y. Nagamatsu, and G. Kimura, *J. Medicin. Chem.*, 1976, **19**, 918.
[333] N. D. Heindel, D. H. Burns, T. Honda, V. R. Risch, and L. W. Brady, *Org. Prep. Proc. Internat.*, 1975, **7**, 291 (*Chem. Abs.*, 1976, **84**, 150 865j).
[334] C. T. Clarke, J. H. Jones, and R. Walker, *J.C.S. Perkin I*, 1976, 1001.

materials described in previous literature were not (138) as reported, but were in fact either (*Z*)-(137) or mixtures of (*E*)- and (*Z*)-(137).

The *N*-acylation of 2-amino-2-deoxy-D-glucose with acetic anhydride in aqueous solvent systems has been carefully examined as a model system for the selective *N*-acylation of chitosan with carboxylic anhydrides.[335] The selective *N*-acylation of amino-sugar components of the gentamicin antibiotics is mentioned in Chapter 20.

A new method for *N*-deacetylation of carbohydrates – particularly polysaccharides containing 2-acetamido-2-deoxyhexoses – involved treatment with sodium hydroxide in aqueous DMSO at *ca.* 100 °C in the presence of sodium thiophenolate as an oxygen scavenger and catalyst.[336] The *N*-benzoyl group was selectively removed from benzyl 4,6-di-*O*-acetyl-2-benzamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside by the sequence of reactions shown in Scheme 34.[337]



Reagents: i, P_2S_5; ii, MeI; iii, 2M-HCl–THF

Scheme 34

Another key step in this synthesis, which was aimed at a hexose analogue of prumycin (see p. 62), necessitated the reduction of the azido-group of benzyl 4-azido-3-*O*-benzyl-2-(benzyloxycarbonyl)amino-2,4-dideoxy-α-D-galactopyranoside without removal of the other protecting groups. This selective reduction was accomplished by careful catalytic hydrogenation over Raney nickel (added in small portions) at room temperature.

2-Acetamido-2-deoxy-D-[2-^{2}H]glucose has been prepared by base-catalysed epimerization of 2-acetamido-2-deoxy-D-mannose in deuterium oxide, the specific labelling by deuterium at C-2 signifying the intermediacy of a 1,2-enol.[338]

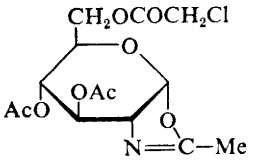
Some 4-nitrophenyl 2-acylamino-2-deoxy-β-D-glucopyranosides – in which the 2-acylamino-group contained various fatty-acid residues – have been prepared and tested as substrates for the β-D-hexosaminidase from *Hohenbuehelia serotina*.[339, 340]

Kinetic measurements have indicated that neighbouring acetamido-group participation occurs in the spontaneous hydrolysis and methanolysis of 2-carboxyphenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and in the spontaneous hydrolysis of 2,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside and

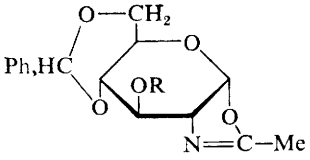
[335] S. Hirano, Y. Ohe, and H. Ono, *Carbohydrate Res.*, 1976, **47**, 315.
[336] C. Erbing, K. Granath, L. Kenne, and B. Lindberg, *Carbohydrate Res.*, 1976, **47**, C5.
[337] H. Kuzuhara, O. Mori, and S. Emoto, *Tetrahedron Letters*, 1976, 379.
[338] W. L. Salo, M. Hamari, and L. Hallcher, *Carbohydrate Res.*, 1976, **50**, 287.
[339] M. G. Vafina and N. V. Molodtsov, *Carbohydrate Res.*, 1976, **47**, 188.
[340] M. G. Vafina, A. Kim, and M. V. Molodtsov, *Khim. prirod. Soedinenii*, 1976, 379 (*Chem. Abs.*, 1976, **85**, 177 874u).

2-acetamido-2-deoxy-β-D-glucopyranosyl fluoride.[341] The methanolyses of these compounds proceeded with predominant retention of configuration, which is also consistent with neighbouring acetamido-group participation.

Syntheses of the oxazoline (139), a useful intermediate in the preparation of β-glycosides from 2-acetamido-2-deoxy-D-glucopyranose,[342] and the disaccharide oxazoline (140),[343] a compound that may prove to be useful in the synthesis of several oligosaccharides found in human milk and the blood-group substances, have been described.

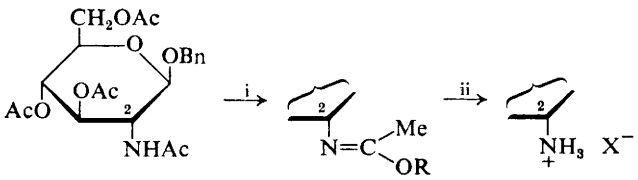


(139)



(140) R = β-D-(AcO)$_4$Gal*p*

Treatment of 2-acetamido(or benzamido)-2-deoxy-D-glucose derivatives with methyl iodide and silver oxide in the presence of silver perchlorate furnished the corresponding methyl imidate [*e.g.* (141)], which was readily hydrolysed in an acidic medium to the amine salt (Scheme 35).[344] 2,3,4-Tri-*O*-chlorosulphonyl-



(141) R = Me

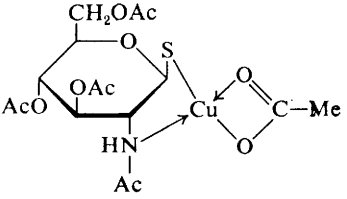
Reagents: i, MeI–Ag_2O–$AgClO_4$; ii, HX

Scheme 35

β-L-fucopyranosyl chloride also reacted with benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside in the presence of silver carbonate and a catalytic amount of silver perchlorate to give the β-L-glycopyranose imidate (141; R = 2,3,4-tri-*O*-chlorosulphonyl-β-L-fucopyranosyl), whereas the corresponding α-anomer was obtained when a large amount of silver perchlorate was used.[345] Methanolysis of the β-L-glycopyranose imidate gave mainly methyl α-L-fucopyranoside (α to β 11.5 : 1) and benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside.

The copper(II) complex (142) was obtained on treatment of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucopyranose with cupric acetate.[346]

[341] F. W. Ballardie, B. Capon, W. M. Dearie, and R. L. Foster, *Carbohydrate Res.*, 1976, **49**, 79.
[342] K. L. Matta and J. J. Barlow, *Carbohydrate Res.*, 1976, **51**, 215.
[343] C. Augé and A. Veyrières, *Carbohydrate Res.*, 1976, **46**, 293.
[344] U. Kraska, J.-R. Pougny, and P. Sinaÿ, *Carbohydrate Res.*, 1976, **50**, 181.
[345] J.-R. Pougny and P. Sinaÿ, *Carbohydrate Res.*, 1976, **47**, 69.
[346] Yu. A. Zhdanov and G. E. Levithan, *Zhur. obshchei Khim.*, 1976, **46**, 937 (*Chem. Abs.*, 1976, **85**, 177 840e).



(142)

The periodate oxidation of 9-*O*-acetyl and 4,9-di-*O*-acetyl derivatives of the methyl ester of *N*-acetyl-β-D-neuraminic acid methyl glycoside has been studied; the 9-*O*-acetyl group was found to hinder the oxidation, thus accounting for the low molar absorbancy coefficient of such derivatives in the periodate-thiobarbituric acid assay.[347] The reactivity of a seven-carbon analogue of *N*-acetylneuraminic acid, namely 5-acetamido-3,5-dideoxy-L-*arabino*-heptulosonic acid, in the thiobarbituric acid and resorcinol assays has also been evaluated and the formation of the various chromophores discussed.[348]

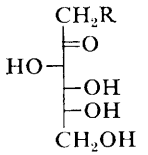
In a synthesis of *N*-acetyl-6-*O*-mycolylmuramic acid, a displacement reaction of a toluene-*p*-sulphonyloxy-group with potassium mycolate was used to introduce the mycoloyl group.[349]

Conformational free energies have been calculated for 2-amino-2-deoxy (hydrochloride) and 2-acetamido-2-deoxy derivatives of D-glucose, D-galactose, and D-mannose using empirical potential functions.[350] The most stable conformations of these amino-sugars were deduced for the α- and β-anomers in the $^4C_1$ and $^1C_4$ chair forms. The results of the calculations indicated that the increased anomeric effect in the protonated and *N*-acetylated amino-sugars in aqueous solution is mainly due to the electrostatic interaction between the positively-charged nitrogen atom at C-2 and the hydroxy-group at C-1.

A detailed investigation of the nature of the thermal decomposition of 2-deoxy-2-(2,4-dinitroanilino)-D-glucose in alkaline borate buffer has been reported.[351] However, the reason why this amino-sugar derivative, but not the isomeric 2-deoxy-2-(2,4-dinitroanilino)-D-galactose, is degraded remains somewhat elusive, although a 3,5-borate complex seems to be involved in the degradation.

^{13}C N.m.r. spectroscopy has been used to study the equilibrium existing between the isomeric forms of D-fructose (143; R = OH) and of each of the Amadori-rearrangement products (143) in [$^2H_5$]pyridine.[352] There was generally 46% of β-D-pyranose, 7% of α-D-pyranose, 30% of β-D-furanose, 12% of α-D-furanose, and 5% of the keto-form at equilibrium, the position of which varied only slightly depending on the substituent at C-1. The vacuum thermolysis of 1-deoxy-1-L-prolino-D-fructose has been examined as part of an investigation aimed at establishing the importance of Amadori compounds as precursors of

[347] J. Haverkamp, R. Schauer, M. Wember, J. P. Kamerling, and J. F. G. Vliegenthart, *Z. physiol. Chem.*, 1975, **356**, 1575 (*Chem. Abs.*, 1976, **84**, 17 673d).
[348] B. P. Peters and N. N. Aronson, jun., *Carbohydrate Res.*, 1976, **47**, 345.
[349] S. Kusumoto, S. Okada, and T. Shiba, *Tetrahedron Letters*, 1976, 4287.
[350] T. Taga and K. Osaki, *Bull. Chem. Soc. Japan*, 1975, **48**, 3250.
[351] R. O. Okotore, *Canad. J. Chem.*, 1975, **54**, 1394.
[352] W. Funcke and A. Klemer, *Carbohydrate Res.*, 1976, **50**, 9.



(143) R = MePhN, NBz_2, NHC_6H_4Me, $C_5H_{10}N$, or $NCH_2CH_2OCH_2CH_2$

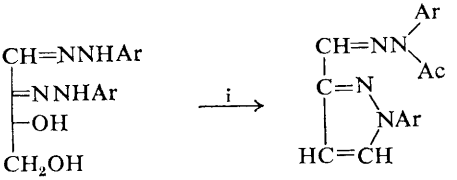
colour and aromas in foods [353] (see Vol. 4, p. 9). Twenty-two compounds, many of them heterocyclic (*e.g.* dihydrofurans, pyrones, furfurylamines, and pyrrolidines), were identified in the distillates, and degradation schemes, based on the products isolated, were proposed. A study of the mechanism of the Amadori rearrangement is referred to in Chapter 10.

A simple method for separating the anomeric ethyl 2-amino-2-deoxy-D-gluco-pyranosides by cation-exchange chromatography has been reported.[118]

[353] F. D. Mills and J. E. Hodge, *Carbohydrate Res.*, 1976, **51**, 9.

9
Hydrazones and Osazones

Aryl- and benzoyl-hydrazones of 2,4-*O*-ethylidene-D-erythrose have been prepared and acetylated.[354] D-*glycero*-Tetrulose phenyl- and 4-substituted-phenyl-osazones (prepared from D-erythrose) gave 1-aryl-3-formylpyrazole *N*-acetylarylhydrazones on boiling with acetic anhydride (Scheme 36). The

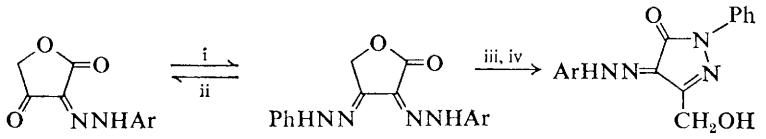


Ar = Ph or *p*-BrC_6H_4

Reagent: i, Ac_2O

Scheme 36

bis(hydrazones) (144) of 2,3-dioxo-γ-butyrolactone afforded 2-hydrazono-3-oxo-γ-butyrolactones (145) on partial hydrolysis with cupric chloride, and they rearranged in alkali to give the 1-aryl-3-hydroxymethylpyrazoline-4,5-dione 4-arylhydrazones (146) (Scheme 37).



(145) (144) (146)

Ar = Phor *p*-ClC_6H_4

Reagents: i, $PhNHNH_2$; ii, $CuCl_2$–EtOH; iii, NaOH; iv, H^+

Scheme 37

D-Glucose, D-mannose, D-galactose, and D-arabinose yielded the corresponding osazones on treatment with 8-hydrazinotheobromine.[355]

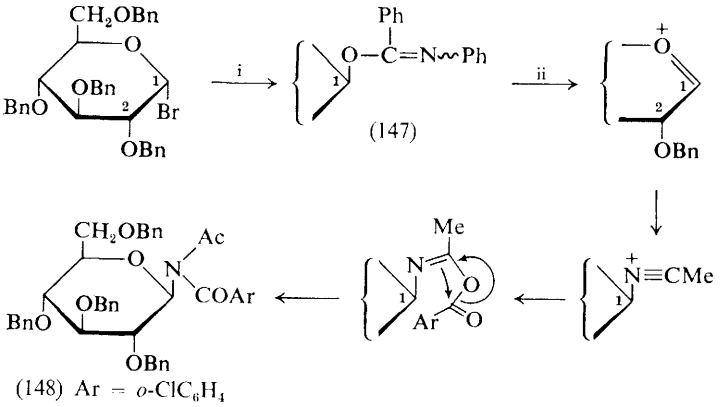
[354] H. S. El Khadem, Z. El Shafei, El S. El Ashry, and M. El Sadek, *Carbohydrate Res.*, 1976, **49**, 185.

[355] K. Dursun, M. Hadzic, and A. Hadzic, *Glas. Hem. Tehnol. Bosne Hercegovine*, 1974, **21–22**, 63 (*Chem. Abs.*, 1976, **85**, 78 288z).

10
Miscellaneous Nitrogen-containing Compounds

Glycosylamines

The reaction of the β-glycopyranosyl imidate (147) with acetonitrile and 2-chlorobenzoic acid gave the di-*N*-acylated β-glycosylamine derivative (148) stereospecifically, presumably by the reactions outlined in Scheme 38.[356] A

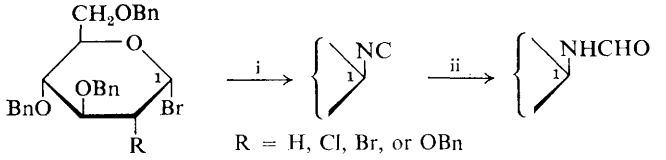


(148) Ar = *o*-ClC_6H_4

Reagents: i, PhCONHPh-Ag_2O; ii, MeCN-$ArCO_2H$

Scheme 38

number of 2-substituted 3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromides reacted with silver cyanide in either toluene or dichloromethane to give mainly, or only, the corresponding β-D-glucopyranosyl isocyanides (in 60—80% yield), which were converted into *N*-glycopyranosylformamide derivatives on hydrolysis with acid (Scheme 39).[357]



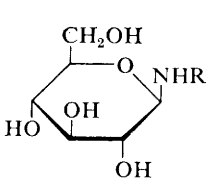
Reagents: i, AgCN; ii, H_3O^+

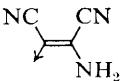
Scheme 39

[356] J. Pougny and P. Sinaÿ, *Tetrahedron Letters*, 1976, 4073.
[357] P. Boullanger and G. Descotes, *Tetrahedron Letters*, 1976, 3427.

D-Glucose reacted with diaminomaleonitrile in acetic acid to yield the *N*-(2-amino-1,2-dicyanoethenyl)-β-D-glucopyranosylamine derivative (149).[358] β-D-Glucopyranosylamine and 2-amino-2-deoxy-D-glucose (isolated as the fully acetylated derivatives) were produced in low yield on u.v.-irradiation of aqueous solutions of D-glucose and glycine or L-lysine.[359]

Treatment of per-*O*-acetylated glycosylamines with 2-chloroethyl isocyanate, followed by nitrosation (sodium nitrite in formic acid) and deacetylation, yielded a series of (2-chloroethyl)nitrosourea derivatives [*e.g.* 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (150)], which showed remarkable anti-



(149) R = 

(150) R = $CONCH_2CH_2Cl$ (NO)

tumour activity against L1210 leukaemia in mice.[360] Various per-*O*-acetylated *N*-arylglycopyranosylamines have been converted into the corresponding acetylated glycopyranosyl α-chlorides with dichloromethyl methyl ether in the presence of zinc chloride.[361]

Polish workers have continued their studies of the chemistry of *N*-arylglycopyranosylamines (see Vol. 9, pp. 71 and 72). *N*-Methyl-*N*-phenyl-β-D-xylopyranosylamine and the corresponding *N*-methyl-*N*-(4-tolyl) and *N*-(4-methoxyphenyl)-*N*-methyl derivatives have been obtained by a conventional route from 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide,[362] and a series of 2,3,5-tri-*O*-benzoyl-*N*-(4-substituted-phenyl)-β-L-arabinofuranosylamines have been prepared[363] and converted into the corresponding *N*-acetyl derivatives.[364] The reactions of D-glucose with *N*-methylaniline and *N*-methyl-*p*-anisidine have been examined by t.l.c., which indicated that the initially formed *N*-aryl-β-D-glucopyranosylamines undergo an Amadori rearrangement, both reactions being catalysed by ammonium chloride.[365] The mutarotation of *N*-(4-tolyl)-D-glucopyranosylamine in simple primary alcohols has been investigated; in general, the β-anomer was found to be more stable than the α-anomer, especially in methanol.[366] The same group has also examined the mutarotation of *N*-(3-bromophenyl)-D-glucopyranosylamine in methanol catalysed by carboxylic acids[367] and by phenols,[367a] which are better catalysts. A linear relationship

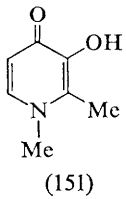
[358] M. Kuwamara, M. Ohchi, and H.-S. Koh, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1889.
[359] L. W. Doner, R. Balicki, and R. L. Whistler, *Carbohydrate Res.*, 1976, **47**, 342.
[360] T. Machinami, S. Nishiyama, K. Kikuchi, and T. Suami, *Bull. Chem. Soc. Japan*, 1975, **48**, 3763.
[361] M. M. Menyhárt, R. Bognár, and I. Farkas, *Carbohydrate Res.*, 1976, **48**, 139.
[362] S. Kolka and J. Sokolowski, *Zeszyty Nauk, Mat., Fiz., Chem.*, 1974, **3**, 107 (*Chem. Abs.*, 1976, **84**, 105 997t).
[363] J. Sokolowski and R. Walczyna, *Roczniki Chem.*, 1976, **50**, 437.
[364] J. Sokolowski and R. Walczyna, *Roczniki Chem.*, 1976, **50**, 597.
[365] S. Kolka, *Roczniki Chem.*, 1976, **50**, 611.
[366] K. Smiataczowa, N. Galicka, and T. Jasinski, *Zeszyty Nauk, Mat., Fiz., Chem.*, 1974, **3**, 101 (*Chem. Abs.*, 1976, **84**, 135 962v).
[367] K. Smiataczowa, N. Galicka, and T. Jasinski, *Zeszyty Nauk, Mat., Fiz., Chem.*, 1974, **3**, 89 (*Chem. Abs.*, 1976, **84**, 135 987g).
[367a] K. Smiataczowa, N. Galicka, and T. Jasinski, *Zeszyty Nauk, Mat., Fiz., Chem.*, 1974, **3**, 95 (*Chem. Abs.*, 1976, **84**, 135 988h).

between $\log k$ and $pK_a(H_2O)$ was established for the acids and for phenols with $pK_a < 7.3$, whereas phenols with $pK_a > 10$ behaved as basic catalysts and those with pK_a 7.3—10 exhibited no catalytic effect.

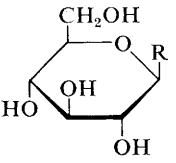
Mössbauer spectra of the ferric chloride complexes of several anomeric *N*-aryl-D-glucopyranosylamines have been reported.[368]

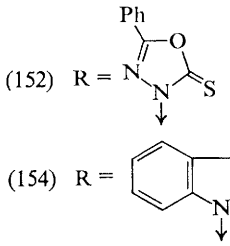
The 4-pyridone (151), an analogue of maltol, has been isolated from the reaction of lactose or maltose with a hot aqueous solution of methylammonium acetate.[369]

The heterocyclic derivative (152)[370] and the 1,2,4-triazine derivatives (153)[371] have been obtained from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide by standard routes. Bischler–Napieralski cyclization of the corresponding *N*-acetyltryptamines and dehydrogenation ($Pd–BaSO_4$) gave the β-D-glucopyranosylcarboline (154) and the related α-L-arabinopyranosylcarboline.[372] The spin-labelled nitroxide derivative (155) has been prepared by reaction of the nicotinamide derivative with 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl bromide.[373]



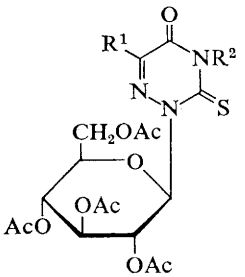
(151)



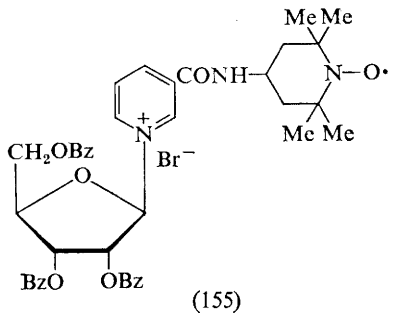


(152) R =

(154) R =



(153) R^1 = Ph or PhCH=CH;
R^2 = Ph or *p*-MeC_6H_4.

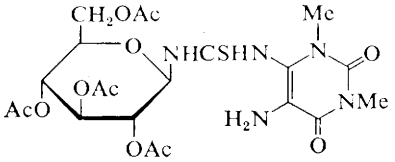


(155)

The formation of *N*-linked carbohydrate–saccharin complexes is reported in Chapter 4 (see Scheme 10).[172]

[368] N. I. Kaletina and B. N. Stepanenko, *Doklady Akad. Nauk S.S.S.R.*, 1975, **223**, 494 (*Chem. Abs.*, 1976, **84**, 5291q).
[369] T. Severin and A. Loidl, *Z. Lebensm.-Untersuch.*, 1976, **161**, 119 (*Chem. Abs.*, 1976, **85**, 143 377u).
[370] G. Wagner and B. Dietzsch, *Pharmazie*, 1976, **31**, 153 (*Chem. Abs.*, 1976, **85**, 33 352z).
[371] A. K. Mansour, Y. A. Ibrahim, and M. M. Eid, *Z. Naturforsch.*, 1976, **31b**, 505 (*Chem. Abs.*, 1976, **85**, 21 754u).
[372] V. N. Tolkachev, M. N. Preobrazhenskaya, V. A. Kudryasheva, and K. F. Turchin, *Zhur. org. Khim.*, 1976, **12**, 1080 (*Chem. Abs.*, 1976, **85**, 124 268t).
[373] N. L. Lifshits, N. I. Mal'tsev, V. A. Yakovlev, E. A. Vorontsov, and N. S. Zakharova, *Khim. geterotsikl. Soedinenii*, 1976, 352 (*Chem. Abs.*, 1976, **85**, 177 804w).

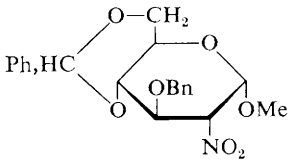
The ^{1}H n.m.r. spectra and rates of periodate oxidation of anomeric 1-*N*-D-glucopyranosylnicotinamide cations have been measured, and they indicate that the anti-anomeric effect stabilizes the conformations in which the aglycone is equatorially oriented.[374] The conformations of *N*-hexopyranosylimidazoles are discussed in Chapter 23. Modified nucleoside derivatives [*e.g.* (156)] have been synthesized by condensation of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate with either 5,6-diamino-1,3-dimethyluracil or *o*-phenylenediamine.[375]



(156)

Nitro-sugars

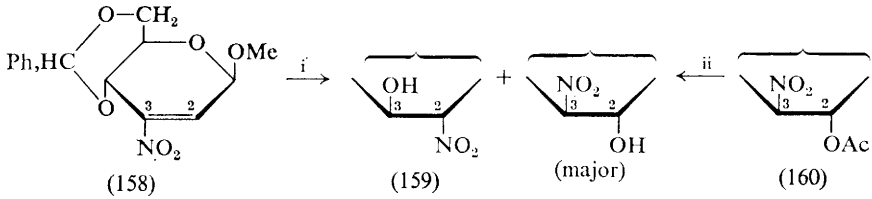
Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-nitro-α-D-glucopyranoside (157) has been prepared by oxidation of the corresponding oxime with trifluoroperacetic acid; (157) appears to adopt a twist-boat conformation ($J_{2,3} \sim 0$ Hz)



(157)

in chloroform, although methyl 4,6-di-*O*-acetyl-3-*O*-benzyl-2-deoxy-2-nitro-α-D-glucopyranoside derived from it assumes a $^4C_1$ chair conformation.[376] Baer's and Sudoh's groups have shown that methyl 4,6-*O*-benzylidene-3-deoxy-3-nitro-α- and -β-D-glucopyranosides and other nitro-sugar derivatives are converted by methanesulphonyl chloride and triethylamine in ether into nitroalkenes [*e.g.* (158)].[377, 377a] In most cases, this method appears to be superior to the customary acetylation–dehydroacetoxylation procedure, although the efficacy of the method depends on the structure and configuration of the nitrosugar, which influence the ease of formation and the stability of the intermediate methanesulphonate. Treatment of the 3-nitroalkene (158) with nitrous acid afforded some of the 2-nitro-sugar (159), which was also prepared by heterogeneous reaction of the 3-nitro-2-acetate (160) with sodium nitrite in the presence of a phase-transfer catalyst (Scheme 40).[378] The 3-acetate of the 2-nitro-sugar (159) afforded methyl 4,6-*O*-benzylidene-2,3-dideoxy-2-nitro-β-D-*erythro*-hex-2-enopyranoside on dehydroacetoxylation, and reacted with nucleophiles (*e.g.*

[374] R. Iff and M. Viscontini, *Helv. Chim. Acta*, 1976, **59**, 2892.
[375] H. Ogura, H. Takahashi, K. Takeda, M. Sakaguchi, N. Nimura, and H. Sakai, *Hukusokan Kagaku Toronkai Koen Yoshishu*, 8*th*, 1975, 154 (*Chem. Abs.*, 1976, **84**, 150 881m).
[376] T. Takamoto and R. Sudoh, *Bull. Chem. Soc. Japan*, 1975, **48**, 3413.
[377] H. H. Baer and F. F. Z. Georges, *J. Org. Chem.*, 1976, **41**, 3474.
[377a] Y. Tachimori, T. Takamoto, and R. Sudoh, *Chem. Letters*, 1976, 483.
[378] T. Sakakibara and R. Sudoh, *Carbohydrate Res.*, 1976, **50**, 197.



Reagents: i, HNO_2; ii, $NaNO_2$–$C_{16}H_{33}P^+Bu_3Br^-$

Scheme 40

ammonia, dimethylamine, or pentane-2,4-dione) to give 3-substituted derivatives that retained the D-*gluco* configuration. Other addition reactions to unsaturated nitro-sugars are dealt with in more detail in Chapter 14.

Treatment of methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-iodo-α-D-glucopyranoside with sodium nitrite and phloroglucinol gave the 6-deoxy-6-nitro-sugar, which was converted, *via* the α-bromide, into the corresponding adenin-9-yl nucleoside.[379]

Heterocyclic Derivatives

Treatment of per-*O*-acetyl-L-fucose benzoylhydrazone with a mixture of mercuric oxide, magnesium oxide, and iodine in diethyl ether furnished the oxadiazole derivative (161), although the L-*rhamno* analogue failed to cyclize,[380] and a number of bis(aroylhydrazones) prepared from 2,3,4,5-tetra-*O*-acetylgalactaryl chloride yielded the corresponding bis(oxadiazole) derivative (162) on treatment with phosphorus oxychloride.[380a] Condensation of the acetylenic sugar 1,2-dideoxy-4,5:6,7-di-*O*-isopropylidene-D-*gluco*-hept-1-ynitol (163) with phenyl azide gave mainly the 1-phenyl-1,2,3-triazole derivative (164) having the sugar chain attached at C-4, together with some of the 5-substituted derivative.[381] The D-*manno* analogue of (163) also gave 4- and 5-substituted 1-phenyl-1,2,3-triazoles on treatment with phenyl azide. The formation of pyrazine derivatives that occurs when D-glucose reacts with ammonia in strongly alkaline solution has been examined.[382]

The reaction of L-arabinose with L-cysteine or of 2,3,4,5-tetra-*O*-acetyl-*aldehydo*-L-arabinose with L-cysteine methyl ester gave mainly thiazolidine derivatives (165) having the (*R*)-configuration at C-2 of the heterocyclic ring, whereas 2,3,4,5-tetra-*O*-acetyl-*aldehydo*-L-arabinose reacted with L-cysteine to give either the (2*S*)-analogue of (165; R^1 = H, R^2 = Ac) or, when an excess of L-cysteine was used, the bicyclic lactone derivative (166).[383] Computer-assisted analysis of the ^1H n.m.r. spectra of (165) (R^1 = Me, R^2 = Ac) and its (*S*)-diastereoisomer showed that the polyacetoxyalkyl side-chain possesses a

[379] T.-S. Lin and R. E. Harmon, *Org. Prep. Proced. Internat.*, 1975, **7**, 165 (*Chem. Abs.*, 1976, **84**, 17 641s).

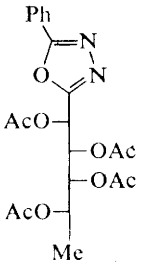
[380] M. Shaban and M. Nassr, *Org. Prep. Proced. Internat.*, 1976, **8**, 107 (*Chem. Abs.*, 1976, **85**, 177 836h).

[380a] M. Shaban and M. Nassr, *Org. Prep. Proced. Internat.*, 1976, **8**, 113 (*Chem. Abs.*, 1976, **85**, 177 837j).

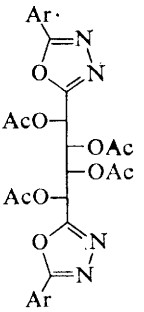
[381] D. Horton and A. Liav, *Carbohydrate Res.*, 1976, **47**, 81.

[382] H. Tsuchida, M. Komoto, H. Kato, and M. Fujimaki, *Nippon Nagei Kagaku Kaishi*, 1976, **50**, 187 (*Chem. Abs.*, 1976, **85**, 160 428d).

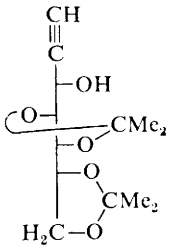
[383] R. Bognár, Z. Györgydeák, L. Szilágyi, G. Horváth, G. Czira, and L. Radics, *Annalen*, 1976, 450.



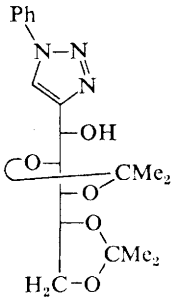
(161)



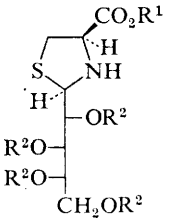
(162) Ar = Ph, *p*-MeC_6H_4, or *p*-ClC_6H_4



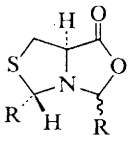
(163)

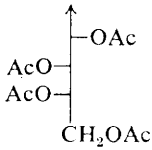


(164)



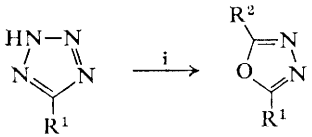
(165) $R^1 = R^2 = H$ or $R^1 = Me$, $R^2 = Ac$





(166) R =

planar zig-zag conformation, which becomes somewhat distorted in certain derivatives of (165).[384] Treatment of the 5-(polyacetoxyalkyl)tetrazoles (167) either with hot acetic anhydride or with benzoyl chloride in pyridine yielded 2-methyl- or 2-phenyl-5-(polyacetoxyalkyl)-1,3,4-oxadiazoles (168) (Scheme 41), respectively.[385] The preferred conformations of the 5-polyacetoxyalkyl side-chains in these derivatives were also deduced using ^1H n.m.r. spectroscopy.



(167) (168)

Reagent: Ac_2O or PhCOCl–py

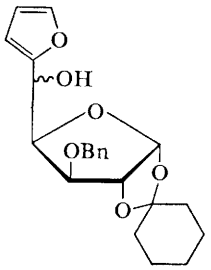
Scheme 41

[384] L. Szilágyi and Z. Györgydeák, *Carbohydrate Res.*, 1976, **48**, 159.
[385] A. M. Seldes, E. G. Gros, I. M. E. Thiel, and J. O. Deferrari, *Carbohydrate Res.*, 1976, **49**, 49.

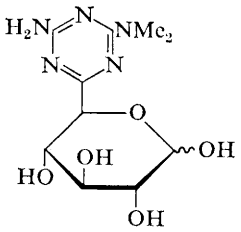
3-*O*-Benzyl-1,2-*O*-cyclohexylidene-α-D-xylodialdo-1,4-furanose reacted with the 2-lithio-derivatives of furan, pyridine, picoline, and benzothiazole to give the corresponding 5-*C*-heterocyclic derivatives [*e.g.* (169)] in low yield.[386]

The methyl esters of D-gluco-, D-galacto-, and D-manno-pyranosyluronic acids reacted with *NN*-dimethylbiguanides to give *s*-triazine derivatives [*e.g.* (170)], which have found use in agriculture.[387]

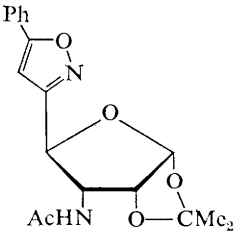
Tronchet's group has extended its studies of the 1,3-dipolar cycloaddition of phenylacetylene to nitrile oxide derivatives of sugars to include the synthesis of 5-phenylisoxazole derivatives of 1,2-substituted 3-azido-3-deoxy-, 3-amino-3-deoxy-, and 3-trifluoroacetamido-3-deoxy-α-D-*xylo*-tetrofuranose, and 3-acetamido-3-deoxy-α-D-*ribo*-tetrofuranose (171).[388] 2-Amino-2-deoxy-D-glucose reacted with substituted-phenyl thiocyanates to yield condensed imidazolidine derivatives (172),[389] and a *spiro*-benzimidazoline prepared from 1,2:5,6-di-*O*-



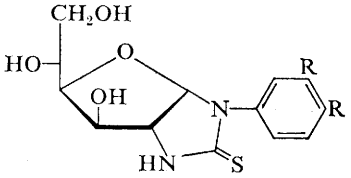
(169)



(170)



(171)

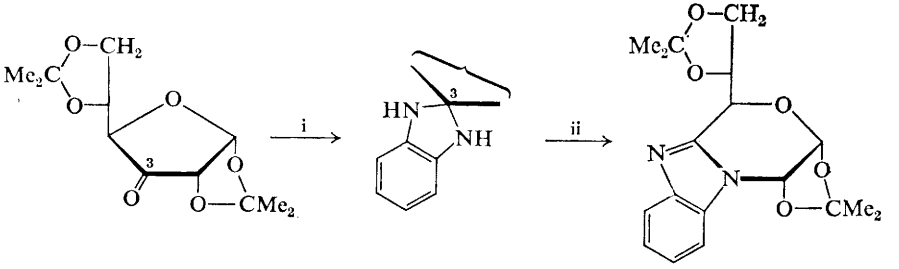


(172) R = H or Cl

sopropylidene-α-D-*ribo*-hexofuranos-3-ulose was shown to rearrange to the condensed benzimidazole derivative (173) in the presence of oxygen (Scheme 42).[390]

Acyclic sugar oxazoles formed on treatment of protected aldonic acid lactones with ethyl isocyanoacetate and base in an aprotic medium are referred to in Chapter 17.

[386] Yu. A. Zhdanov, V. G. Alekseeva, and E. L. Korol, *Doklady Akad. Nauk S.S.S.R.*, 1975, **225**, 1336 (*Chem. Abs.*, 1976, **84**, 150 848f).
[387] C. S. Lee and K. Maekawa, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 785.
[388] J. M. J. Tronchet and J. Poncet, *Carbohydrate Res.*, 1976, **46**, 119.
[389] F. Garcia Gonzalez, J. Fernandez Bolanos, and M. A. Pradera de Fuentes, *Anales de Quim.*, 1974, **70**, 57 (*Chem. Abs.*, 1976, **84**, 90 457r).
[390] J. M. J. Tronchet and B. Gentile, *Helv. Chim. Acta*, 1976, **59**, 1380.



(173)

Reagents: i,  ; ii, O_2

Scheme 42

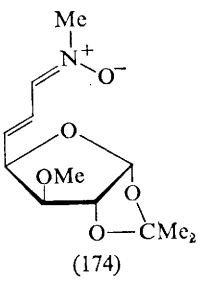
Oximes

The oximes of L-arabinose, D-ribose, and D-xylose have been prepared and the ratio of *syn-* to *anti*-forms in each of them in solution determined by ^{1}H n.m.r. spectroscopy.[391] The conversion of *ω-aldehydo*-sugar oximes into hydroximoyl chlorides, *via gem*-chloronitroso-derivatives and their dimers, has been investigated using a combination of ^{1}H n.m.r. spectroscopy and kinetic methods.[392] A reaction scheme was proposed.

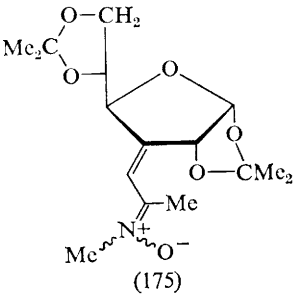
Miscellaneous Compounds

Examples of azido-sugars obtained by ring-opening of oxirans or by displacement of sulphonyloxy-groups are mentioned in Chapter 8.

New sugar nitrones, obtained by condensation of *N*-methylhydroxylamine with carbonyl derivatives of suitably protected sugars, have been reported by Tronchet's group.[393] In most instances, only one of the two possible geometric isomers was isolated [*e.g.* (174)], although both geometric isomers of (175) were obtained in crystalline form. Treatment of 3-amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (176) with 4-nitrobenzenediazonium tetrafluoroborate afforded a mixture of tautomeric triazines (Scheme 43), and



(174)

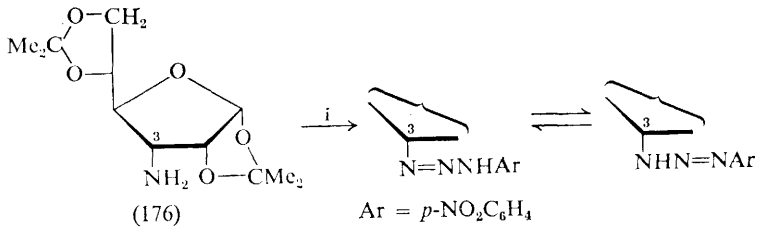


(175)

[391] M. Iio, T. Shimotokube, and H. Omura, *J. Fac. Agric., Kyushu Univ.*, 1975, **20**, 1 (*Chem. Abs.*, 1976, **84**, 44 563z).

[392] J. M. J. Tronchet and F. Barbalat-Rey, *Pharm. Acta Helv.*, 1975, **50**, 404 (*Chem. Abs.*, 1976, **84**, 122 181q).

[393] J. M. J. Tronchet and E. Mihaly, *Carbohydrate Res.*, 1976, **46**, 127.



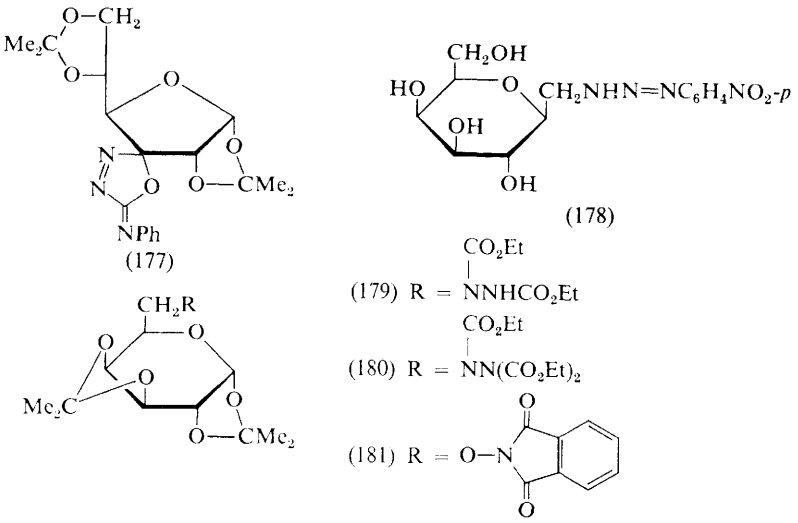
(176) Ar = *p*-$NO_2C_6H_4$

Reagent: i, *p*-$NO_2C_6H_4N_2^+ BF_4^-$

Scheme 43

1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexos-3-ulose phenylsemicarbazone gave the *spiro*-2-phenylimino-1,3,4-oxadiazole (177) when treated with mercuric oxide, magnesium oxide, and iodine.[394] 1-β-D-Galactopyranosylmethyl-3-(4-nitrophenyl)triazine (178), similarly prepared from the corresponding amine, acted as an irreversible inhibitor of the β-D-galactosidase from *Escherichia coli*, presumably by generation *in situ* of the D-galactosylmethyl carbocation, which attacks residues at the active site of the enzyme.[395]

1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose reacted with either diethyl or dibutyl azodicarboxylate and triphenylphosphine in refluxing THF to give the corresponding 6-[1,2-bis(alkoxycarbonyl)hydrazino]-6-deoxy- and 6-[1,2,2-tris(alkoxycarbonyl)hydrazino]-6-deoxy-derivatives, [*e.g.* (179) and (180)], respectively,[396] and with *N*-hydroxyphthalimide, triphenylphosphine, and diethyl azodicarboxylate in THF at room temperature to give the 6-*O*-phthalimido-derivative (181).[397] Other *O*-phthalimidohexoses were also reported.



(177) (178)

(179) R = $NNHCO_2Et$, CO_2Et

(180) R = $NN(CO_2Et)_2$, CO_2Et

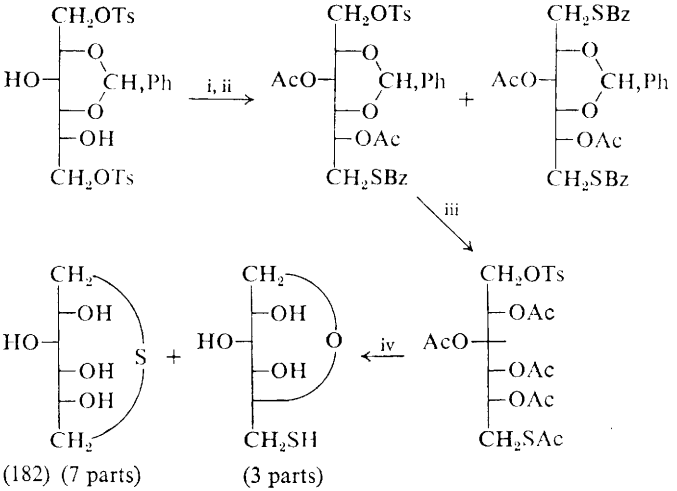
(181) R = O–N

[394] J. M. J. Tronchet and F. Rachidzadeh, *Helv. Chim. Acta*, 1976, **59**, 2855.
[395] M. L. Sinnott and P. J. Smith, *J.C.S. Chem. Comm.*, 1976, 223.
[396] G. Grynkiewicz, J. Jurczak, and A. Zamojski, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1976, **24**, 83 (*Chem. Abs.*, 1976, **85**, 33 313n).
[397] E. Grochowski and J. Jurczak, *Carbohydrate Res.*, 1976, **50**, C15.

11
Thio- and Seleno-sugars

A review of thio-sugars has discussed the conformations of various dialkyl dithioacetals and the use of thio-sugars in the synthesis of deoxynucleosides.[398]

1,6-Thioanhydro-D-glucitol (182) has been synthesized from 2,4-*O*-benzylidene-1,6-di-*O*-toluene-*p*-sulphonyl-D-glucitol by the route shown in Scheme 44.[399]



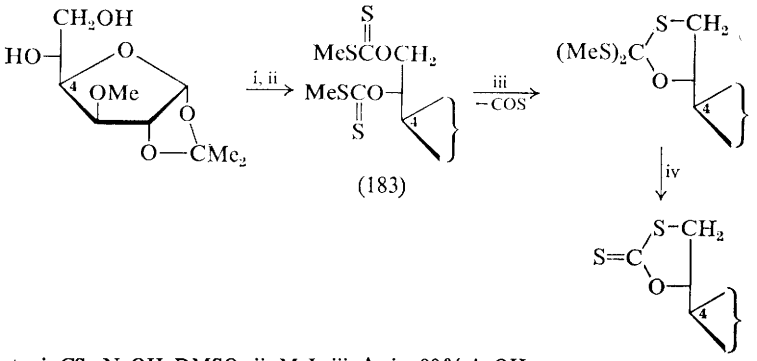
Reagents: i, Ac_2O–py; ii, KSBz–Me_2CO; iii, Ac_2O–AcOH–H_2SO_4; iv, MeONa

Scheme 44

A convenient synthesis of 1,2-*O*-isopropylidene-3-*O*-methyl-6-thio-α-D-gluco-furanose 5,6-dithiocarbonate, a versatile intermediate in the synthesis of unsaturated sugars and thio- and deoxy-sugars (see Vol. 8, p. 84), is based on thermal decomposition of the bis(dithiocarbonate) (183) (Scheme 45).[400]

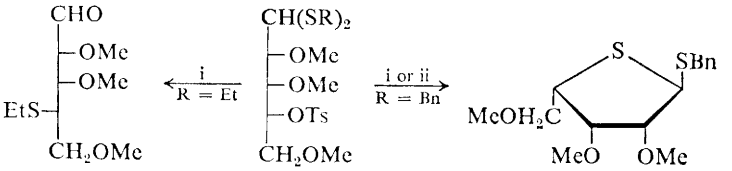
Further examples of rearrangements involving cyclic sulphonium ions have been reported. Thus 2,3,5-tri-*O*-methyl-4-*O*-toluene-*p*-sulphonyl-D-ribose diethyl dithioacetal (184) and dibenzyl dithioacetal (185) gave 4-*S*-ethyl-2,3,5-tri-*O*-methyl-4-thio-L-lyxose (186) and benzyl 2,3,5-tri-*O*-methyl-1,4-dithio-α-L-lyxo-furanoside (187) (Scheme 46) when heated either in aqueous pyridine or with sodium iodide in acetone; similar rearrangements to the 4-thiofuranosides were observed with 2,3,5-tri-*O*-methyl-4-*O*-toluene-*p*-sulphonyl-D-xylose and -lyxose

[398] D. Horton, *Pure Appl. Chem.*, 1975, **42**, 301.
[399] J. Kuszmann and P. Sohár, *Carbohydrate Res.*, 1976, **48**, 23.
[400] G. Descotes and A. Faure, *Synthesis*, 1976, 449.



Reagents: i, CS_2–NaOH–DMSO; ii, MeI; iii, Δ; iv, 80% AcOH

Scheme 45



Reagents: i, py–H_2O; ii, NaI–Me_2CO

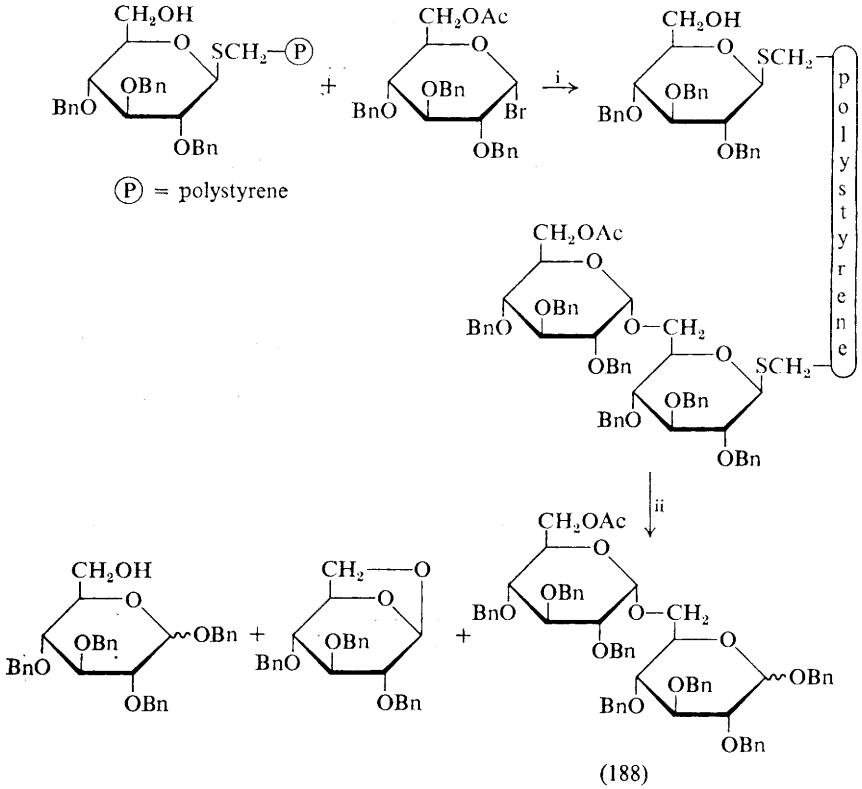
Scheme 46

dibenzyl dithioacetals.[401] These reactions clearly proceed by intramolecular displacement of the toluene-*p*-sulphonyloxy-group with the formation of cyclic 1,4-sulphonium ions, which undergo ring-opening at C-1 and hydrolysis when derived from (184) or $\overset{+}{S}$—Bn bond cleavage when derived from (185). However, the 5-toluene-*p*-sulphonates of 2,3,4-tri-*O*-methyl-D-ribose and -xylose dibenzyl dithioacetals gave 2,5-anhydro-3,4-di-*O*-methyl-D-ribose or -xylose dibenzyl dithioacetal, respectively, when heated with sodium iodide in acetone. Details have been published of the acid-catalysed reaction of both 5-thio-D-ribose and 5-thio-D-xylose with acetone and 2,2-dimethoxypropane to give 1,2:3,4-di-*O*-isopropylidene-5-thio-α-D-ribo- or -xylo-pyranose, respectively.[402] No furanoid products were detected, thus demonstrating the exceedingly strong preference for the formation of those diacetals with a six-membered, sulphur-containing ring, despite the considerable steric strain of a cyclic acetal derived from a vicinal *trans*-diol in the 5-thio-D-xylopyranose derivative and of a *cis-syn-cis* arrangement of the cyclic acetal rings in the 5-thio-D-ribopyranose derivative.

Anderson's group has reported conventional syntheses of 2,3,4- and 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranose [403] and 2,3,4- and 2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranose [404] in connection with an approach to the solid-phase

[401] T. Van Es, *Carbohydrate Res.*, 1976, **46**, 237.
[402] N. A. Hughes and C. J. Wood, *Carbohydrate Res.*, 1976, **49**, 225.
[403] S. A. Holick, S.-H. L. Chiu, and L. Anderson, *Carbohydrate Res.*, 1976, **50**, 215.
[404] M. A. Nashed and L. Anderson, *Carbohydrate Res.*, 1976, **51**, 65.

synthesis of oligosaccharides in which the first sugar residue is attached to the solid support by a thioglycosidic linkage. This approach was used in the synthesis of derivatives (188) of isomaltose (Scheme 47).[405] Quantitative cleavage of the



Reagents: i, 2,6-lutidine–PhH at 65 °C; ii, MeI–BnOH–PhH

Scheme 47

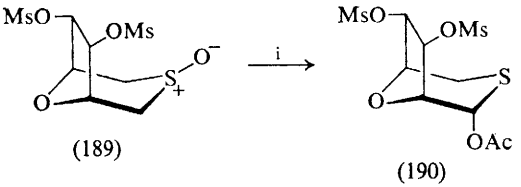
sugars from a partially coupled polystyrene support was accomplished by the action of methyl iodide and benzyl alcohol in refluxing benzene, yielding, *inter alia*, the disaccharide benzyl glycosides (188), which gave isomaltose following deprotection and purification.

1,6-Anhydro-1(6)-thio-β-maltotriose has been synthesized from 1,6-anhydro-β-maltotriose nona-acetate by a sequence of reactions analogous to that employed in the preparation of 1,6-anhydro-1(6)-thio-β-D-disaccharides[406] (see Vol. 8, p. 85). Fully acetylated derivatives of 1-β-thio- and 6-thio-α-maltotriose were also reported.

Full details have appeared on the synthesis of racemic 4-thiotetrose derivatives by Pummerer rearrangement of esters of thiolan-3,4-diol 1-oxides (*i.e.*

[405] S.-H. L. Chiu and L. Anderson, *Carbohydrate Res.*, 1976, **50**, 227.
[406] K. Takeo and T. Kuge, *Carbohydrate Res.*, 1976, **48**, 282.

sulphoxides); the protecting and directing properties of 3,4-benzeneboronic esters were found to be particularly useful.[407] Pummerer rearrangement of sulphoxides derived from hexitols containing a 1,4-oxathian ring [*e.g.* (189)] gave only one (190) of the four possible isomers (Scheme 48), but the sulphoxide derived from (190) did not undergo a Pummerer rearrangement.[408]

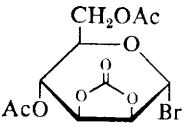


(189) (190)

Reagent: i, Ac_2O at 50 °C

Scheme 48

Condensation of the appropriate per-*O*-acetylated glycopyranosyl bromide with 4-aminobenzenethiol in the presence of sodium methoxide has been used to prepare 4-aminophenyl 1-thio-β-D-gluco-, -galacto-, and -xylo-pyranosides and 4-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside.[409] 4-Aminophenyl 1-thio-β-D-glucopyranosiduronic acid was synthesized by condensation of methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate with 4-aminobenzenethiol, followed by saponification with sodium hydroxide. An attempt to obtain the 1,2-*cis*-1-thioaldopyranoside by reaction of 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide (191) with sodium 4-nitrobenzene-



(191)

thioxide in DMF gave both the α- and β-1-thioaldopyranosides, which were isolated by chromatography and fractional recrystallization.[410] By contrast, the α-bromide (191) reacted with sodium 4-nitrophenoxide to give mainly the 1,2-*cis*-aldopyranoside. However, 1,2-*cis*-(4-nitrophenyl) 1-thioaldopyranosides (including 4-nitrophenyl 1-thio-β-D-mannopyranoside), which are potential ligands for the purification of glycosidases by affinity chromatography, have been synthesized stereoselectively by reaction of the appropriate per-*O*-acetylated 1,2-*trans*-aldopyranosyl bromide (*e.g.* 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide) with 4-nitrobenzenethiol in HMPT in the presence of either aqueous potassium carbonate at room temperature or sodium hydride at 70 °C.[411] The reaction of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride with sodium

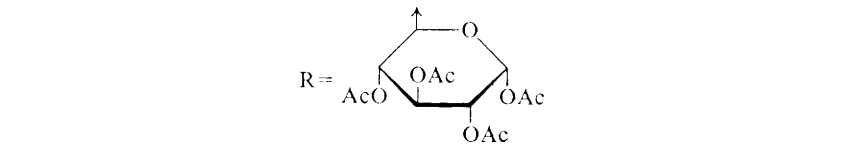
[407] J. E. McCormick and R. S. McElhinney, *J.C.S. Perkin I*, 1976, 2533.
[408] J. Kuszmann and P. Sohár, *Acta Chim. Acad. Sci. Hung.*, 1976, **88**, 167 (*Chem. Abs.*, 1976, **85**, 33 309r).
[409] N. Iino and K. Yoshida, *Carbohydrate Res.*, 1976, **51**, 223.
[410] K. L. Matta and J. J. Barlow, *Carbohydrate Res.*, 1976, **48**, 294.
[411] M. Blanc-Muesser, J. Defaye, and H. Driguez, *Tetrahedron Letters*, 1976, 4307.

sulphide in HMPT gave, after deacetylation and purification, αα-1-thiotrehalose. These reactions, which appear to involve S_N2 displacement of halide ion at the anomeric centre, can also be used to obtain 1,2-*trans*-(4-nitrophenyl) 1-thio-aldopyranosides from per-*O*-acetylated 1,2-*cis*-aldopyranosyl bromides.

A new, high-yield synthesis of 6-thio-β-D-fructopyranose has utilized the isomerization of 6-thio-D-glucose by D-glucose isomerase.[412] Treatment of 6-thio-β-D-fructopyranose with acetic anhydride and zinc chloride afforded the acyclic penta-acetate, whereas 1,3,4,5-tetra-*O*-acetyl-6-thio-β-D-fructopyranose resulted when acetic anhydride in pyridine was used.

Uridine 5′-(5-thio-α-D-glucopyranosyl pyrophosphate) has been synthesized and found to be a potent activator of rat-liver glycogen synthase *a*.[413]

The reactions outlined in Scheme 49 have been used to prepare 1,2,3,4-tetra-*O*-acetyl-6-*Se*-benzoyl-6-seleno-α-D-glucopyranose (192), 6,6′-diselenobis(1,2,3,4-



Reagents: i, BzSeK–Me_2CO; ii, I_2–$CHCl_3$; iii, $(Me_2As)_2$–CH_2Cl_2

Scheme 49

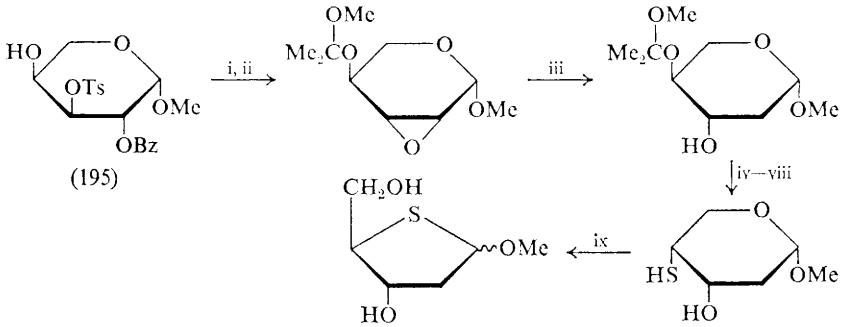
tetra-*O*-acetyl-α-D-glucopyranose) (193), and 1,2,3,4-tetra-*O*-acetyl-6-*Se*-dimethylarsino-6-seleno-α-D-glucopyranose (194).[413a] All attempts to prepare thio- and *NN*-dimethylseleno-pseudoureido derivatives by the reaction of 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-iodo-α-D-glucopyranose with thiourea or *NN*-dimethylselenourea, respectively, were unsuccessful (*cf.* the β-anomer, Vol. 9, p. 80).

The synthesis of purine and pyrimidine 2′-deoxynucleosides from a 1,2-dithio-sugar precursor [414] is noted in Chapter 21, which also refers to the alkylation of seleno- and thio-substituted nucleosides using a mixture of a dialkyl disulphide (or diselenide) and tributylphosphine in DMF.[415] Other thio-sugar derivatives are mentioned in Chapter 12.

[412] M. Chmielewski, M. Chen, and R. L. Whistler, *Carbohydrate Res.*, 1976, **49**, 479.
[413] T. L. Graham and R. L. Whistler, *Biochemistry*, 1976, **15**, 1189.
[413a] G. C. Chen, J. R. Daniel, and R. A. Zingaro, *Carbohydrate Res.*, 1976, **50**, 53.
[414] D. Horton and M. Sakata, *Carbohydrate Res.*, 1976, **48**, 41.
[415] C.-Y. Shiue and S.-H. Chu, *J. Org. Chem.*, 1976, **41**, 1847.

12
Derivatives with Sulphur in the Sugar Ring

The sequence of reactions shown in Scheme 50 was used to convert methyl 2-*O*-benzoyl-3-*O*-toluene-*p*-sulphonyl-β-L-arabinopyranoside (195) into a mixture



Reagents: i, MeONa–MeOH; ii, CH_2=C(Me)OMe–HCl; iii, $LiAlH_4$; iv, BnBr–NaH–DMSO; v, 80% AcOH; vi, TsCl–py; vii, AcSK–DMF; viii, Na–NH_3; ix, MeOH–H^+

Scheme 50

of methyl 2-deoxy-4-thio-α- and -β-D-*erythro*-pentofuranosides (196), which were previously available only by a longer route (see Vol. 4, p. 87).[416] The acetonation of 5-thio-D-ribose and 5-thio-D-xylose [402] is mentioned in Chapter 11, which also reports rearrangements giving rise to derivatives of benzyl 4-thio-pentofuranosides [401] and racemic 4-thiotetroses.[407] Nucleosides containing 4-thio-D-ribofuranosyl residues are dealt with in Chapter 21.

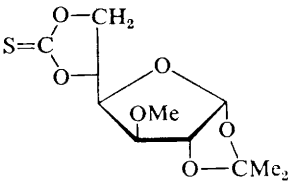
The acetalation of 1,6-anhydro-1(6)-thio-D-glucitol with acetone, formaldehyde, or benzaldehyde to give 2,3:4,5-diacetals has been noted in Chapter 5.[189]

No reports have appeared during the past year of derivatives of sugars containing nitrogen or phosphorus in a five- or six-membered ring.

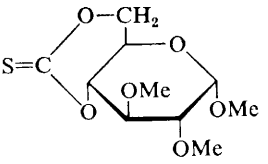
[416] Y.-L. Fu and M. Bobek, *J. Org. Chem.*, 1976, **41**, 3831.

13
Deoxy-sugars

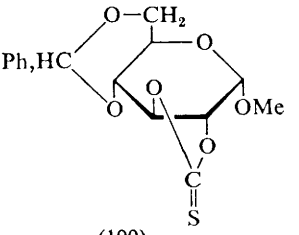
Barton and Subramanian have reported a procedure for the synthesis of deoxy-sugars and -nucleosides based on opening of diol thiocarbonates in a radical fashion using tributyltin hydride (*cf.*, Vol. 9, p. 86).[264] For example, the thiocarbonates (197) and (198) were cleaved regioselectively (secondary radical more stable than primary radical) to give, after alkaline hydrolysis, 5-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-*xylo*-hexofuranose (57%) and methyl 4-deoxy-2,3-di-*O*-methyl-α-D-*xylo*-hexopyranoside (61%), respectively, whereas the 2,3-thiocarbonates (199) and (200) afforded mixtures of the corresponding 2-deoxy-(30% and 60%, respectively) and 3-deoxy-sugars (60% and 40%, respectively).



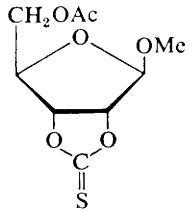
(197)



(198)



(199)



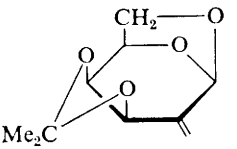
(200)

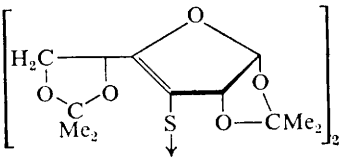
(201) RO

(202)



R=

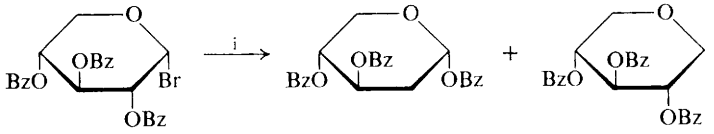




(203)

An efficient method for the deoxygenation of protected keto-sugars to give deoxy-sugars involved treatment with phosphorus pentasulphide in pyridine and reductive desulphurization of the products, whose nature varied with the

substrate; for example, 1,6-anhydro-3,4-*O*-isopropylidene-β-D-*lyxo*-hexopyranosulose (201) yielded the bridged derivative (202), whereas 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose gave the disulphide (203), presumably *via* the thioketone.[417] Hydrogenolysis of per-*O*-benzoylated *cis*-1,2-glycopyranosyl bromides over palladized charcoal in the presence of triethylamine gave the benzoylated 2-deoxypyranose as well as the 1,5-anhydroalditol derivative (see Scheme 51).[418] This reaction was also used to prepare

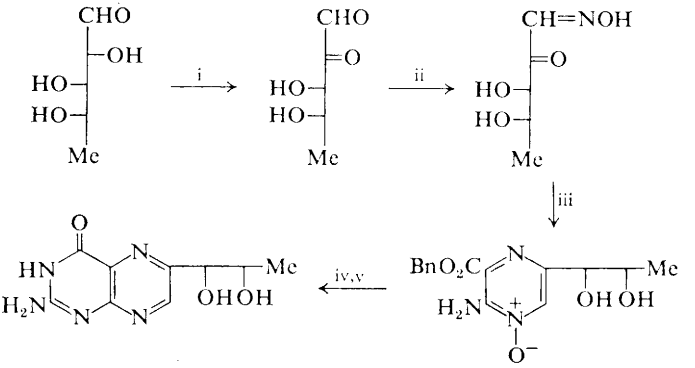


Reagent: i, H_2-Pd-Et_3N

Scheme 51

benzoylated derivatives of 2-deoxy-D-*lyxo*-hexopyranose, 2,6-dideoxy-L-*arabino*-hexopyranose, and 2-deoxy-D-lactose. The synthesis of deoxy-sugars by reductive dechlorination of dichlorodideoxy-sugars has been noted in Chapter 7,[286] and the reductive dehalogenation of halogeno-nucleosides is referred to in Chapter 21.

The yield of 5-deoxy-L-arabinose obtained by degradation of L-rhamnose diethyl dithioacetal was significantly improved when oxidation to the intermediate bis-sulphone was carried out with 3-chloroperbenzoic acid in anhydrous *p*-dioxan.[419] The reactions outlined in Scheme 52 were then used to convert



Reagents: i, $Cu(OAc)_2$; ii, $Me_2C{=}NOH$ (pH 3.5); iii, $BnO_2CCH(NH_2)CN$-EtOH; iv, $H_2NC(=NH)NH_2$-NaOMe; v, $Na_2S_2O_4$

Scheme 52

5-deoxy-L-arabinose into L-*erythro*-biopterin, a naturally occurring pteridine that is widely distributed in micro-organisms, insects, algae, amphibia, and mammals. 6-(D-*arabino*-Tetrahydroxybutyl)- and 6-(D-*threo*-trihydroxypropyl)-

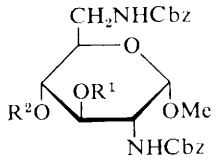
[417] P. Köll, R.-W. Rennecke, and K. Heyns, *Chem. Ber.*, 1976, **109**, 2537.
[418] I. Lundt and C. Pedersen, *Acta Chem. Scand.*, 1976, **B30**, 680.
[419] E. C. Taylor and P. A. Jacobi, *J. Amer. Chem. Soc.*, 1976, **98**, 2301.

pterins were also prepared by an analogous route from D-glucose and D-xylose, respectively. Methyl 6-deoxy-α-DL-altro-, -gluco-, -galacto-, and -ido-hexopyranosides have been synthesized *via* epoxidation of methyl 2,3,6-trideoxy-DL-hex-2-enopyranosides followed by alkaline or acidic hydrolysis,[420] and D-fucose has been obtained from D-galactose by a route that involved reduction of methyl 6-*O*-toluene-*p*-sulphonyl-α-D-galactopyranoside with lithium aluminium hydride.[420a]

The preparation of methyl β-L-rhamnopyranoside [54] and the synthesis of (1 -> 2)-linked disaccharides containing non-reducing residues of 6-deoxy-D-glucopyranose [72] and of 3-*O*-α-L-rhamnopyranosyl-D-glucose [73] have been noted in Chapter 3.

A new, three-step synthesis of 1-deoxy-D-fructose (27% overall yield) is based on reductive desulphurization of 2-amino-2-deoxy-D-glucose diethyl dithioacetal to give 2-amino-1,2-dideoxy-D-glucitol, which was oxidatively deaminated using 3,5-di-t-butyl-1,2-benzoquinone.[421] The metabolism of 1-deoxy-D-fructose and of its reduction products was investigated. The first example of a naturally occurring 1-deoxypentulose is reported in Chapter 20.

The 3-sulphonate (204) has been shown to yield a 1 : 5 mixture of methyl 2,6-di(benzyloxycarbonylamino)-2,3,6- and -2,4,6-trideoxy-α-D-*ribo*-hexopyranosides on treatment with sodium borohydride in DMSO at 80 °C, while the

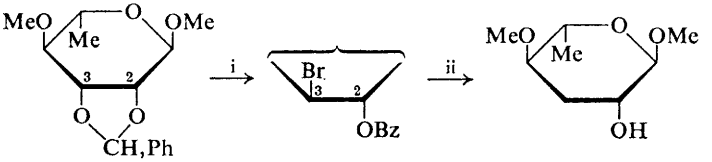


(204) R^1 = Ts, R^2 = H
(205) R^1 = H, R^2 = Ts

4-sulphonate (205) was transformed into methyl 2,6-di(benzyloxycarbonylamino)-2,3,6-trideoxy-α-D-*xylo*-hexopyranoside *via* ring-opening of the D-*galacto*-oxiran with lithium aluminium hydride.[422] Surprisingly, neither this nor the D-*allo*-oxiran derived from (204) reacted with sodium borohydride in DMSO, implying that an oxiran is not an intermediate in the deoxygenation reaction on (204). References to the synthesis of 4-dimethylamino-2,3,4,6-tetradeoxy-D-*erythro*-hexose (D-forosamine),[295] a racemic 7-deoxylincosamine derivative,[300] 3-amino-2,3,6-trideoxy-D-*ribo*-hexose [301] and its L-enantiomer (ristosamine),[302] 2-amino-2,6-dideoxy-D-allose and -altrose hydrochlorides,[305] and the derivatives of several 3-amino-2,3,6-trideoxyhexopyranoses [307] and of racemic 3-amino-3,4-dideoxypentopyranoses [308] can be found in Chapter 8.

Methyl 3,6-dideoxy-4-*O*-methyl-α-L-*arabino*-hexopyranoside (a derivative of ascarylose) has been synthesized from methyl 2,3-*O*-benzylidene-4-*O*-methyl-α-L-rhamnopyranoside by the route shown in Scheme 53.[290] Treatment of

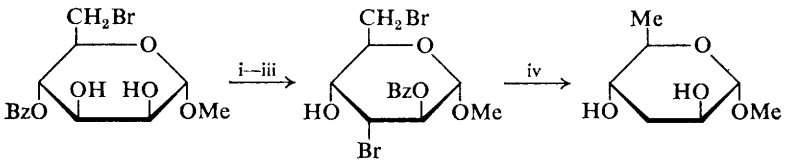
[420] O. Achmatowicz, jun., and B. Szechner, *Roczniki Chem.*, 1976, **50**, 729.
[420a] V. L. Montalvo, R. C. Fuentes, and O. A. Ching Puente, *Bol. Soc. quim. Peru*, 1975, **41**, 75 (*Chem. Abs.*, 1976, **84**, 74 536k).
[421] W. L. Dills, jun., and W. L. Meyer, *Biochemistry*, 1976, **15**, 4506.
[422] H. Saeki, N. Takeda, Y. Shimada, and E. Ohki, *Chem. and Pharm. Bull.* (*Japan*), 1976, **24**, 724.



Reagents: i, NBS–CCl_4; ii, $LiAlH_4$–THF

Scheme 53

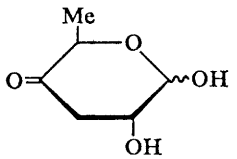
methyl 2,3:4,6-di-*O*-benzylidene-α-D-mannopyranoside with NBS in carbon tetrachloride afforded a mixture of stereoisomeric 3,6- and 4,6-dibromodibenzoates – due to participation by the benzoyl group introduced at C-4 – which on reduction with lithium aluminium hydride gave methyl 3,6-dideoxy-α-D-*arabino*-hexopyranoside (methyl α-tyveloside) and methyl 4,6-dideoxy-α-D-*arabino*-hexopyranoside, respectively. An alternative synthesis of methyl α-tyveloside is shown in Scheme 54. Steven's group has reported two syntheses



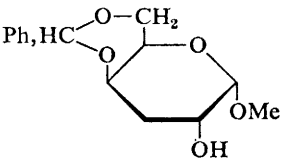
Reagents: i, PhCHO–$ZnCl_2$; ii, EtOK; iii, NBS–CCl_4; iv, $LiAlH_4$–THF

Scheme 54

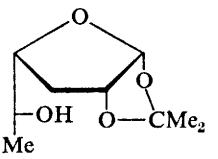
of 3,6-dideoxy-D-*erythro*-hexos-4-ulose (206), which as its cytidine diphosphate nucleotide conjugate is an intermediate in the biosynthesis of 3,6-dideoxyhexoses in such micro-organisms as *Pasteurella pseudotuberculosis*; one of the routes was developed from methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*ribo*-hexopyranoside and the other from the α-D-*xylo*-analogue (207).[423] It is notable that acid-catalysed acetonation of 3,6-dideoxy-D-*xylo*-hexose afforded mainly 3,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuranose (208) rather than the desired 3,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexopyranose, which had therefore to be prepared by a somewhat longer route from (207).



(206)



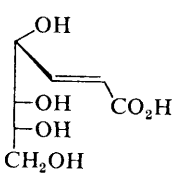
(207)



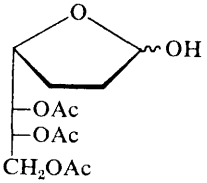
(208)

Standard procedures have been used to convert the 2,3-unsaturated heptonic acid (209) (prepared from 3,4,5,6,7-penta-*O*-acetyl-1-deoxy-1-diazo-D-*gluco*-heptulose) into 5,6,7-tri-*O*-acetyl-2,3-dideoxy-D-*arabino*-heptofuranose (210),

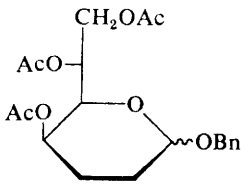
[423] C. L. Stevens, K. W. Schultze, D. J. Smith, and P. M. Pillai, *J. Org. Chem.*, 1975, **40**, 3704.



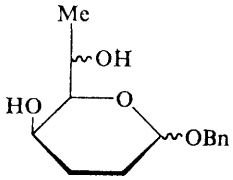
(209)



(210)



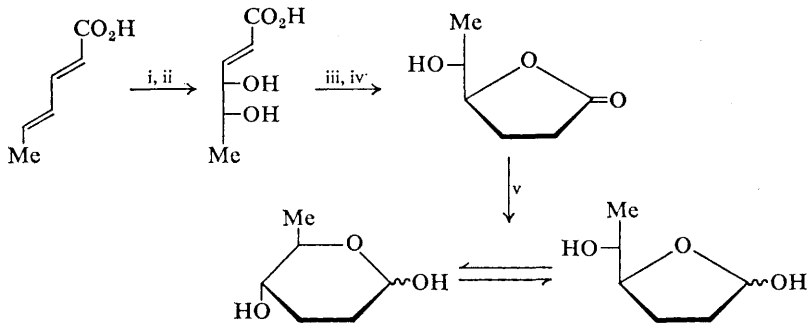
(211)



(212)

which gave the anomeric heptopyranosides (211) with benzyl alcohol and boron trifluoride.[424] Treatment of (211) in turn with sodium methoxide, periodate ion, and methyl magnesium iodide afforded the anomeric benzyl 2,3,7-trideoxy-D-*arabino*- and -L-*xylo*-heptopyranosides (212).

A new synthesis of racemic amicetose (2,3,6-trideoxy-DL-*erythro*-hexose) from sorbic acid is outlined in Scheme 55.[425]



Reagents: i, $MeCO_3H$; ii, H_3O^+; iii, H_2–Pd–C; iv, H^+; v, Bu^i_2AlH

Scheme 55

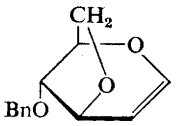
Chapters 14, 21, and 23 also contain references to deoxy-sugars and their derivatives.

[424] L. Baumeister, I. Dyong, and H. Luftmann, *Chem. Ber.*, 1976, **109**, 1245.
[425] I. Dyong and N. Jersch, *Chem. Ber.*, 1976, **109**, 896.

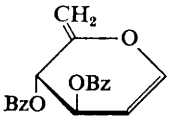
14
Unsaturated Derivatives

Glycals

3,4-Di-*O*-benzyl-6-deoxy-6-fluoro-D-glucal has been synthesized from 6-*O*-t-butyldimethylsilyl-D-glucal by the route shown in Scheme 56.[426] This route was devized because a seemingly more direct route involving benzylation of 6-*O*-toluene-*p*-sulphonyl-D-glucal gave the 3,6-anhydro-derivative (213). More-

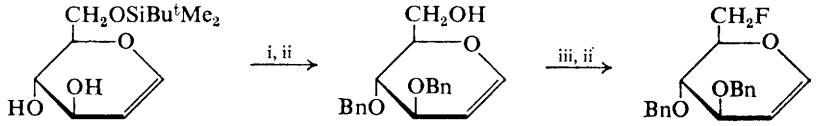


(213)



(214)

over, attempts to introduce the 6-fluoro-substituent by reaction of 3,4-di-*O*-benzoyl-6-*O*-toluene-*p*-sulphonyl-D-glucal with caesium fluoride in DMF yielded the diene (214). An analogous series of reactions to those outlined in Scheme 56



Reagents: i, BnBr–Ag_2O–DMF; ii, $Bu_4N^+F^-$–DMF; iii, TsCl–py

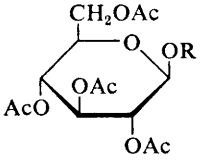
Scheme 56

was also used to convert 6-*O*-t-butyldimethylsilyl-3-deoxy-D-glucal into 4-*O*-benzyl-3,6-dideoxy-6-fluoro-D-glucal.

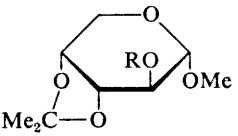
The stereoselectivity of formation of 1,2-dideoxy-1,2-dihalogeno-α-D-glucopyranose derivatives when bromine and chlorine are added to 3,4,6-tri-*O*-acetyl- and 3,4,6-tri-*O*-benzyl-D-glucal has been shown to be influenced both by the substituents and the polarity of the solvent.[427] Similar results were observed for halogenomethoxylations.

The reaction of 3,4,6-tri-*O*-acetyl-D-glucal with appropriately protected monosaccharide derivatives in the presence of boron trifluoride etherate has been used to prepare modified disaccharides (215)—(218) containing αβ-(1 → 1)-, α- and β-(1 → 2)-, α-(1 → 3)-, and α-(1 → 4)-linkages, respectively.[428] Both the

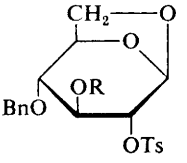
[426] I. D. Blackburne, P. M. Fredericks, and R. D. Guthrie, *Austral. J. Chem.*, 1976, **29**, 381.
[427] P. Boullanger and G. Descotes, *Carbohydrate Res.*, 1976, **51**, 55.
[428] J. Thiem and J. Schwentner, *Tetrahedron Letters*, 1976, 3117.



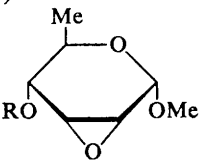
(215)



(216)

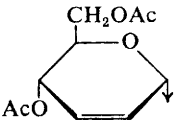


(217)



(218)

R =

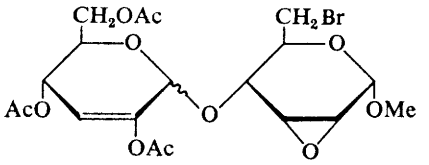


α- and β-(1 → 4)-linked disaccharides (219) were obtained from a similar reaction with 2,3,4,6-tetra-*O*-acetyl-2-hydroxy-D-glucal.

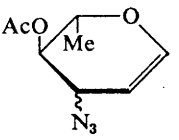
The photochemical addition of formamide to 2,3,4,6-tetra-*O*-acetyl-2-hydroxy-D-glucal is mentioned in Chapter 3.[137]

3,4-Di-*O*-acetyl-L-rhamnal (115) gave a mixture of (220) (60%), (221) (5%), and (222) (22%) when treated with sodium azide in acetonitrile in the presence of boron trifluoride etherate.[304] The conversion of the epimeric 3-azides (220) into derivatives of L-acosamine and L-ristosamine is noted in Chapter 8.

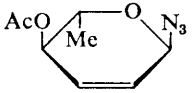
Dehydrochlorination of 2,3,5-tri-*O*-benzyl-α-D-arabinofuranosyl chloride using a molecular sieve gave the benzylated 2-hydroxyglycal (223).[105] A mixture of three substituted furans, namely 2-[(isobutylthio)methyl]furan (224) and its 3- and 4-isobutylthio-derivatives [(225) and (226), respectively] was obtained when 2,5-anhydro-3,4-di-*O*-toluene-*p*-sulphonyl-D-xylose di-isobutyl dithioacetal (227) reacted with sodium iodide–zinc in DMF (the Tipson–Cohen reagent) at 150 °C.[429]



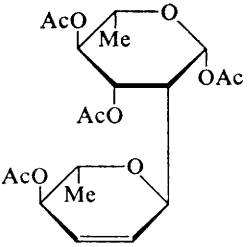
(219)



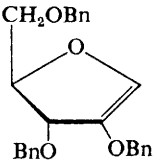
(220)



(221)

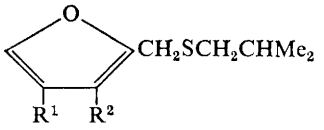


(222)

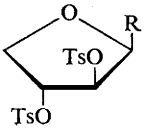


(223)

[429] P. Angibeaud, J. Defaye, H. Franconie, and M. Blanc-Muesser, *Carbohydrate Res.*, 1976, **49**, 209.



(224) $R^1 = R^2 = H$
(225) $R^1 = H$; $R^2 = SCH_2CHMe_2$
(226) $R^1 = SCH_2CHMe_2$; $R^2 = H$



(227) $R = CH(SCH_2CHMe_2)_2$



(228) R =

By contrast, 2,5-anhydro-3,4-di-*O*-toluene-*p*-sulphonyl-D-xylose ethylene dithioacetal (228), its D-ribose epimer, and the analogous L-arabinose di-isobutyl dithioacetal all reacted with the Tipson–Cohen reagent by an *E*2-type mechanism with formation of the anticipated 3-alkenes.

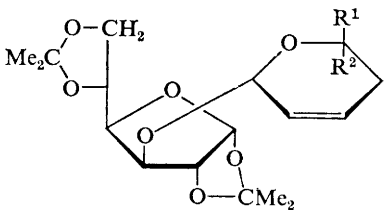
The reactions of glycals resulting in the formation of branched-chain sugar derivatives are discussed in Chapter 15, and the conformations of acetylated glycals are discussed in Chapter 23.

Other Unsaturated Derivatives

The preparation of methyl 4,6-*O*-benzylidene-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranoside from the 2,3-disulphonates (84) using the Tipson–Cohen reaction has been noted in Chapter 6.[257]

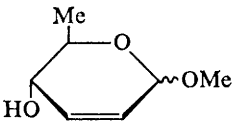
Further examples of the synthesis of disaccharides by the sequence (i) synthesis of a dienyl ether of a monosaccharide, (ii) cycloaddition, and (iii) functionalization (see Vol. 8, p. 19 and Vol. 9, p. 18) have appeared.[430] Thus *O*-α-D- and -L-altropyranosyl-(1 → 3)-D-glucose and *O*-β-L-altropyranosyl-(1 → 3)-D-glucose have been synthesized by functionalization of either (229) or (230), which were obtained *via* cycloaddition of butyl glyoxylate to 3-*O*-(buta-1,3-dienyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose. Configurational inversion in one step allowed the preparation of *O*-α-D-galactopyranosyl-(1 → 3)-D-glucose and *O*-α-D-allopyranosyl-(1 → 3)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose.

Racemic methyl 2,3,6-trideoxy-α- or -β-hex-2-enopyranoside (231) reacted with phthalimide, triphenylphosphine, and diethyl azodicarboxylate to give 4-deoxy-4-phthalimido-derivatives, *e.g.* (232), having the configuration at C-4 inverted.[431] However, products arising from both direct and allylic substitution

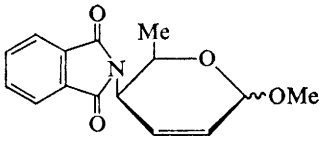


(229) $R^1 = CH_2OH$; $R^2 = H$
(230) $R^1 = H$; $R^2 = CH_2OH$

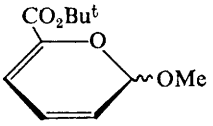
[430] S. David, A. Lubineau, and J.-M. Vatèle, *J.C.S. Perkin I*, 1976, 1831.
[431] A. Banaszek, B. Szechner, J. Mieczkowski, and A. Zamojski, *Roczniki Chem.*, 1976, **50**, 105.



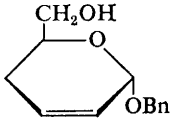
(231)



(232)



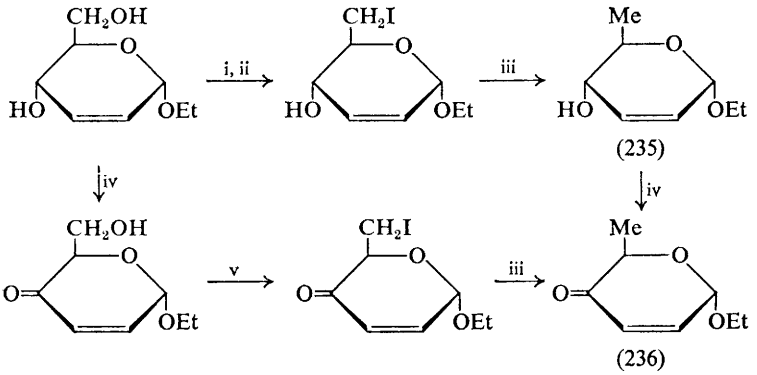
(233)



(234)

were obtained when suitably protected methyl 3,4-dideoxyhex-3-enopyranosides were treated with these reagents, which also yielded 2,6-disubstituted pyran derivatives (233) with t-butyl (methyl 3,4-dideoxyhex-3-enopyranosid)uronates. The epoxidation of methyl 2,3,6-trideoxy-DL-hex-2-enopyranosides has been referred to in Chapter 13.[420] The *cis*-hydroxylation of racemic dihydropyran derivatives [*e.g.* (234)] using alkaline permangate has been reported.[432]

The formation, *inter alia*, of methyl 2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranoside when methyl 2,3-*O*-benzylidene-α-L-rhamnopyranoside reacted with butyl-lithium has been mentioned in Chapter 5 (see Scheme 17).[190] A high-yield route (70% overall) to ethyl 2,3,6-trideoxy-α-D-*erythro*-hex-2-enopyranoside (235) is shown in Scheme 57.[433] Oxidation of the allylic alcohol (235) with activated



Reagents: i, TsCl–py; ii, NaI–MeCOEt–py; iii, H_2–Ni; iv, MnO_2; v, $(PhO)_3\overset{+}{P}MeI^-$–DMF

Scheme 57

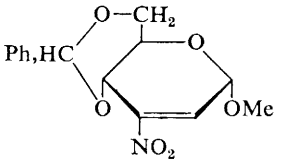
manganese dioxide gave ethyl 2,3,6-trideoxy-α-D-*glycero*-hex-2-enopyranosid-4-ulose (236), which was more conveniently prepared by the alternative route shown in Scheme 57. Apparent anomalies in the ^1H n.m.r. spectrum of ethyl

[432] V. B. Mochalin, A. N. Kornilov, I. S. Varpa Khovskaya, and A. N. Vul'fson, *Zhur. org. Khim.*, 1976, **12**, 58 (*Chem. Abs.*, 1976, **84**, 150 849g).

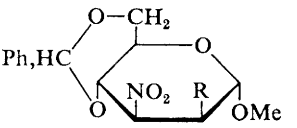
[433] M. B. Yunker, S. Y.-K. Tam, D. R. Hicks, and B. Fraser-Reid, *Canad. J. Chem.*, 1976, **54**, 2411.

6-*O*-benzoyl-4-iodo-2,3,4-trideoxy-α-D-*threo*-hex-2-enopyranoside have been resolved following determination of the crystal structure, which showed that the six-membered ring adopts a half-chair ($^0H_5$) conformation.[434]

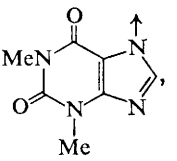
An efficient procedure for the synthesis of nitroalkenes is mentioned in Chapter 10.[377, 377a] Methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-α-D-*erythro*-hex-2-enopyranoside (237) reacted stereoselectively with hydrazoic acid, hydrogen cyanide, theophylline, and 2,6-dichloropurine under weakly acidic or neutral conditions to give addition products (238) having the thermodynamically less stable D-*manno* configuration, whereas it reacted with more basic reagents to yield mainly products having the more stable D-*gluco* configuration.[435] The rate and stereoselectivity of the reaction of the 3-nitroalkene (237) with hydrazoic acid was found to be markedly dependent on the solvent used: thus the rate of the reaction increased progressively in the series chloroform < acetonitrile < THF ~ [$^2H_6$]DMSO and the stereoselectivity increased according to the sequence chloroform < acetonitrile ~ THF < [$^2H_6$]DMSO. The reaction in [$^2H_6$]-DMSO was complete within 10 min and gave methyl 2-azido-4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-α-D-mannopyranoside (238; R = N_3) as the principal product, whereas that in chloroform was incomplete after *ca.* 32 h and gave a mixture of (238; R = N_3) and its D-glucose analogue. The reaction of the 3-nitroalkene (237) with hydrogen cyanide in acetonitrile, which was catalysed



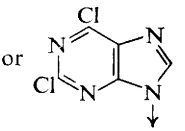
(237)

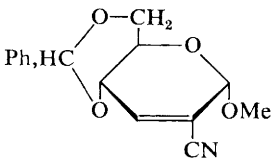


(238) R = N_3, CN,



or





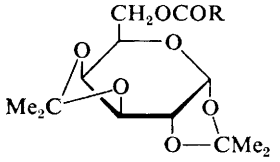
(239)

by potassium cyanide, furnished the 2-cyanoalkene (239) as well as the addition product (238; R = CN). References to nitro-sugars also appear in Chapters 10 and 15.

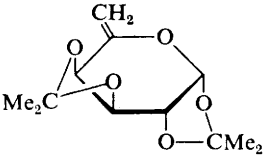
Photolysis of various aryl-substituted 6-esters of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (240) yielded a mixture of 6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (0—50%) and the terminal alkene (241) (0—26%).[436] Sucrose-5- (242) and -5′-enes (243) have been synthesized by reaction of 6-deoxy-6-iodo- or 6′-deoxy-6′-iodo-sucrose hepta-acetate, respec-

[434] R. Stokhuyzen and C. Chieh, *J.C.S. Perkin II*, 1976, 481.
[435] T. Sakakibara and R. Sudoh, *Carbohydrate Res.*, 1976, **50**, 191.
[436] R. W. Binkley and J. L. Meinzer, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 465.

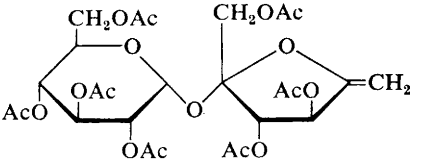
tively, with silver fluoride in pyridine.[437] Catalytic hydrogenation of the 5-ene (242) gave β-D-fructofuranosyl 6-deoxy-β-L-idopyranoside hepta-acetate (45% yield), whereas similar hydrogenation of the 5′-ene (243) gave 6′-deoxysucrose



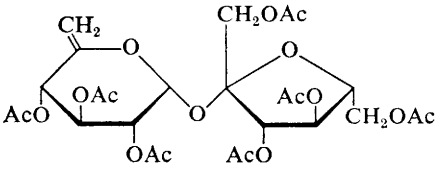
(240) R = Ph, Bn, CH_2CH_2Ph, $CHPh_2$, or $CMePh_2$



(241)



(243)



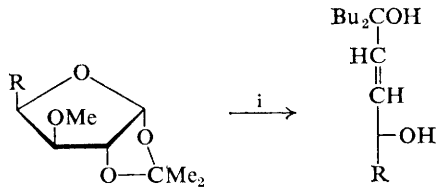
(242)

hepta-acetate (99% yield). The reactions of methyl 3,4-anhydro-6-deoxy-β-L-*arabino*-hex-5-enopyranoside (38) with ammonia and azide and cyanide ions are discussed in Chapter 4,[177] and the ready hydrolysis of a disaccharide 5′-ene is noted in Chapter 15.

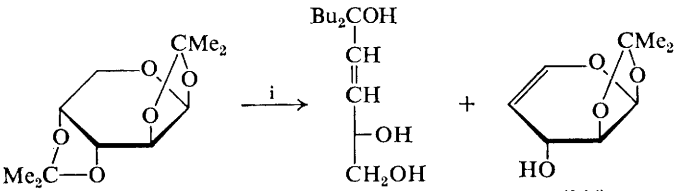
In continuing their investigations of the reactions of carbohydrate derivatives with butyl-lithium, Klemer and her colleagues have examined the reactions of various mono- and di-*O*-isopropylidene acetals, which gave rise to unsaturated derivatives (Scheme 58), albeit in low yield.[438, 438a] It was suggested that these reactions occur by abstraction of the proton at C-1 and loss of acetone to give a 2-deoxy-1-enolate anion, which reverts to the 2,3-unsaturated lactone with expulsion of the substituent at C-3: further attack by butyl-lithium would then give the acyclic products observed. 1,2:3,4-Di-*O*-isopropylidene-β-D-arabino-pyranose also undergoes a competitive reaction, initiated by abstraction of one of the protons at C-5, to give the 4-ene derivative (244).

The (*Z*)-isomer (245) of 1-*O*-acetyl-2,3:4,5-di-*O*-isopropylidene-D-*erythro*-pent-1-enitol was obtained on heating 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-D-arabinose or the D-ribose analogue with an excess of acetic anhydride containing sodium acetate.[439] Acetic anhydride and sodium acetate also converted 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-D-xylose into the (*Z*)-D-*threo*-pent-1-enitol (246), which, like (245), could be photoisomerized to the (*E*)-isomer.

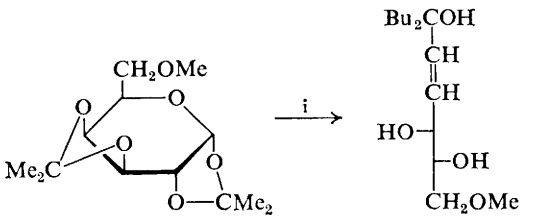
[437] R. Khan and M. R. Jenner, *Carbohydrate Res.*, 1976, **48**, 306.
[438] G. Rodemeyer and A. Klemer, *Chem. Ber.*, 1976, **109**, 1708.
[438a] A. Klemer, G. Rodemeyer, and F.-J. Linnenbaum, *Chem. Ber.*, 1976, **109**, 2849.
[439] A. Ducruix, C. Pascard-Billy, S. J. Eitelman, and D. Horton, *J. Org. Chem.*, 1976, **41**, 2652.



R = CH_2OMe or $CHOMeCH_2OMe$



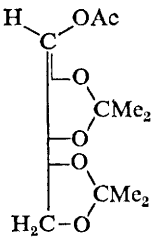
(*E*)- and (*Z*)-isomers (244)



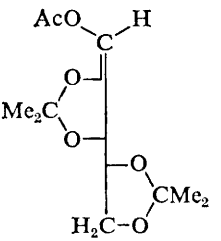
(*E*)- and (*Z*)-isomers

Reagent: i, BuLi–THF

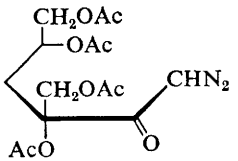
Scheme 58



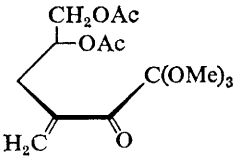
(245)



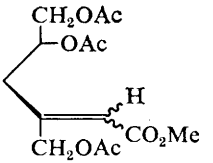
(246)



(247)



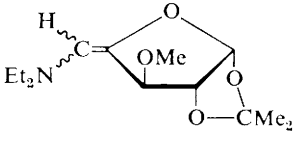
(248)



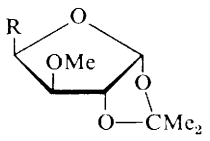
(249)

The stereochemistry about the double bond in (245) and (246) was established by *X*-ray crystallography.

A further study of the use of the Arndt–Eistert reaction in carbohydrate chemistry examined the reactions of the diazoketone (247) (prepared from α-D-glucoisosaccharinic acid), which gave mainly the orthoester (248) and both the (*E*)- and (*Z*)-isomers (249) of a 2,3-unsaturated methyl hexonate derivative on treatment with silver oxide in methanol.[440] The 3-*C*-methylene derivative (248) is a useful precursor of 4-deoxy-3-*C*-methyl-D-hexoses.



(250)



(251) R = CHO
(252) R = $CH{=}CCl_2$
(253) R = $C{\equiv}CCl$

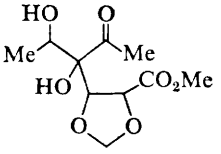
The enamine (250) was formed when 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (251) reacted with diethylamine.[145] Dehydrochlorination of the terminal alkene (252) [prepared by a Wittig reaction on (251)] using the anion derived from *N*-methylaniline gave the alkyne (253). Other alkynic derivatives of sugars are noted in Chapter 3.

[440] D. Glittenberg and I. Dyong, *Chem. Ber.*, 1976, **109**, 3115.

15
Branched-chain Sugars

Natural Products

Methyl eurekanate, one of the products obtained on methanolysis of the antibiotic flambamycin, has been shown to have the structure (254), although the stereochemistry remains to be determined.[441] D-Apiose has been identified as



(254)

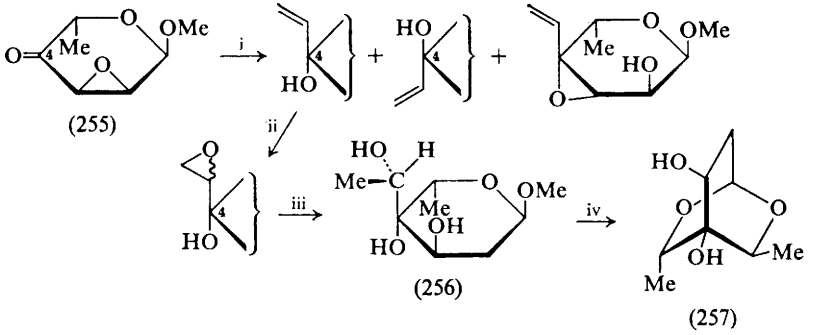
the non-reducing, terminal residue of a tetrasaccharide occurring in platycodin D, the principal saponin from the roots of *Platycodon grandiflorum* de Candolle.[442] This is the first reported example of a saponin containing apiose.

An unsaturated branched-chain sugar found in an antibiotic is referred to in Chapter 20.

Compounds with an $R^1—C—OR^2$ Branch

Paulsen's group has reported the synthesis of derivatives (256) and (257) of the hydroxyethyl-branched octose that is found as a component of the quinocycline complexes isolated from *Streptomyces aureofaciens*.[443] The route from the epoxyketone (255) is outlined in Scheme 59. An identical route was used to prepare branched-chain analogues in the D-series, in which it was shown that acid-catalysed methanolysis of the hydroxyethyl-branched derivative (258) yielded the more stable isomer (259) *via* the anhydro-sugar D-(257). Paulsen's group has also described a synthesis of a derivative (260) of pillarose (see Vol. 9, p. 99), a component of the antibiotic pillaromycin, using the dianion (261) prepared from 2-hydroxymethyl-1,3-dithiane to introduce the branch at C-4 (Scheme 60).[444] A similar approach, using the anion derived from 2-methyl-1,3-dithiane, and subsequent desulphurization and reduction, *etc.*, was adopted in a synthesis of a derivative (262) of aldgarose (4,6-dideoxy-3-*C*-[(*S*)-1-hydroxyethyl]-D-*ribo*-hexopyranose $3,3^1$-cyclic carbonate) [445] (*cf.* Vol. 8, p. 100).

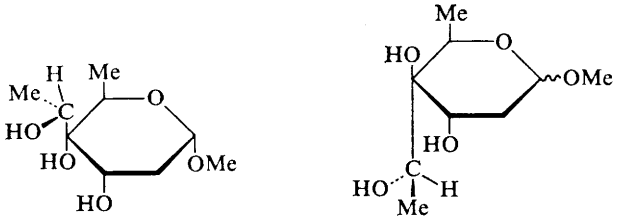
[441] W. D. Ollis, C. Smith, and D. E. Wright, *J.C.S. Chem. Comm.*, 1976, 347.
[442] A. Tada, Y. Kaneiwa, J. Shoji, and S. Shibata, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2965.
[443] H. Paulsen and V. Sinnwell, *Angew. Chem. Internat. Edn.*, 1976, **15**, 438.
[444] H. Paulsen, K. Roden, V. Sinnwell, and W. Koebernick, *Angew. Chem. Internat. Edn.*, 1976, **15**, 439.
[445] H. Paulsen, B. Sumfleth, and H. Redlich, *Chem. Ber.*, 1976, **109**, 1362.



(255) (256) (257)

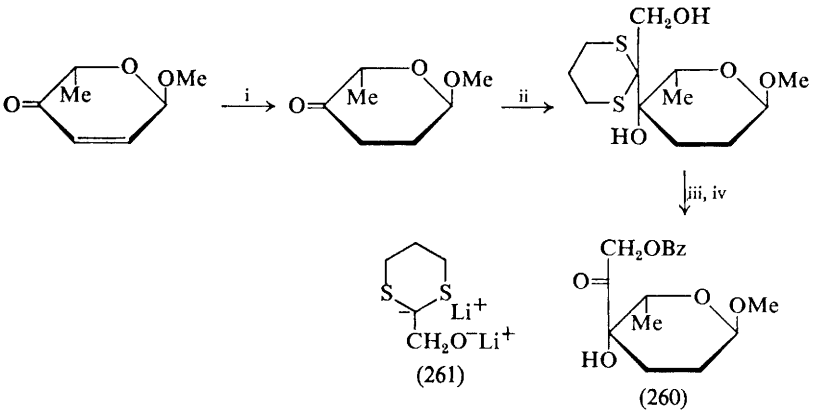
Reagents: i, CH_2=CHLi; ii, *m*-$ClC_6H_4CO_3H$; iii, $LiAlH_4$; iv, H_3O^+

Scheme 59



(258) (259)

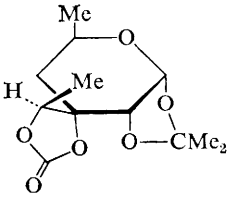
The formation of 4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-2-*C*-methyl-D-*glycero*-D-*talo*-octitol (28) as a by-product in the photoamidation of 2,3,4,6-tetra-*O*-acetyl-2-hydroxy-D-glucal has been noted in Chapter 3.[137] The same paper also reported that acetone-initiated photoamidation of the 3-*C*-(methoxycarbonyl)methylene derivative (263) gave a mixture of two amides, which were transformed into a



(261) (260)

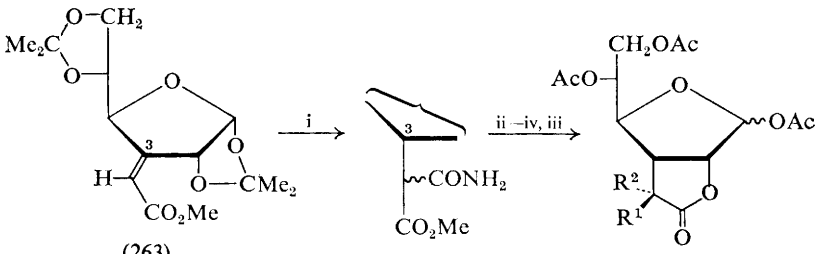
Reagents: i, H_2–cat.; ii, (261); iii, BzCl–py; iv, HgO–BF_3,Et_2O

Scheme 60



(262)

separable mixture of the γ-lactones (264) and (265) by the sequence of reactions shown in Scheme 61. The (*R*)- or (*S*)-chirality assigned to the γ-lactones (264) and (265), respectively, is based on ^{1}H n.m.r. spectroscopy.



(263)

(264) $R^1 = CO_2Me$; $R^2 = H$
(265) $R^1 = H$; $R^2 = CONH_2$

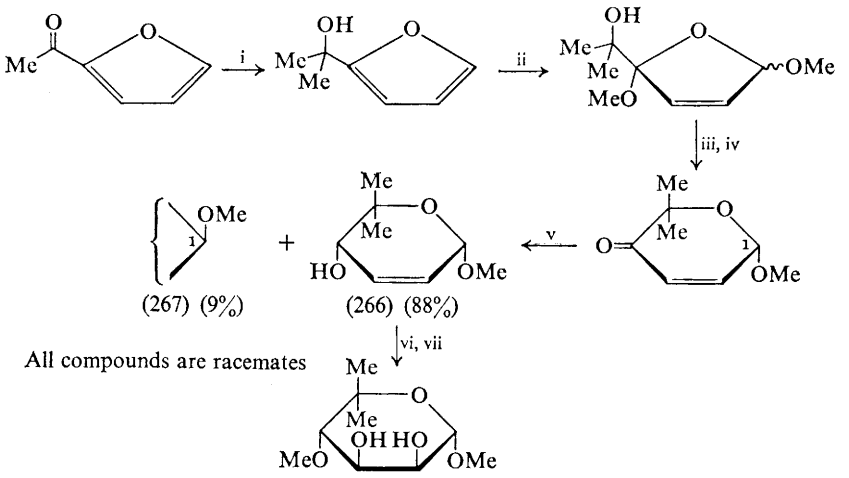
Reagents: i, $HCONH_2$–Me_2CO–*hν*; ii, AcOH; iii, Ac_2O-py; iv, CF_3CO_2H

Scheme 61

A synthesis of racemic methyl α-DL-novioside (268) from 2-acetylfuran is outlined in Scheme 62.[446] Angyal[446a] has contested the authors' contention – based on ^{1}H n.m.r.-spectroscopic examination of the unsaturated derivatives (266) and (267) – that the anomeric configurations originally assigned to the methyl noviosides and novobiocin should be reversed. The configurations shown in Scheme 62 are those assigned by Angyal. A novel route to branched-chain sugars involved the condensation of 2,3-*O*-isopropylidene-D-glyceraldehyde with 4,5-dimethyldioxaphospholen to give the corresponding dioxaphospholan, which furnished a mixture of glycosides of 1-deoxy-3-*C*-methyl-D-*ribo*-hexulose following hydrolysis at pH 5.6 and treatment with acidified methanol (Scheme 63).[447] It is noteworthy that a single configuration, with three chiral centres, was formed in >53% yield starting from a precursor with a single chiral centre.

Methyl α- and β-coumerosides [(269) and (270), respectively], obtained on methanolysis of coumermycin A_1, have been converted into their C-2 epimers by an oxidation–reduction sequence (Scheme 64).[448] The thermodynamic equilibrium between the anomeric coumerosides was shown to differ appreciably from that between the C-2 epimers. The positions of the equilibria indicated that

[446] O. Achmatowicz, jun., G. Grynkiewicz, and B. Szechner, *Tetrahedron*, 1976, **32**, 1051.
[446a] S. J. Angyal, in correspondence with O. Achmatowicz, jun.
[447] S. David, M.-C. Lepine, G. Aranda, and G. Vass, *J.C.S. Chem. Comm.*, 1976, 747.
[448] A. E. Wick, J. F. Blount, and W. Leimgruber, *Tetrahedron*, 1976, **32**, 2057.

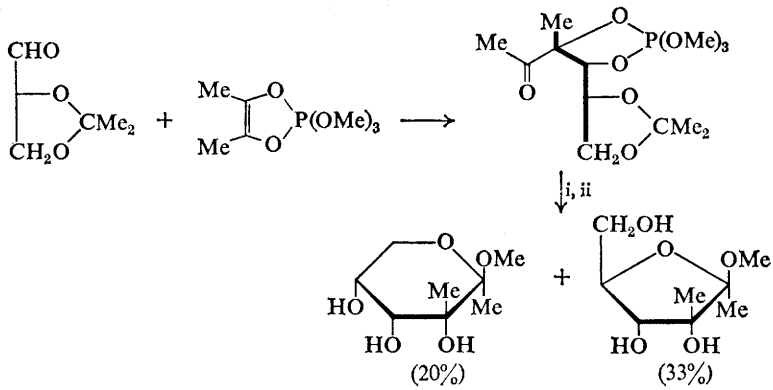


Reagents: i, MeMgBr; ii, Br_2–MeOH; iii, H_3O^+; iv, $(MeO)_3CH$–BF_3; v, $LiAlH_4$; vi, Me_2SO_4–NaOH; vii, OsO_4–H_2O

Scheme 62

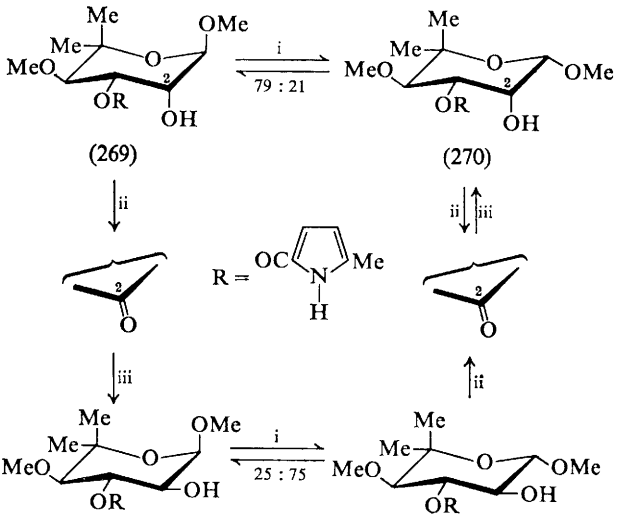
the Δ2 and anomeric effects are of similar magnitude (*ca.* 1.4 kcal mole^{-1}) and that their sum is roughly equal to the free energy of a 1,3-diaxial MeO//Me interaction.

Treatment of 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose with sodium cyanide in ethanol, followed by the addition of an equimolar amount of methyl nitroacetate, gave mainly 3-*C*-cyano-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, whereas the D-allose analogue (85%) was obtained when the keto-sugar was added to a mixture of sodium cyanide and methyl nitroacetate



Reagents: i, H_2O; ii, MeOH–*aci*–resin

Scheme 63



Reagents: i, MeOH–HCl; ii, DMSO–DCC–H^+; iii, $NaBH_4$

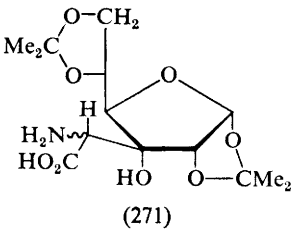
Scheme 64

in ethanol.[449] No explanation was advanced for these interesting results. Rosenthal's group has also converted 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranos-3-ulose into the glycos-3-ylglycine derivatives (271) [3^1-(*S*), 67%; 3^1-(*R*), 6%] by treatment with methyl nitroacetate, catalytic reduction of the resulting nitro derivatives, and alkaline hydrolysis.[450] This 3-ulose and other carbonyl derivatives of monosaccharides have been converted into *C*-allyl and *C*-propargyl derivatives by the action of allyl or propargyl bromide and zinc dust in THF.[451]

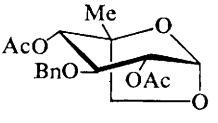
Details of the stereoselective synthesis of derivatives of 3- and 5-*C*-methyl-D-glucose and 5-*C*-methyl-L-idose have appeared (see Vol. 8, p. 106); the configurations were established by conversion of the branched-chain sugars into acetylated pyranoses, namely 1,2,4,6-tetra-*O*-acetyl-3-*C*-methyl-β-D-glucopyranose, 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-5-*C*-methyl-β-D-glucopyranose, and 2,4-di-*O*-acetyl-1,6-anhydro-3-*O*-benzyl-5-*C*-methyl-β-L-idopyranose (272), respectively.[452] The stereoselectivities of the addition of methyl magnesium iodide, diazomethane, and nitromethane to methyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-*arabino*-hexopyranosidulose have been investigated.[453] Whereas the Grignard reagent furnished a 2-*C*-methyl derivative having the D-*gluco* configuration (attack from the less-hindered side), diazomethane and nitromethane gave a spiro-oxiran or a 2-*C*-nitromethyl derivative, respectively, having the D-*manno* configuration (attack from the more-hindered side).

[449] A. Rosenthal and B. L. Cliff, *Canad. J. Chem.*, 1976, **54**, 543.
[450] A. Rosenthal and B. L. Cliff, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 263.
[451] Yu. A. Zhdanov, Yu. E. Alekseev, and V. A. Tyumenev, *Doklady Akad. Nauk S.S.S.R.*, 1976, **226**, 1334 (*Chem. Abs.*, 1976, **85**, 33 285e).
[452] M. Funabashi, H. Sato, and J. Yoshimura, *Bull. Chem. Soc. Japan*, 1976, **49**, 788.
[453] J. Yoshimura, K. Mikami, K. Sato, and C. Shin, *Bull. Chem. Soc. Japan*, 1976, **49**, 1686.

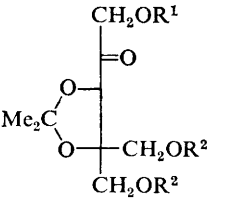
In last year's Report (Vol. 9, p. 102) it was noted that an attempt to synthesize derivatives of L-dendroketose [4-*C*-(hydroxymethyl)-L-*glycero*-pentulose] led to racemization when an intermediate in the synthesis, 1,5-di-*O*-benzoyl-4-*C*-(benzoyloxymethyl)-3,4-*O*-isopropylidene-L-*glycero*-pentulose (273) ($R^1 = R^2 =$ Bz), was debenzoylated with methanolic sodium methoxide. This problem has



(271)



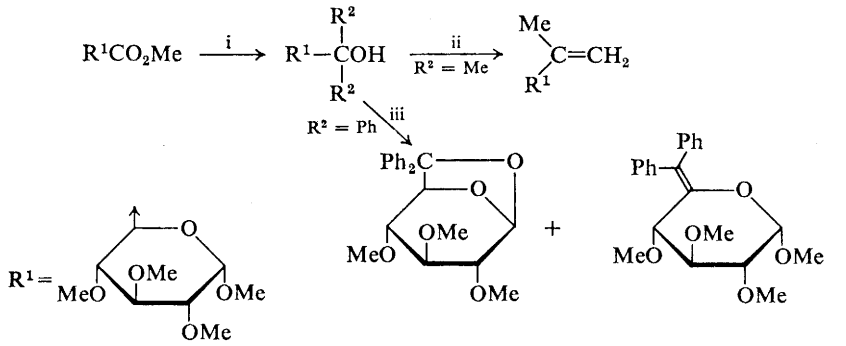
(272)



(273)

now been overcome using a derivative (273) (R^1 = Tr; R^2 = Bn) protected with trityl and benzyl groups, which were removed by catalytic hydrogenolysis to give 3,4-*O*-isopropylidene-L-dendroketose.[454]

Aspinall's group has explored the possibility of modifying the hexuronic acid (or ester) residues in acidic polysaccharides by converting them into hex-5-enopyranosides, which might then undergo selective fragmentation.[455] Model studies showed that methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)-uronate reacted with methyl or phenyl magnesium halides to give the corresponding tertiary alcohols, which, on treatment with thionyl chloride in pyridine or on heating with DMSO, gave either the hex-6-enopyranoside or the 1,6-anhydro derivative and some of the desired hex-5-enopyranoside, respectively (Scheme 65).



Reagents: i, MeMgI or PhMgBr–Et_2O; ii, $SOCl_2$–py; iii, DMSO at 165 °C

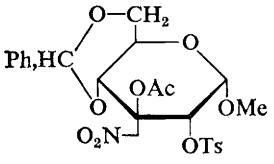
Scheme 65

[Methyl 6-*O*-(6-deoxy-2,3,4-tri-*O*-methyl-β-L-*arabino*-hex-5-enopyranosyl)-2,3,4-tri-*O*-methyl-β-D-glucopyranoside – which was prepared from methyl 2,2′,3,3′,4,4′-hexa-*O*-methyl-β-melibioside by reaction with triphenylphosphite methiodide and dehydroiodination of the 6′-deoxy-6′-iodo derivative – underwent selective

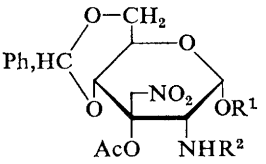
[454] E. B. Rathbone and G. R. Woolard, *Carbohydrate Res.*, 1976, **46**, 183.
[455] G. O. Aspinall, O. Igarashi, T. N. Krishnamurthy, W. Mitura, and M. Funabashi, *Canad. J. Chem.*, 1976, **54**, 1709.

hydrolysis with acid at room temperature with the liberation of methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranoside.]

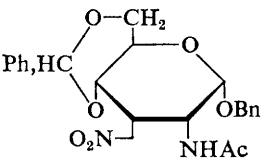
Some examples of the addition of nitromethane to alkyl 2-acylamino-4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranosid-3-uloses and a 2-toluene-*p*-sulphonyloxy analogue have been reported.[456] The 3-acetates (274) and (275), prepared by acetylation of the resulting adducts, gave nitroalkane-branched derivatives (276) and (277) on reduction with sodium borohydride in acetonitrile, *via* the exocyclic nitroalkenes. Treatment of the D-*gluco*-compound (274) with methanolic ammonia yielded a mixture of exocyclic (278) and endocyclic (279)



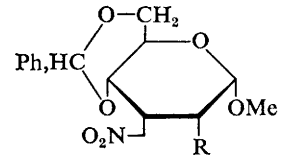
(274)



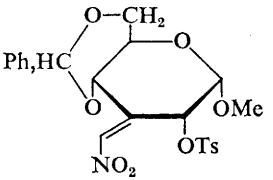
(275) R^1 = Me; R^2 = Bz or R^1 = Bn; R^2 = Ac



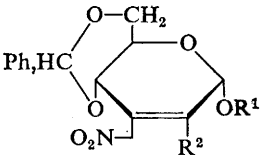
(276)



(277) R = OTs or NHBz



(278)



(279) R^1 = Me; R^2 = OTs or R^1 = Bn; R^2 = NHAc

(R^1 = Me, R^2 = OTs) nitroalkenes; the preference for the endocyclic double bond was demonstrated by isomerization of (278) to (279) (R^1 = Me; R^2 = OTs) (60% yield) in boiling pyridine. The D-*allo*-compound (275) (R^1 = Bn; R^2 = Ac) gave only the endocyclic nitroalkene (279) (R^1 = Bn; R^2 = NHAc) on dehydroacetoxylation with potassium t-butoxide in THF, whereas the exocyclic nitroalkene (278) was obtained on similar treatment of the D-*gluco*-compound (274).

Oxirans derived from branched-chain sugars are mentioned in Chapter 4, while the m.s. of some 2,6-dideoxy-3-*C*-methylhexopyranose derivatives is referred to in Chapter 24.

Compounds with an $R^1—C—R^2$ Branch

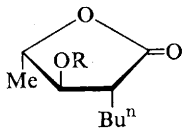
Syntheses of naturally occurring (+)-blastmycinone (280) and its C-3 and C-4 stereoisomers have been reported.[457] The *C*-butyl branch was introduced by reaction of methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside with n-butyl magnesium chloride to give methyl 4,6-*O*-benzylidene-3-*C*-butyl-3-deoxy-α-D-altropyranoside, which was degraded and transformed into the L-*arabino* isomer (280) (see Vol. 9, p. 204).

Treatment of methyl 2,3-anhydro-5-*O*-trityl- or -benzyl-α-D-ribofuranoside with 2-lithio-1,3-dithiane, followed by desulphurization of the dithiane adducts,

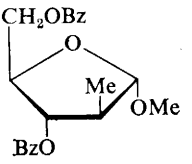
[456] J. H. Jordaan, J. J. Nieuwenhuis, and G. J. Lourens, *Carbohydrate Res.*, 1976, **51**, 195.
[457] M. Kinoshita, S. Aburaki, and N. Konishi, *Asahi Garasu Kogyo Gijutsu Shoreikai Kenkyu Hokoku*, 1974, **25**, 103 (*Chem. Abs.*, 1976, **84**, 5268n).

has provided a regioselective route to novel 2-*C*-alkyl-2-deoxy-D-arabinofuranose derivatives [*e.g.* (281)].[458]

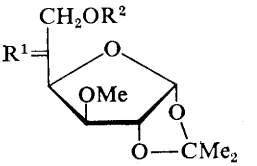
Methylene dimagnesium bromide can be used to convert appropriately protected keto-sugars [*e.g.* (282)] into the corresponding *C*-methylene derivatives [*e.g.* (283)], and offers a useful alternative to the Wittig reaction.[459] Tronchet's group has used the Wittig reaction to prepare 3-*C*-difluoromethylene and 3-*C*-chlorofluoromethylene derivatives of 1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*- and -*xylo*-hexofuranos-3-uloses [*e.g.* (284) and (285)].[460] The 3-*C*-chlorofluoromethylene derivatives [*e.g.* (285)] were reductively dechlorinated with lithium aluminium hydride, yielding the corresponding 3-*C*-fluoromethylene derivatives [*e.g.* (286)] with inversion of configuration at the double bond.



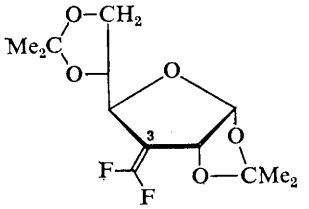
(280) R = $COBu^t$



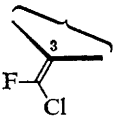
(281)



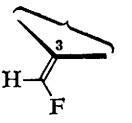
(282) R^1 = O; R^2 = Bn or Tr
(283) R^1 = CH_2; R^2 = Bn or Tr



(284)

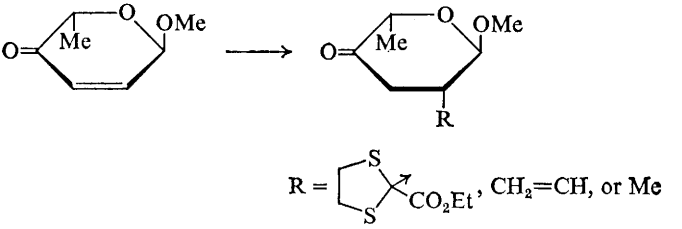


(285)



(286)

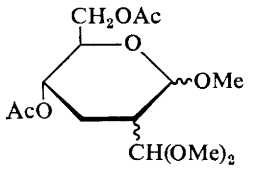
Derivatives of unsaturated sugars have been used as starting materials for the synthesis of a number of branched-chain sugars. Pyranosidic enones, for example, were shown to undergo 1,4-additions with lithium dimethyl cuprate, vinyl magnesium bromide, and 2-lithio-2-ethoxycarbonyl-1,3-dithiane, with the carbanion adding *trans* to the glycosidic substituent (Scheme 66).[461] 4,6-Di-*O*-



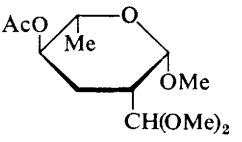
Scheme 66

[458] A. Yamashita and A. Rosowsky, *J. Org. Chem.*, 1976, **41**, 3422.
[459] J. Yoshimura, K. Sato, H. Wakai, and M. Funabashi, *Bull. Chem. Soc. Japan*, 1976, **49**, 1169.
[460] J. M. J. Tronchet, D. Schwarzenbach, and F. Barbalat-Rey, *Carbohydrate Res.*, 1976, **46**, 9.
[461] H. Paulsen, W. Koebernick, and H. Koebernick, *Tetrahedron Letters*, 1976, 2297.

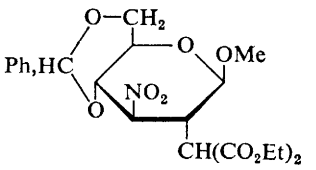
acetyl-1,5-anhydro-2,3-dideoxy-D-*erythro*-hex-1-enitol (4,6-di-*O*-acetyl-3-deoxy-D-glucal) reacted with trimethyl orthoformate in the presence of boron trifluoride etherate to give three of the four possible stereoisomers (287), namely methyl 4,6-di-*O*-acetyl-2,3-dideoxy-2-dimethoxymethyl-α- and -β-D-*arabino*-hexopyranosides and the α-D-*ribo* analogue, in the ratio 7 : 6 : 10.[462] A similar reaction with 4-*O*-acetyl-1,5-anhydro-2,3,6-trideoxy-L-*erythro*-hex-1-enitol (4-*O*-acetyl-3-deoxy-L-rhamnal) gave a single stereoisomer (288). However, orthoesters of acetic and benzoic acids failed to give related branched-chain derivatives. Following their earlier studies in the α-series (Vol. 9, p. 104), Sudoh and his co-workers have examined the alkylation of methyl 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-β-D-*erythro*- and -*threo*-hex-2-enopyranosides with a series of active



(287)



(288)



(289)

methylene compounds using a phase-transfer process; for example, the β-D-*erythro*-compound reacted with diethyl malonate in the presence of hexadecyltributylphosphonium bromide as a phase-transfer catalyst to give methyl 4,6-*O*-benzylidene-2,3-dideoxy-2-bis(ethoxycarbonyl)methyl-3-nitro-β-D-glucopyranoside (289), whereas similar treatment of the β-D-*threo*-compound gave the D-galactose analogue of (289).[463]

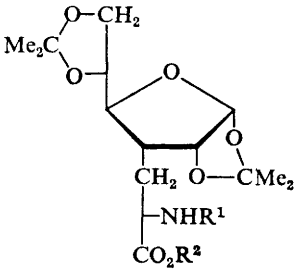
L-3-(3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranos-3-yl)alanine (290) ($R^1 = R^2 = H$) has been synthesized by a route that involved the addition of the carbanion derived from methyl (methylthio)methyl sulphoxide to 3-*C*-cyanomethyl-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose.[464] Subsequent treatment of the resulting (glycosyl)enamino sulphoxide with acetic anhydride, base-catalysed ester-exchange, and reductive desulphurization afforded the *N*-acetyl-3-(glycos-3-yl)alanate (290) (R^1 = Ac; R^2 = Me), which was hydrolysed to (290) ($R^1 = R^2 = H$) with base.

Other derivatives of branched-chain sugars are referred to in Chapters 14, 17, and 21.

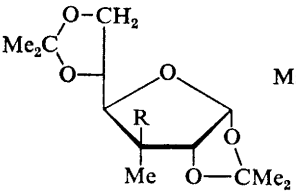
Compounds with an R—C—N Branch

Addition of mercuric azide to 1,2:5,6-di-*O*-isopropylidene-3-*C*-methylene-α-D-*ribo*-hexofuranose yielded, after reductive demercuration, the branched-chain azide (291), which could be transformed into the corresponding acetamido derivative (292).[465] The conversion of (291) into the 3,6-acetylepimino derivative (293) established that it has the D-*gluco* configuration (see Vol. 9, p. 106).

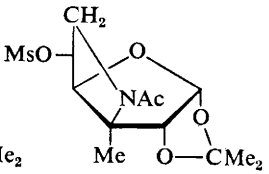
[462] K. Heyns, R. Hohlweg, J. I. Park, and J. Thiem, *Tetrahedron Letters*, 1976, 1481.
[463] T. Sakakibara, M. Yamada, and R. Sudoh, *J. Org. Chem.*, 1976, **41**, 736.
[464] A. Rosenthal and A. J. Brink, *Carbohydrate Res.*, 1976, **46**, 289.
[465] J. S. Brimacombe, J. A. Miller, and U. Zakir, *Carbohydrate Res.*, 1976, **49**, 233.



(290)



(291) R = N_3
(292) R = NHAc



(293)

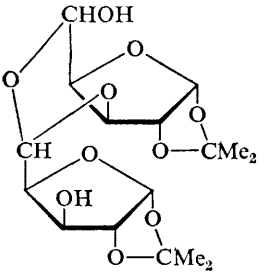
16

Aldehydo-sugars, Aldosuloses, Dialdoses, and Diuloses

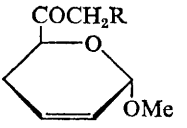
The occurrence of 6-deoxy-D-*arabino*-hexofuranos-5-ulose as a component of an antibiotic is noted in Chapter 20.

γ-Irradiation of crystalline β-D-fructose yielded 6-deoxy-D-*threo*-2,5-hexodiulose *via* a chain reaction, but the G-value for the formation of 6-deoxy-D-*threo*-2,5-hexodiulose decreased with increasing dose.[466, 466a] γ-Irradiation of D-fructose is a convenient method for preparing this diulose (a yield of 6% is obtained at a dose of 10^{21} eV g^{-1}).[466] The chemical modifications induced in polycrystalline cycloamylose hydrates during γ-irradiation have been investigated by using g.l.c.–m.s. to analyse the monosaccharides released on subsequent enzymic and acidic hydrolysis; 3-deoxy-D-*erythro*-hexos-4-ulose, D-*xylo*-hexos-5-ulose, 6-deoxy-D-*xylo*-hexos-5-ulose, 5-deoxy-D-*xylo*-hexodialdose, a 2,6-dideoxyhexos-5-ulose, and a 3-deoxypentulose were among the products identified.[467] D-*threo*-2,5-Hexodiulose, a metabolic product of acetic acid bacteria utilizing D-fructose or L-sorbose, has been shown to form a dimer between a furanose form and a pyranose form in the crystalline state.[468] Methyl α- and β-D-[5-^{18}O]xylopyranosides have been prepared by way of oxygen exchange between [^{18}O]water and the dimeric product (294) obtained on oxidation of 1,2-*O*-isopropylidene-α-D-glucofuranose with periodate.[56a]

Levoglucosenone (1,6-anhydro-3,4-dideoxy-β-D-*glycero*-hex-3-enopyranosulose) has been identified as a product of the pyrolysis of phenyl β-D-glucopyrano-



(294)



(295) R = Me, Et, Bu, *etc.*

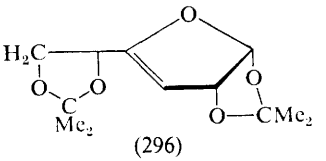
[466] M. Dizdaroglu, J. Leitich, and C. von Sonntag, *Carbohydrate Res.*, 1976, **47**, 15.
[466a] S. Kawakishi, Y. Kito, and M. Namiki, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1897.
[467] P. J. Baugh, J. I. Goodall, G. O. Phillips, C. von Sonntag, and M. Dizdaroglu, *Carbohydrate Res.*, 1976, **49**, 315.
[468] L. K. Hansen, A. Hordvik, and R. Hove, *J.C.S. Chem. Comm.*, 1976, 572.

side, levoglucosan, and cellulose and its chlorinated and phosphorylated derivatives.[469] The yield of levoglucosenone was appreciably reduced at higher temperatures, indicating that decomposition of levoglucosenone occurred under the pyrolytic conditions, although it was substantially improved when an acidic catalyst (*e.g.* zinc chloride or diphenyl hydrogen phosphate) was added.

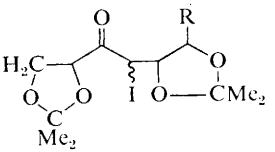
Syntheses of methyl 2,6-dideoxy-4-*O*-methyl-α-L-*erythro*-hexopyranosid-3-ulose [190] and 3,6-dideoxy-D-*erythro*-hexos-4-ulose [423] are referred to in Chapters 5 and 13, respectively.

A series of racemic 6-acyl-2-methoxy-5,6-dihydro-(2*H*)-pyrans (295) has been prepared from the corresponding 6-alkoxycarbonyl derivatives by Claisen condensation with alkyl esters and subsequent decarboxylation of the resulting β-keto-esters.[470]

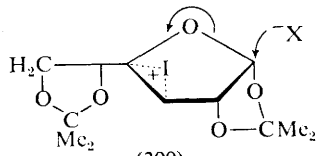
3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enofuranose (296) reacted with iodine and thallium(I) fluoride, isocyanate (and methanol), or acetate in an inert solvent to give the acyclic ketones (297)—(299) in high yield.[293] This unusual opening of the furanose ring presumably involves the iodonium ion (300) as an intermediate.



(296)

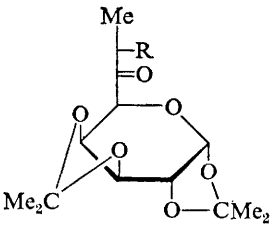


(297) R = F
(298) R = $NHCO_2Me$
(299) R = OAc

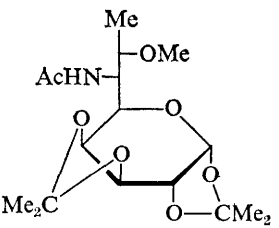


(300)

The configuration of a keto-ether (301; R = OMe) obtained in 28% yield on methanolysis of the diazoketone (301; R = N_2) has been shown to have the L-*glycero*-D-*galacto* configuration, rather than the D- or L-*glycero*-L-*altro* configuration previously suggested by c.d. (see Vol. 8, p. 135), by formation of the *N*-acetyl-lincosamine derivative (302) and its C-6 epimer.[471]



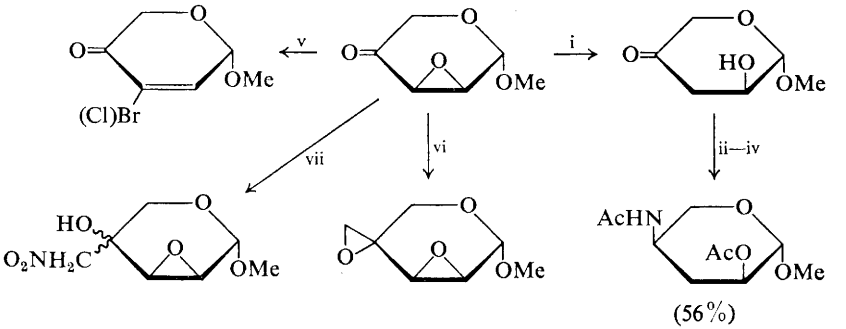
(301)



(302)

[469] F. Shafizadeh and P. P. S. Chin, *Carbohydrate Res.*, 1976, **46**, 149.
[470] A. Konowal, K. Belniak, J. Jurczak, M. Chmielewski, O. Achmatowicz, jun., and A. Zamojski, *Roczniki Chem.*, 1976, **50**, 505.
[471] S. David and J.-C. Fischer, *Carbohydrate Res.*, 1976, **46**, 273.

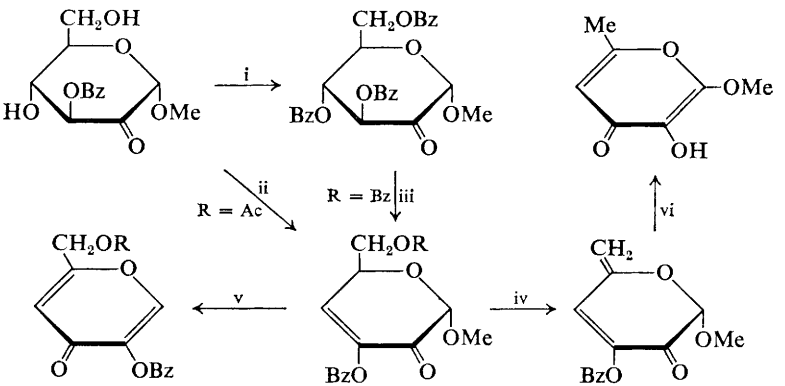
Details have appeared on the synthesis and ring-opening reactions (*e.g.* with halide ions) of a number of methyl anhydropyranosiduloses [472] (see Vol. 8, pp. 58 and 94). Related studies by Overend's group are summarized in Scheme 67.[473] The uses of glycopyranosiduloses as synthetic intermediates are covered in Chapters 13 and 15.



Reagents: i, H_2–Pd; ii, NH_2OH; iii, H_2–Pt; iv, Ac_2O–py; v, HCl or HBr; vi, CH_2N_2 or $Me_2\overset{+}{S}OCH_2^-$; vii, $MeNO_2$–MeONa

Scheme 67

The diacylated 4-deoxyhex-3-enopyranosiduloses (303), prepared from methyl 3-*O*-benzoyl-α-D-*arabino*-hexopyranosidulose, were converted in basic media and, less readily, in acidic media into the γ-pyrone system (Scheme 68).[474] Synthetic routes to ethyl 2,3,6-trideoxy-α-D-*glycero*-hex-2-enopyranosid-4-ulose (236) are noted in Chapter 14 (see Scheme 57).[433]



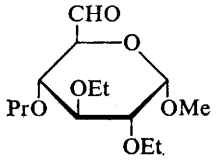
(303) R = Ac or Bz

Reagents: i, BzCl–py; ii, Ac_2O–py; iii, H^+; iv, py; v, H^+ or base; vi, H_2O

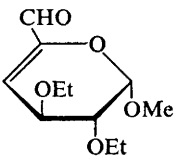
Scheme 68

[472] H. Paulsen and K. Eberstein, *Chem. Ber.*, 1976, **109**, 3907.
[473] G. S. Hajivarnava, W. G. Overend, and N. R. Williams, *Carbohydrate Res.*, 1976, **49**, 93.
[474] F. W. Lichtenthaler, K. Strobel, and G. Reidel, *Carbohydrate Res.*, 1976, **49**, 57.

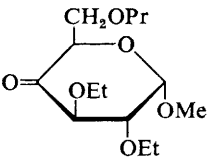
Degradation of methyl 2,3-di-*O*-ethyl-4-*O*-propyl-α-D-*gluco*-hexodialdo-1,5-pyranoside (304) with sodium ethoxide yielded the methyl β-L-*threo*-hex-4-enopyranoside derivative (305), which lost most of the remaining alkoxy-groups on heating with aqueous acetic acid.[475] The isomeric hexopyranosid-4-ulose (306) also rapidly lost a substantial proportion of its alkoxy-groups on treatment either with sodium butoxide or with sodium butoxide followed by aqueous acetic acid. Both (304) and (306) also released methanol, ethanol, and propanol



(304)



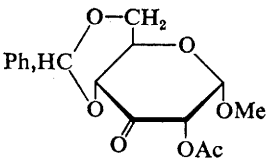
(305)



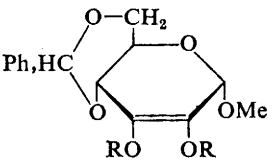
(306)

on heating at 100 °C with aqueous acetic acid. These model experiments provide an understanding of the degradation of methylated polysaccharides containing oxidized residues under basic and acidic conditions. The alkaline degradation of a partially methylated disaccharide containing an oxidized residue is also discussed in Chapter 3.[112]

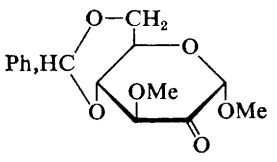
Several reports have dealt with the stereochemistry of the reduction of alkyl or aryl 4,6-*O*-benzylidenehexopyranosid-2- and -3-uloses. Reduction of methyl 2-*O*-acetyl-4,6-*O*-benzylidene-α-D-*ribo*-hexopyranosid-3-ulose (307) with sodium borodeuteride in moist methanol gave methyl 4,6-*O*-benzylidene-α-D-[3-^{2}H]-allopyranoside (equatorial attack by the reductant), whereas a mixture of the



(307)



(308)



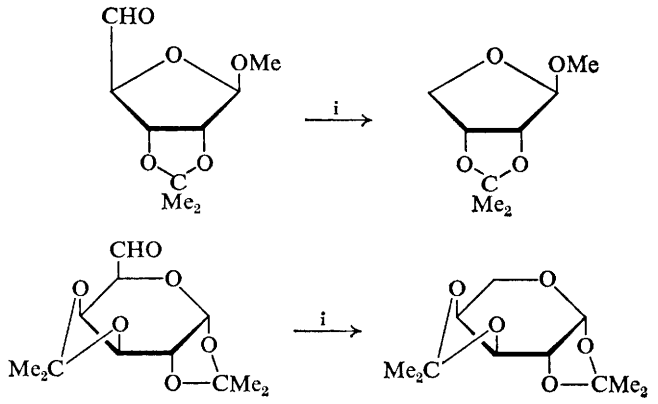
(309)

α-D-[3-^{2}H]allopyranoside derivative and methyl 4,6-*O*-benzylidene-α-D-[2-^{2}H]-glucopyranoside was obtained following reduction with sodium borodeuteride in dry propan-2-ol and subsequent deacetylation.[476] Formation of the 2-deuteriated D-glucopyranoside derivative may involve stereospecific attack of the reductant at C-2 of a 2,3-enediol intermediate (308; R = H), and the ready generation of a 2,3-enediol from (307) was demonstrated by the preparation of the enediol diacetate (308; R = Ac). These results emphasize the need for caution when labelling experiments are used to locate a carbonyl group in sugar derivatives, at least when dry alcoholic media are used. Reduction of methyl 4,6-*O*-benzylidene-2-*O*-methyl-α- and -β-D-*ribo*-hexopyranosid-3-ulose and methyl 4,6-*O*-benzylidene-3-*O*-methyl-β-D-*arabino*-hexopyranosidulose with sodium borohydride gave principally or exclusively the products arising from equatorial

[475] P.-E. Jansson, L. Kenne, B. Lindberg, and S. Svensson, *Acta Chem. Scand.* (*B*), 1976, **30**, 631.
[476] D. C. Baker, J. Defaye, A. Gadelle, and D. Horton, *J. Org. Chem.*, 1976, **41**, 3834.

attack by the reductant, whereas similar reduction of methyl 4,6-*O*-benzylidene-3-*O*-methyl-α-D-*arabino*-hexopyranosidulose (309) gave only the related D-glucopyranoside (*i.e.* axial attack by the reductant).[477] These results are consistent with the operation of steric approach control during the reductions. The conversion of aryl 3-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosides into D-mannose analogues by an oxidation–reduction sequence is noted in Chapter 3.[98]

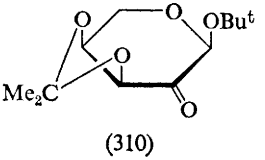
Suitably protected ω-*aldehydo*-sugar derivatives can be decarbonylated using tris(methyldiphenylphosphine)rhodium chloride (Scheme 69).[478]



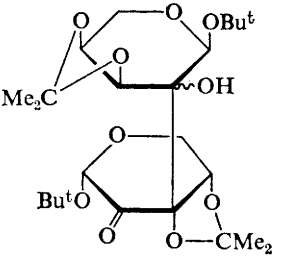
Reagent: i, $(MePh_2P)_3RuCl$–PhMe–PhCN

Scheme 69

An attempt to anomerize t-butyl 3,4-*O*-isopropylidene-α-L-*erythro*-pentopyranosidulose (310) by heating with DBU in dichloromethane yielded instead the dimer (311), which is the product of an aldol condensation.[479] Formation of



(310)



(311)

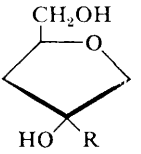
the dimer (311) indicated that enolization of (310) involves abstraction of the proton at C-3, the carbon atom that carries the smaller number of oxygen substituents.

[477] Y. Kondo, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2251.
[478] D. J. Ward, W. A. Szarek, and J. K. N. Jones, *Chem. and Ind.*, 1976, 162.
[479] P. M. Collins and R. Iyer, *Carbohydrate Res.*, 1976, **46**, 277.

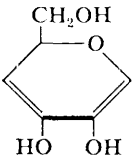
17
Sugar Acids and Lactones

Aldonic Acids

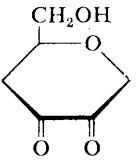
A 1,4-anhydroisosaccharinic acid (312) has been identified as one of the principal products formed by end-wise degradation of cellulose with alkali; it is presumably formed by way of a benzylic acid-type rearrangement on the *keto*-form (314) of the pyran derivative (313).[480] Characterization of (312) was accomplished



(312) R = CO_2H
(315) R = H



(313)

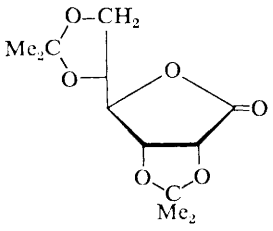


(314)

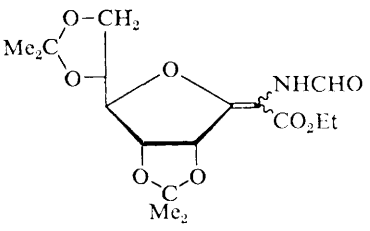
by its conversion into (315), following reduction of the methyl ester, oxidation with periodate, and reduction of the resulting ketone with potassium borohydride. Reference to the products formed on alkaline degradation of cellobiose is made in Chapter 2.[41a]

Unsaturated derivatives of aldonic acids are mentioned in Chapters 13 and 14, while related amino derivatives are referred to in Chapter 8.

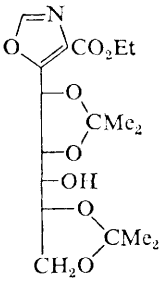
Protected aldono-1,4- or -1,5-lactones [*e.g.* (316)] reacted with ethyl isocyanoacetate and base in an aprotic medium to give either α-(formylamino)acrylic ester derivatives [*e.g.* (317)] or, where base-catalysed elimination reactions can be suppressed, acyclic oxazole derivatives [*e.g.* (318)].[481]



(316)



(317)



(318)

[480] G. Petersson and O. Samuelson, *Acta Chem. Scand.* (*B*), 1976, **30**, 27.
[481] S. J. Eitelman, R. H. Hall, and A. Jordaan, *J.C.S. Chem. Comm.*, 1976, 923.

A procedure for separating mixtures of D-ribono- and D-arabinono-1,4-lactones involved acid-catalysed benzylidenation and precipitation of the 2,3-*O*-benzylidene-D-ribono-1,4-lactone formed.[482]

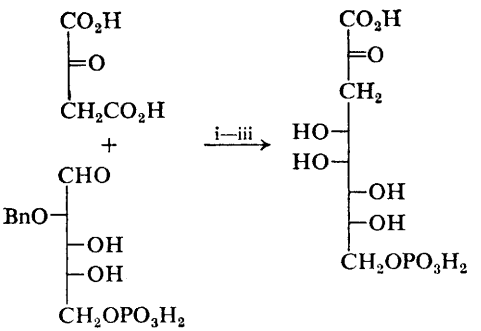
Aldonic acids (*e.g.* melibionic and gentiobionic acids) derived from reducing disaccharides have been coupled to the amino-groups of proteins with the aid of a water-soluble carbodi-imide.[483] The immunochemical properties of some of the synthetic glycoproteins were assessed using plant agglutinins and antibodies raised towards the conjugates.

The hydrolysis of D-galactono-1,4-lactone has been examined using pH, optical rotation, and conductance methods,[484] and the abilities of a series of aldonic, alduronic, and aldaric acids to form complexes with Fe^{3+}, Fe^{2+}, Mn^{2+}, Cu^{2+}, Al^{3+}, Ni^{2+}, and Co^{2+} ions have been investigated using paper chromatography.[485] Bivalent metal ions have been shown to influence the proportions of D-*ribo*- and D-*arabino*-cyanohydrins obtained when D-erythrose reacts with sodium cyanide.[486]

2,3:5,6-Di-*O*-cyclohexylidene-D-mannono-1,4-lactone furnished the corresponding 1,2-unsaturated 3-deoxy-4-nonulose derivatives on treatment with either allyl or propargyl bromide in THF in the presence of zinc dust.[451]

Ulosonic Acids

3-Deoxy-D-*manno*-octulosonic acid 8-(dihydrogen phosphate) has been synthesized by base-catalysed condensation of 2-*O*-benzyl-D-arabinose 5-phosphate with oxalacetate, followed by hydrogenolytic removal of the protecting benzyl group (Scheme 70).[487] It is necessary to use the 2-*O*-benzyl derivative, since D-arabinose 5-phosphate undergoes spontaneous isomerization to D-*erythro*-pentulose 5-phosphate. The 9-*O*-acetyl and 4,9-di-*O*-acetyl derivatives of the



Reagents: i, HO^-; ii, H_2–Pd–C; iii, ion-exchange chromatography

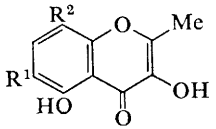
Scheme 70

[482] V. T. Novikov, E. V. Gromova, I. A. Avrutskaya, and M. Ya. Fioshin, *Khim.-Farm. Zhur.*, 1976, **10**, 89 (*Chem. Abs.*, 1976, **85**, 63 264q).
[483] J. Lönngren, I. J. Goldstein, and J. E. Niederhuber, *Arch. Biochem. Biophys.*, 1976, **175**, 661.
[484] S. K. Dutta, *Indian J. Chem.*, 1975, **13A**, 980 (*Chem. Abs.*, 1976, **84**, 90 441f).
[485] M. E. Shishniashvili, M. I. Goguadze, and A. Ya. Khorlin, *Khelaty Met. prirod. Soedinenii Ikh. Primen.*, 1974, **1**, 26 (*Chem. Abs.*, 1976, **84**, 74 545n).
[486] J. Stepinski and J. Swiderski, *Roczniki Chem.*, 1976, **50**, 1991.
[487] D. Charon and L. Szabó, *J.C.S. Perkin I*, 1976, 1628.

methyl ester of *N*-acetyl-β-D-neuraminic acid methyl glycoside have been prepared (see also p. 67).[347]

Uronic Acids

Two new chromones, (319) and (320), have been isolated and identified after heating D-glucuronic or D-galacturonic acids in slightly acidic aqueous solutions at 96 °C.[488] The chromone (320) was also formed when D-xylose was similarly

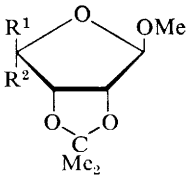


(319) R^1 = OH; R^2 = H
(320) R^1 = H; R^2 = OH

treated. The rates of periodate oxidation of methyl (methyl 4-*O*-methyl-α-D-glucopyranosid)uronate, methyl 4-*O*-methyl-α-D-glucopyranoside, and several complex polyuronides have been measured; the D-glucopyranosyluronic acid residues in hyaluronic acid, chondroitin 4- and 6-sulphates, and chondroitin sulphate D exhibited anomalously low rates of oxidation.[489] Polarographic reduction of D-glucuronic acid has been shown to involve the formation of D-glucofuranurono-6,3-lactone, which is then reduced in two stages.[490] The reactions of D-glucofuranurono-6,3-lactone have been reviewed by Dax and Weidmann.[491]

The g.l.c. of TMS ethers of hexuronic acid derivatives is referred to in Chapter 26.

Brief treatment of methyl (methyl 2,3-*O*-isopropylidene-β-D-ribofuranosid)-uronate (321) with an equimolar proportion, or a slight excess, of sodium methoxide did not yield the 3,4-unsaturated 3-deoxypentofuranosiduronate, but



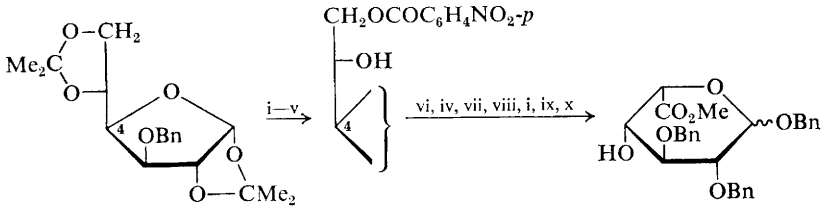
(321) R^1 = CO_2Me; R^2 = H
(322) R^1 = H; R^2 = CO_2Me

gave instead the α-L-lyxose analogue (322) (52%).[492] [However, treatment of (321) with a large excess of sodium methoxide in methanol resulted in loss of the acetal group and formation of the unsaturated pentofuranosiduronate derivative

[488] T. Popoff and O. Theander, *Acta Chem. Scand.* (*B*), 1976, **30**, 705.
[489] J. E. Scott and M. J. Tigwell, *Biochem. Soc. Trans.*, 1975, **3**, 662.
[490] M. Bhaduri, F. H. Chowdhury, and N. B. Fouzder, *Indian J. Chem.*, 1976, **14A**, 284 (*Chem. Abs.*, 1976, **85**, 160 445g).
[491] K. Dax and H. Weidmann, *Adv. Carbohydrate Chem. Biochem.*, 1976, **33**, 190.
[492] M. P. Kotick and D. L. Leland, *Carbohydrate Res.*, 1976, **46**, 299.

(see R. S. Schmidt, D. Heermann, and K.-H. Jung, *Annalen*, 1974, 1856)]. 1,2-Acetals of D-glucofuranurono-6,3-lactone reacted with 2,3,4,6-tetra-*O*-acetyl-α-D-hexopyranosyl bromides under Koenigs–Knorr conditions to give β-(1 → 5)-linked derivatives, which could be reduced to the corresponding disaccharides [*e.g.* 5-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,2-*O*-isopropylidene-α-D-glucofuranose], using sodium borohydride in a protic solvent, or to the unsaturated derivative (323), using sodium borohydride in an aprotic solvent.[493] The derived disaccharide uronamides were converted into 6-amino-derivatives (*e.g.* 6-amino-6-deoxy-5-*O*-β-D-glucopyranosyl-D-glucofuranose) by standard reactions.

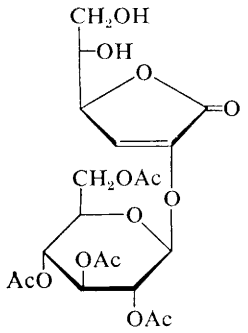
Methyl (benzyl 2,3-di-*O*-benzyl-α- and -β-L-idopyranosid)uronates have been synthesized from 3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (Scheme 71).[494] ^{1}H N.m.r. spectroscopy indicated that both anomers adopt



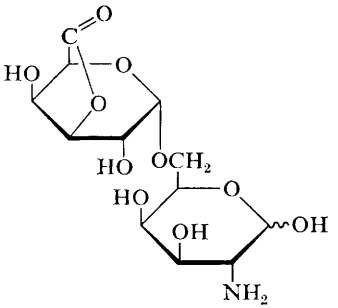
Reagents: i, H^+; ii, TsCl–py; iii, AcOK–Ac_2O; iv, MeONa; v, *p*-$NO_2C_6H_4COCl$–py; vi, BnOH–H^+; vii, PhCHO–$ZnCl_2$; viii, BnCl–KOH; ix, O_2–Pt; x, CH_2N_2

Scheme 71

a $^1C_4$ conformation almost exclusively. Methyl (1,2,3-tri-*O*-acetyl-4-*O*-methyl-β-D-glucopyranosyl)uronate has been obtained from 1,2,3-tri-*O*-acetyl-β-D-glucopyranose by a series of straightforward transformations.[495] The advantages of using glycosyl acetates in the synthesis of β-D-glucuronides are mentioned in Chapter 3.[89]



(323)

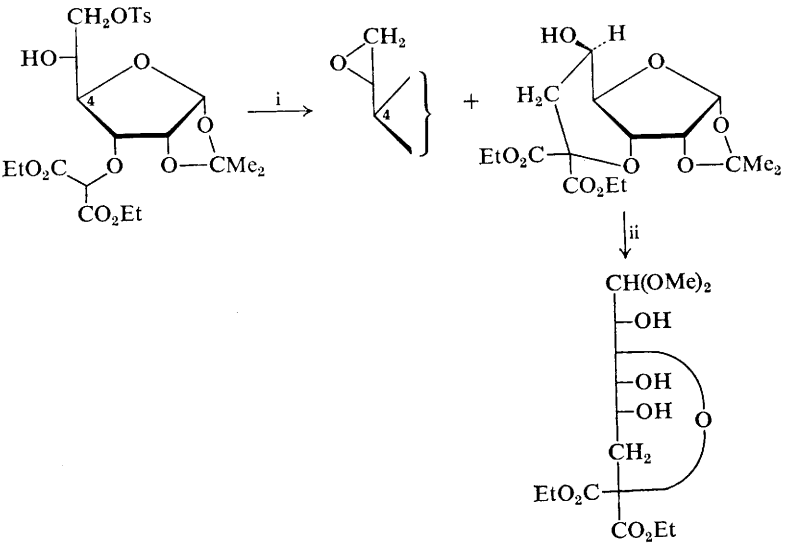


(324)

[493] H. Weidmann, M. Appenroth, R. Leipert-Klug, K. Dax, and P. Stöckl, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 235.
[494] J. Kiss and P. C. Wyss, *Tetrahedron*, 1976, **32**, 1399.
[495] P. Kováč, R. Brežný, V. Mihálov, and R. Palovčík, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 445.

The disaccharide (324), which contains D-galactopyranosylurono-6,3-lactone as its non-reducing residue, has been obtained in 40% yield by condensation of D-galactopyranosyluronic acid with 2-amino-2-deoxy-D-galactopyranose in 6M-hydrochloric acid at 40 °C under reduced pressure.[496]

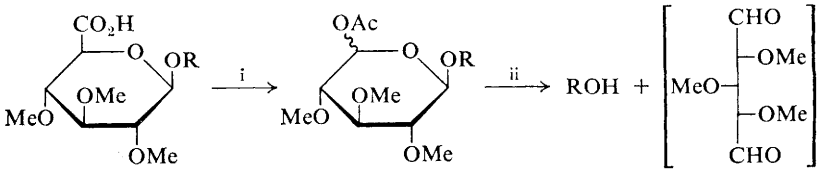
Some 3,7-anhydro-6-deoxyoctose derivatives related to octosyl acids A and B (isolated from the fermentation broth of *Streptomyces cacaoi* var. *asoensis*) have been synthesized by the route shown in Scheme 72.[497]



Reagents: i, NaH; ii, MeOH–H^+

Scheme 72

A new method for selectively cleaving the D-glucuronidic linkages of saponins is based on oxidative decarboxylation of per-*O*-methylated derivatives with lead tetra-acetate in benzene, followed by release of the aglycone with base (Scheme 73).[498]



R = sapogenol derivative

Reagents: i, $Pb(OAc)_4$–PhH; ii, MeONa–MeOH

Scheme 73

[496] A. Klemer, F. Rohde, and W. Funcke, *Carbohydrate Res.*, 1976, **49**, C5.
[497] K. Ansai and T. Saita, *J.C.S. Chem. Comm.*, 1976, 681.
[498] I. Kitagawa, M. Yoshikawa, Y. Ikenishi, Kwang Sik Im, and I. Yosioka, *Tetrahedron Letters*, 1976, 549.

The condensation of hexuronic acid derivatives with hexosaminides to give amide-linked disaccharides is referred to in Chapter 8.[319, 320]

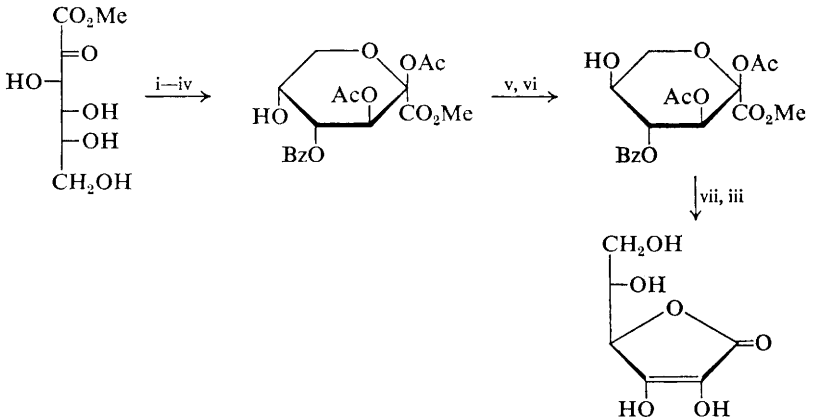
D-Galacturonic acid has been identified as a component of *Pneumococcus* type 25 capsular polysaccharide,[499] and 2-*O*-methyl-D-glucuronic acid has been isolated for the first time in Nature from an extracellular polysaccharide of a species (*Porphyridium cruentum*) of red alga.[500] The acidic sugar component in *Klebsiella* type 37 capsular polysaccharide has been identified as 4-*O*-[(*S*)-1-carboxyethyl]-D-glucuronic acid.[501] Antibiotic substances and nucleosides that contain uronic acid components are referred to in Chapters 20 and 21, respectively.

Other Acid Derivatives

Mucic acid reacted with triethyl phosphite to give the bis(cyclic phosphite) (79).[248]

L-Ascorbic Acid

L-Ascorbic acid has been synthesized from methyl D-*arabino*-hexulosonate, a readily available fermentation product, by the reactions outlined in Scheme 74;



Reagents: i, $Me_2C(OMe)_2$–H^+; ii, Ac_2O–py; iii, H^+; iv, BzCl(1 mole)–py; v, CrO_3–py; vi, $NaBH_4$; vii, MeONa–MeOH

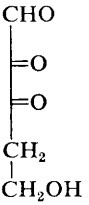
Scheme 74

the key step in this transformation involved the inversion of configuration at C-5 by an oxidation–reduction sequence.[502] L-[6-^{3}H]Sorbose (prepared by oxidation of D-[1-^{3}H]glucitol at HO-5 with *Acetobacter suboxydans*) has been transformed into L-[6-^{3}H]ascorbic acid by a standard series of reactions,[502a] and

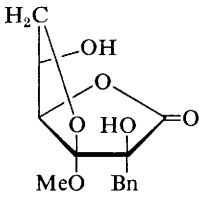
[499] A. Das, M. Heidelberger, and R. Brown, *Carbohydrate Res.*, 1976, **48**, 304.
[500] J. H. Kieras, F. J. Fieras, and D. V. Bowen, *Biochem. J.*, 1976, **155**, 181.
[501] B. Lindberg, B. Lindqvist, J. Lönngren, and W. Nimmich, *Carbohydrate Res.*, 1976, **49**, 411.
[502] T. Ogawa, K. Taguchi, N. Takasaka, M. Mikata, and M. Matsui, *Carbohydrate Res.*, 1976, **51**, C1.
[502a] N. Flueck and J. Wuersch, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 273.

L-ascorbic acid 2-[^{35}S]sulphate having a high specific activity has been obtained by sulphation of 5,6-*O*-isopropylidene-L-ascorbic acid with [^{35}S]sulphur trioxide in DMF; the presence of pyridine promoted considerable side-reactions, perhaps initiated by hydrolysis of the 5,6-*O*-isopropylidene group.[262]

Dehydro-L-ascorbic acid afforded the 1,2-bis(phenylhydrazone) of the tricarbonyl compound (325), in addition to 3-hydroxy-2-pyrone, on heating with phenylhydrazine hydrochloride.[503] Similar treatment of L-*threo*-pentosulose (L-xylosone) gave the same bis(phenylhydrazone), but it was established that L-xylosone is not the main precursor of (325) in the oxidative degradation of dehydro-L-ascorbic acid.[504]



(325)



(326)

The crystal structure of the *C*-benzyl derivative (326) of a *keto*-form of L-ascorbic acid has been determined.[505]

Several reports have discussed the oxidation of L-ascorbic acid with metal cations. *Ab initio* studies on the conformations of L-ascorbic acid and its monoanion gave results in good agreement with the known crystal structures.[506] It appears that L-ascorbic acid is able to assume a conformation having the six oxygen atoms in a 'bucket-seat' arrangement, which enhances its ability to reduce metal cations (*e.g.* Cu^{2+} or Hg^{2+}). Although the overall mechanism for the participation of L-ascorbic acid in physiological processes is unknown, there is evidence suggesting that five species of L-ascorbic acid may be involved.

The kinetics of autoxidation of L-ascorbic acid catalysed by Cu^{+} ions have been determined.[507, 508] Important features of the autoxidation, including the formation of hydrogen peroxide as a stable intermediate and of a ternary complex containing oxygen, copper(I), and ascorbate, were discussed. Reagents that complexed Cu^{+} ions were shown to inhibit the autoxidation. Cu^{+} apparently remains formally univalent throughout the entire reaction cycle and acts as an electron carrier between two substrate molecules. Other workers have shown that the metal-ion-catalysed oxidation of L-ascorbic acid at alkaline pH values is inhibited by superoxide dismutase.[509] The kinetics and mechanism of the oxida-

[503] T. Kurata and M. Fujimaki, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1287.
[504] T. Kurata and M. Fujimaki, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1429.
[505] J. Hvoslef and S. Nordenson, *Acta Cryst.*, 1976, **B32**, 1665.
[506] G. L. Carlson, H. Cable, and L. G. Pedersen, *Chem. Phys. Letters*, 1976, **38**, 75.
[507] E. Schwertnerová, D. M. Wagnerová, and J. Vepřek-Šiška, *Coll. Czech. Chem. Comm.*, 1976, **41**, 2463.
[508] D. M. Wagnerová, E. Schwertnerová, and J. Vepřek-Šiška, *Coll. Czech. Chem. Comm.*, 1976, **41**, 2473.
[509] B. Halliwell and C. H. Foyer, *Biochem. J.*, 1976, **155**, 697.

tion of L-ascorbic acid by tris(1,10-phenanthroline)iron(III) complexes have also been examined in detail.[510]

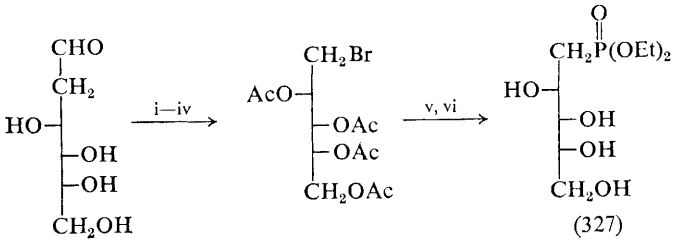
Quantitative analysis of the products of oxidation of L-[1- and 6-^{14}C]ascorbic acid with periodate at pH 7.5 showed that 2 moles of formate (from C-4 and C-5) and 1 mole each of carbon dioxide (from C-3), oxalate (from C-1 and C-2), and formaldehyde (from C-6) are formed, together with carbon dioxide (*ca.* 17%) liberated from a side-reaction at C-1.[511]

[510] E. Pelizzetti, E. Mentasti, and E. Pramauro, *Inorg. Chem.*, 1976, **15**, 2898.
[511] R. J. Harkrader, L. M. Plunkett, and B. M. Tolbert, *Analyt. Biochem.*, 1976, **72**, 310.

18
Inorganic Derivatives

Carbon-bonded Compounds

The phosphonate (327), a phosphorus analogue of desonoic acid, has been synthesized *via* application of the Borodin–Hunsdiecker reaction to 2-deoxy-D-*arabino*-hexose (Scheme 75).[512]



Reagents: i, Br_2–H_2O; ii, $BaCO_3$; iii, Ac_2O–py; iv, Br_2–HgO; v, $(EtO)_3P$; vi, HO^-

Scheme 75

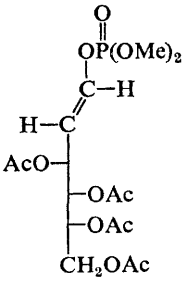
The ^1H– and ^{13}C–^{199}Hg couplings in specifically mercurated sugars are referred to in Chapter 23.

Oxygen-, Sulphur-, and Selenium-bonded Compounds

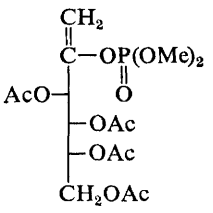
In continuing their investigations on phosphorus-containing sugars, Paulsen's group has applied the Perkow reaction to a number of α-acyloxycarbonyl sugars.[236] 2,3,4,5,6-Penta-*O*-acetyl-*aldehydo*-D-glucose, for example, reacted with trimethyl phosphite at 100 °C to give the enol phosphate (328) (15%), which gave 2-deoxy-D-*arabino*-hexose on hydrolysis with acid. A Perkow reaction on 1,3,4,5,6-penta-*O*-acetyl-*keto*-D-fructose yielded the enol phosphates (329) (28%) and (330) (57%), which afforded 1-deoxy-D-fructose and 3-deoxy-D-*erythro*-hexulose, respectively, on hydrolysis with acid. Similar reactions with suitably protected 2(3)-acyloxy-(sulphonyloxy)hexopyranosid-3(2)-uloses gave, depending on the leaving group, either the corresponding enol phosphates or mixtures of the enol phosphates and α-hydroxyphosphonates [*e.g.* (331)].

The joint action of tris(dimethylamino)phosphine and carbon tetrachloride on vicinal diols furnished either oxirans or spirophosphoranes; 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-gluco- and -allo-furanose, for example, yielded

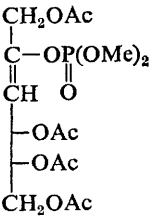
[512] Yu. A. Zhdanov, L. A. Uzlova, Z. I. Glebova, and G. K. Kist'yan, *Zhur. obshchei Khim.*, 1975, **45**, 1614 (*Chem. Abs.*, 1976, **84**, 5294t).



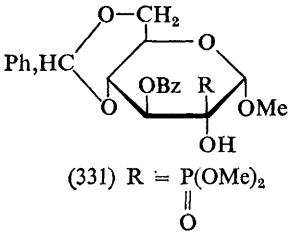
(328)



(329)



(330)

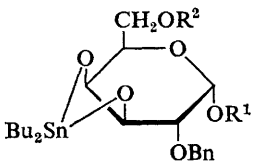


(331) R = $P(OMe)_2$ (O)

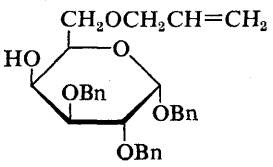
mixtures of stereoisomeric (*cis,cis*; *cis,trans*; and *trans,trans*) spirophosphoranes (see Vol. 9, p. 47), which were examined by ^{31}P and ^{1}H n.m.r. spectroscopy.[513]

The use of tributylstannyl alkoxides in glycoside synthesis is reported in Chapter 3,[60] and several tributylstannyl ethers of simple sugars and their derivative have also been prepared;[201] D-glucose, for example, reacted with tributyltin oxide to give a trisubstituted derivative. The reactions of tributylstannyl derivatives of sugars with NBS and aldehydes in carbon tetrachloride are mentioned in Chapter 6.[202]

The reaction of dibutyltin oxide with pyranoid *cis*-1,2-diols has attracted interest, since the resulting cyclic dibutylstannylene derivatives are substituted regioselectively at the equatorial oxygen-atom when treated with an acyl chloride or an active alkyl halide. Thus the cyclic dibutylstannylene derivative (332;



(332) R^1 = Bn; R^2 = $CH_2CH{=}CH_2$
or R^1 = $CH_2CH{=}CH_2$; R^2 = Bn



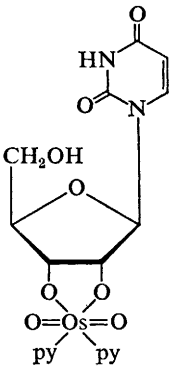
(333)

R^1 = Bn; R^2 = $CH_2CH{=}CH_2$) reacted with benzyl bromide in DMF to give a 66% yield of benzyl 6-*O*-allyl-2,3-di-*O*-benzyl-α-D-galactopyranoside (333),[199] and 3-*O*-substituted derivatives were also formed regioselectively when the structural isomer (332; R^1 = $CH_2CH{=}CH_2$; R^2 = Bn) was treated with allyl bromide, benzoyl chloride, or methyl iodide in an aprotic medium.[200] Benzylation

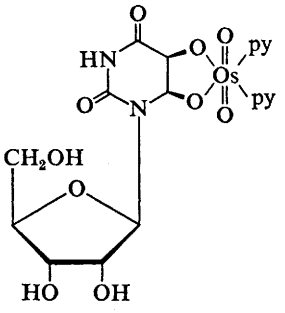
[513] R. Boigegrain and B. Castro, *Tetrahedron*, 1976, **32**, 1283.

of methyl 4,6-*O*-benzylidene-2,3-*O*-dibutylstannylene-α-D-mannopyranoside also occurred at the equatorial O-3 position.[200]

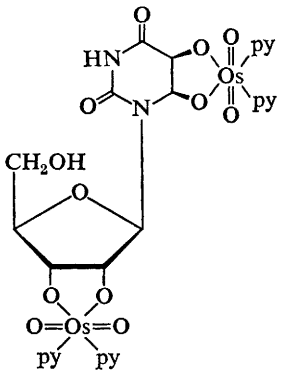
Further detailed work on the interactions of free sugars with metal cations in aqueous solutions has appeared. ^{1}H N.m.r. measurements showed that D-lyxose and D-ribose form 1 : 1 complexes with Ca^{2+} and La^{3+} ions in aqueous solution.[52] Complexation of the metal ions occurred with the β-pyranose form of D-lyxose and with the α- and β-pyranose and α-furanose forms of D-ribose, in agreement with Angyal's findings (see Vol. 8, p. 123) that sugars that are able to adopt an *ax.,eq.,ax.* arrangement of adjacent hydroxy-groups on a pyranoid ring or a *cis,cis* arrangement on a furanoid ring readily form metal-ion complexes in aqueous solution. In extending their own work on cationic complexes of sugars, Angyal and his co-workers have attributed the lanthanide-induced shifts in the ^{1}H n.m.r. spectra of *epi*-inositol and other cyclitols to a combination of diamagnetic interactions caused by the metal cation, pseudocontact interactions operating through space, and contact interactions operating through bonds.[514] Contact interactions show stereospecificity and are greatest when the bonds connecting the protons to the cation form a planar zig-zag arrangement. Lanthanide-induced shifts in the ^{1}H n.m.r. spectra of some methyl glycosides and



(334)



(335)



(336)

[514] S. J. Angyal and D. Greeves, *Austral. J. Chem.*, 1976, **29**, 1223.

1,6-anhydro-β-D-hexopyranoses were also examined in detail.[515] The approximate location of the cation in the complexes was determined and an attempt was made to explain the shifts in terms of contact and pseudocontact interactions.

The usefulness of osmium derivatives in nucleic acid chemistry has prompted an investigation of the synthesis and reactions of osmium ligand complexes [*e.g.* (334)—(336)] of common nucleosides.[516] Such dipyridylosmium esters underwent relatively rapid transesterification reactions with glycols. Other groups have reported n.m.r. studies on the complexes formed between nucleosides and transition-metal carbonyls {*e.g.* $[Rh(CO_2)Cl]_2$ and $W(CO)_6$}[517] and on the ionic binding of Li^+ and Cl^- ions to nucleosides (based on ^7Li and ^{35}Cl nuclear magnetic relaxation times).[518]

The temperature- and concentration-dependent increases in the line-width of the ^{23}Na resonance when small amounts of sugars (D-fructose, D-glucose, D-galactose, and L-sorbose) were added to sodium perchlorate in pyridine indicated that weak complexes were formed.[519] The stability constant was determined for an L-sorbose–Na^+ complex, which was suggested to have three pyridine nitrogen atoms and two (or three) oxygen atoms arranged around the sodium cation.

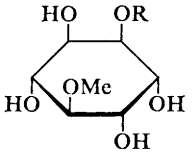
The synthesis of 6-thio- and 6-seleno-α-D-glucopyranose esters of dimethylarsinous acid is referred to in Chapter 11 (see Scheme 49).[413a] Related 1- and 6-thio- and 1- and 6-seleno-D-glucopyranose esters of dialkyl-phosphinous and -antimonous acids have also been prepared [520] (see also Vol. 9, p. 80).

[515] S. J. Angyal, D. Greeves, L. Littlemore, and V. A. Pickles, *Austral. J. Chem.*, 1976, **29**, 1231.
[516] F. B. Daniel and E. J. Behrman, *J. Amer. Chem. Soc.*, 1975, **97**, 7352.
[517] W. Beck and N. Kottmair, *Chem. Ber.*, 1976, **109**, 970.
[518] A. C. Plaush and R. R. Sharp, *J. Amer. Chem. Soc.*, 1976, **98**, 7973.
[519] C. Detellier, J. Grandjean, and P. Laszlo, *J. Amer. Chem. Soc.*, 1976, **98**, 3375.
[520] R. A. Zingaro, *Chemica Scripta*, 1975, **8A**, 51 (*Chem. Abs.*, 1976, **84**, 180 485c).

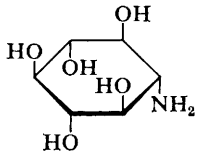
19
Cyclitols

D-Pinitol (from *Mimosa pudica*) and pinpollitol (from *Pinus radiata*) have been identified as 4-*O*-methyl- and 1,4-di-*O*-methyl-D-*chiro*-inositol [(337)[521] and (338)[522]], respectively.

A highly phosphorylated, monomeric form of *neo*-inositol ('pre-mannose') has been detected on the cell surface of a strain of *Amoeba discoides*.[523]



(337) R = H
(338) R = Me



(339)

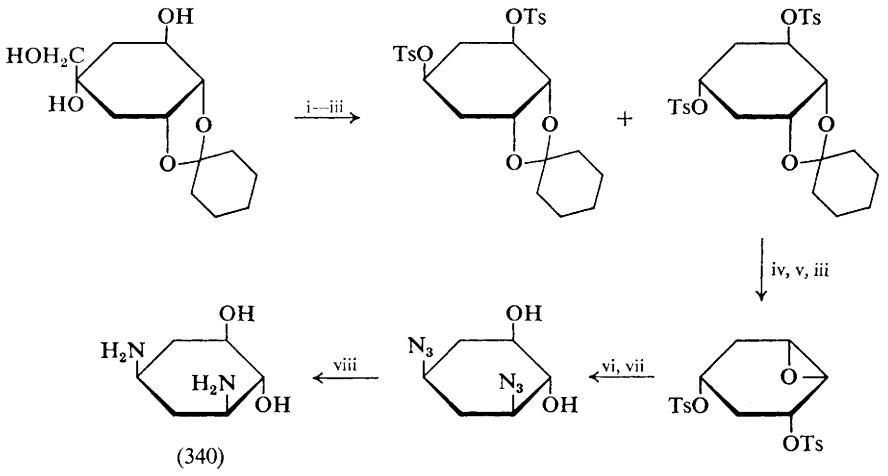
α-L-Fucopyranosyl-*myo*-inositol has been isolated from the urine of ABH secretors.[126]

1-Amino-1-deoxy-*scyllo*-inositol (339) has been prepared by hydrogenolytic reduction of the corresponding 3,4-di-*O*-benzyl-1-deoxy-1-nitro-derivative, which was obtained by base-catalysed cyclization of 2,3-di-*O*-benzyl-6-deoxy-6-nitro-D-glucose.[524]

A semisynthetic approach to novel aminoglycoside antibiotics has been described.[525] Thus D-(+)-2,6-dideoxystreptamine (340) was synthesized (Scheme 76) and then incorporated into 6-deoxyneomycins and 6-deoxyparomomycins using mutant organisms. Pseudo-β-DL-galactopyranose penta-acetate (341) and pseudo-α-DL-altropyranose penta-acetate (342) have been derived from 1,2-*O*-cyclohexylidene-5-deoxy-DL-*chiro*-inositol, as shown in Scheme 77.[525a]

Racemic (1,3/2)-1-benzyloxycarbonylamino-3-*O*-ethyl- and (1,2/3)-3-benzyloxycarbonylamino-1-*O*-ethyl-cyclohexanediols (prepared from 3-ethoxycyclohexene) have been condensed under Koenigs–Knorr conditions with 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,4-dinitrophenyl)amino-α-D-glucopyranosyl bromide to yield

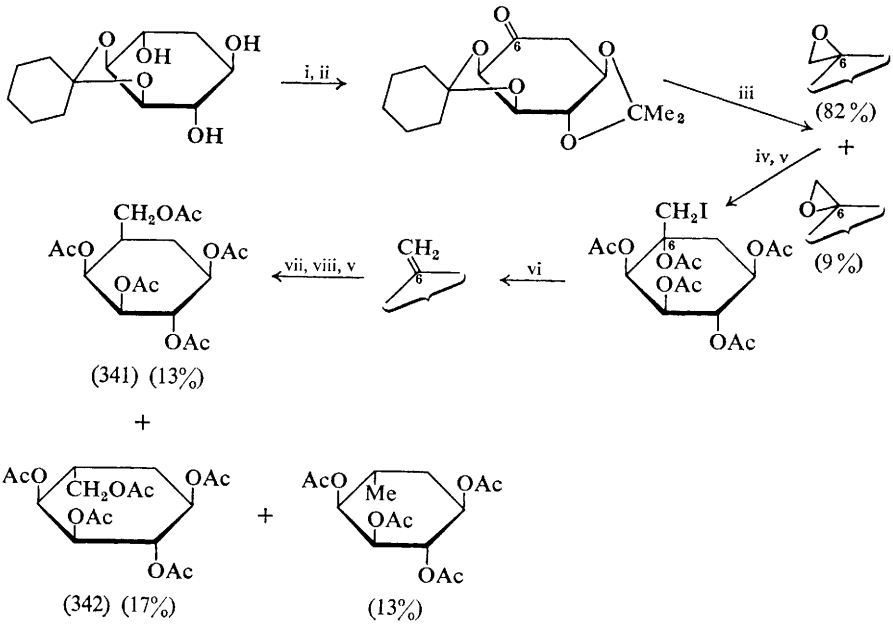
[521] H. Schildknecht and D. S. P. Iyengar, *Naturwiss.*, 1975, **62**, 533 (*Chem. Abs.*, 1976, **84**, 59 903j).
[522] S. J. Angyal, R. T. Gallacher, and P. M. Pojer, *Austral. J. Chem.*, 1976, **29**, 219.
[523] M. H. Laird, H. J. Allen, J. F. Danielli, and R. J. Winzler, *Arch. Biochem. Biophys.*, 1976, **175**, 384.
[524] V. D. Gusev, T. K. Mitrofanova, O. N. Tolkachev, and R. P. Evstigneeva, *Bioorg. Khim.*, 1975, **1**, 898 (*Chem. Abs.*, 1976, **84**, 105 942w).
[525] J. Cleophax, S. D. Gero, J. Leboul, M. Akhtar, J. E. G. Barnett, and C. J. Pearce, *J. Amer. Chem. Soc.*, 1976, **98**, 7110.
[525a] T. Suami, S. Ogawa, T. Ishibashi, and I. Kasahara, *Bull. Chem. Soc. Japan*, 1976, **49**, 1388.



(340)

Reagents: i, $NaIO_4$; ii, $LiBH_4$; iii, TsCl–py; iv, AcOH; v, MeONa; vi, H^+; vii, NaN_3–DMF; viii, H_2–cat.

Scheme 76

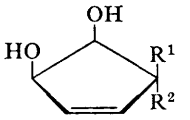


Reagents: i, $Me_2C(OMe)_2$–H^+; ii, DMSO–Ac_2O; iii, CH_2N_2; iv, NaI–HI; v, Ac_2O; vi, Zn–AcOH; vii, BH_3; viii, H_2O_2–HO^-

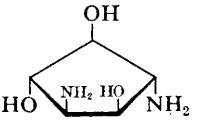
Scheme 77

the corresponding α-glycosides.[526] ^{1}H N.m.r. studies of the *N*-acetylated derivatives of these glycosides and of penta-*N*-acetylkanamycin B showed that equatorial substituents adjacent to the glycosidic linkage cause the signal of the anomeric proton to shift to higher field. N.m.r. investigations of inositol derivatives are also referred to in Chapter 23.

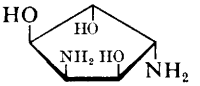
Several papers have reported the synthesis of polyhydroxy-cyclopentanes. The relative stereochemistry of the 4,5-dihydroxycyclopenten-3-ylaminomethyl side-chain in nucleoside Q from *Escherichia coli* tRNA has been established by synthesis of the related cyclopentenyl derivatives (343) and (344) and careful



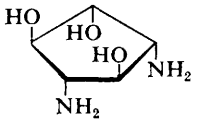
(343) R^1 = H; R^2 = NHBn
(344) R^1 = NHBn; R^2 = H



(345)



(346)



(347)

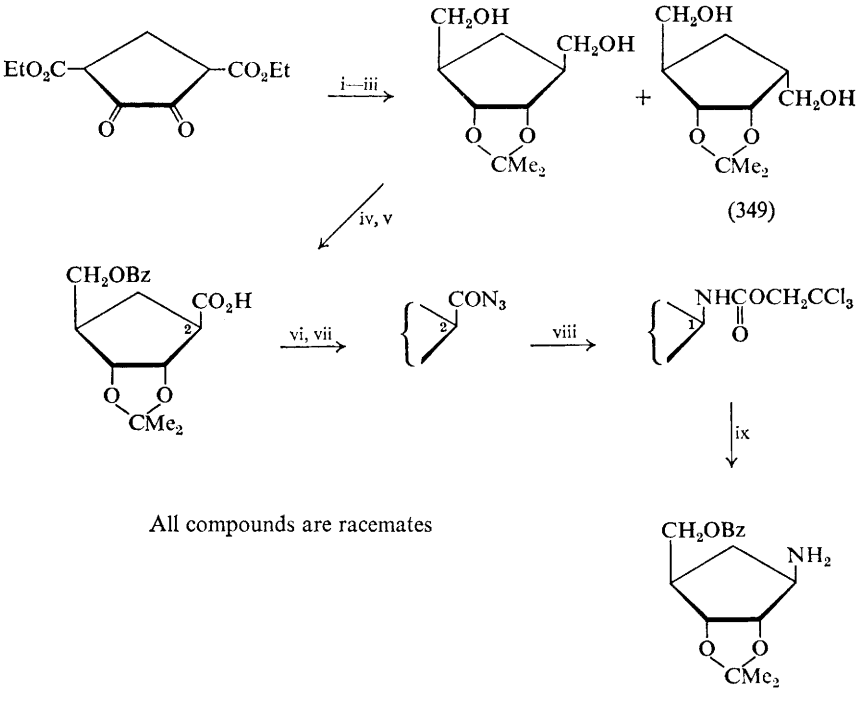
analysis of their ^{1}H n.m.r. spectra, which indicated that the *cis,trans*-compound (343) has the same relative stereochemistry as that of the nucleoside Q side-chain.[526a] Three hitherto unknown 2,5-diamino-1,3,4-cyclopentanetriols, namely (345) (1,2,4/3,5), (346) (1,2,3/4,5), and (347) (1,3/2,4,5), have been prepared by essentially standard procedures involving either base-catalysed cyclization with nitromethane or azide displacements.[527]

Derivatives [*e.g.* (348)] of pseudo-β-DL-ribofuranosylamine have been synthesized by the reactions outlined in Scheme 78, and a related series of reactions on the *trans*-bishydroxymethyl derivative (349) afforded isomeric pseudo-α-DL-ribofuranosyl and pseudo-α-DL-lyxofuranosyl derivatives.[528]

The phosphatidylinositol (350) has been synthesized from *myo*-inositol 1,2,4,5,6-penta-acetate[529] and the phosphatidylinositols (351) and (352) from 1,2:4,5-di-*O*-cyclohexylidene-*myo*-inositol.[530] A transesterification reaction on 3,4,6-tri-*O*-acetyl-β-D-mannopyranosyl 1,2-(ethyl orthoacetate) using 3,6-di-*O*-benzyl-1,2-*O*-cyclohexylidene-*myo*-inositol has been reported.[531]

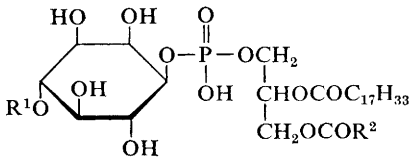
Equilibration of the δ-*O*-methyl-deoxynitroinositols [*e.g.* (353)] at pH 8.2 revealed that the order of stability is *myo*-5 ⩾ *epi*-1 ~ *epi*-6 > *allo*-5 >

[526] A. Hasegawa and F. Kinoshita, *Gifu Daigaku Nogakubu Kenkyu Hokoku*, 1975, **38**, 175 (*Chem. Abs.*, 1976, **84**, 165 152y).
[526a] T. Ohgi and T. Goto, *Tetrahedron Letters*, 1976, 367.
[527] K. Tadano, Y. Emori, M. Ayabe, and T. Suami, *Bull. Chem. Soc. Japan*, 1976, **49**, 1108.
[528] A. Holý, *Coll. Czech. Chem. Comm.*, 1976, **41**, 647.
[529] A. I. Lyutik, V. A. Sukhanov, V. I. Shvets, and R. P. Evstigneeva, *Bioorg. Khim.*, 1975, **1**, 684 (*Chem. Abs.*, 1976, **84**, 59 900f).
[530] V. P. Shevchenko, M. L. Tsirenina, Yu. G. Molotkovskii, and L. D. Bergel'son, *Doklady Akad. Nauk S.S.S.R.*, 1975, **223**, 504 (*Chem. Abs.*, 1976, **84**, 5286s).
[531] V. N. Krylova, V. M. Kornitskaya, V. I. Shvets, and R. P. Evstigneeva, *Zhur. org. Khim.*, 1975, **11**. 2034 (*Chem. Abs.*, 1976, **84**, 105 941v).

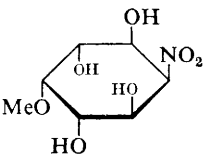


Reagents: i, H_2–cat.; ii, $Me_2C(OMe)_2$–H^+; iii, $LiAlH_4$; iv, BzCN; v, RuO_4; vi, $ClCO_2Et$; vii, LiN_3; viii, Δ-Cl_3CCH_2OH; ix, Zn–AcOH

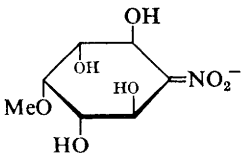
Scheme 78



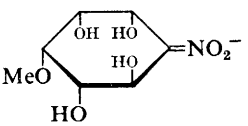
(350) R^1 = H; R^2 = $C_{17}H_{35}$
(351) R^1 = H; R^2 = $C_{15}H_{31}$
(352) R^1 = PO_3H_2; R^2 = $C_{15}H_{31}$



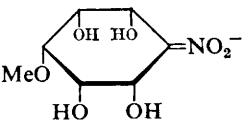
(353)



(354)



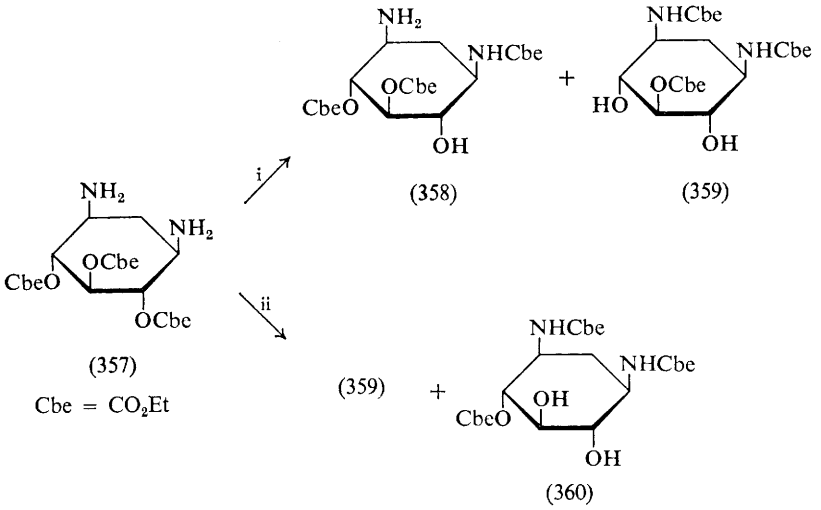
(355)



(356)

neo-2 ≫ *cis* (see also Vol. 9, p. 124).[532] Good agreement was obtained between the relative thermodynamic stabilities revealed by the equilibration studies and those based on calculations of the conformational free energies. At pH > 12, the order of thermodynamic stability of the corresponding sodium nitronates was assessed as (354) > (355) > (356), in good agreement with the order predicted from the calculated conformational free energies.

One or two O → N acyl migrations yielding (358) and (359), respectively, occurred when 2-deoxy-4,5,6-tri-*O*-ethoxycarbonylstreptamine (357) was heated in pyridine at 50 °C, and an O → O acyl migration yielding (360) accompanied the O → N migrations at 100 °C (Scheme 79).[533]



Reagents: i, py at 50 °C; ii, py at 100 °C

Scheme 79

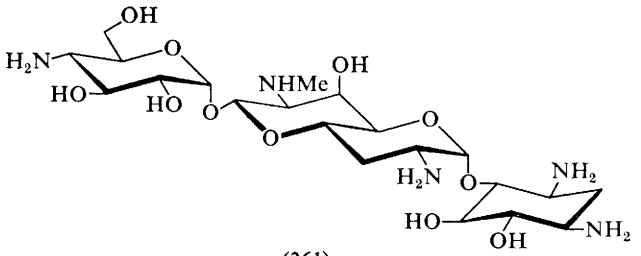
[532] H. H. Baer and J. Kovář, *Canad. J. Chem.*, 1976, **54**, 2038.
[533] T. Kurisu, M. Yamashita, Y. Nishimura, T. Miyake, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1976, **49**, 285.

20
Antibiotics

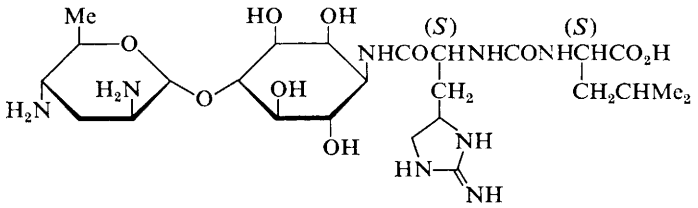
Aminoglycoside Antibiotics

The biosynthesis of the aminoglycoside antibiotics has been reviewed.[534]

Apramycin, a broad-spectrum aminoglycoside antibiotic produced by a strain of *Streptomyces tenebrarius*, has been assigned the structure (361), which contains residues of 4-amino-4-deoxy-D-glucose and an octadiose that exists as a rigid bicyclic system.[535] The structure (361), which was first derived from chemical and spectroscopic evidence, was confirmed by *X*-ray crystallographic analysis.



(361)



(362)

Further reports on the structures of destomycin B[536] (see Vol. 9, p. 131) and hikizimycin[537] (see Vol. 9, p. 134) have appeared. The structure of minosaminomycin (362), an aminoglycoside antibiotic obtained from a *Streptomyces* culture, has been established by degradation studies and partial synthesis.[538] The aminoglycoside antibiotic G-52 produced by *Micromonospora zionensis* has been

[534] K. L. Rinehart, jun., and R. M. Stroshane, *J. Antibiotics*, 1976, **29**, 319.
[535] S. O'Connor, L. K. T. Lam, N. D. Jones, and M. O. Chaney, *J. Org. Chem.*, 1976, **41**, 2087.
[536] M. Shimura, Y. Sekizawa, K. Iinuma, H. Naganawa, and S. Kondo, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 611.
[537] K. Uchida, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 395.
[538] K. Iinuma, S. Kondo, K. Maeda, and H. Umezawa, *J. Antibiotics*, 1975, **28**, 613.

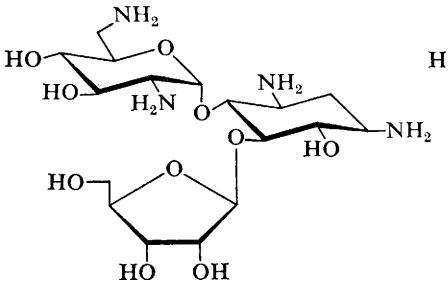
shown to be identical to 6′-*N*-methylsisomicin by its synthesis from sisomicin [539] (see Vol. 8, p. 132).

The carbohydrate components of actinridins A and B have been identified as D-mannose, L-actinosamine (3-amino-2,3,6-trideoxy-4-*O*-methyl-L-*arabino*-hexopyranose), and 2-*O*-(3-amino-2,3,6-trideoxy-L-*arabino*-hexopyranosyl)-D-glucopyranose.[539a]

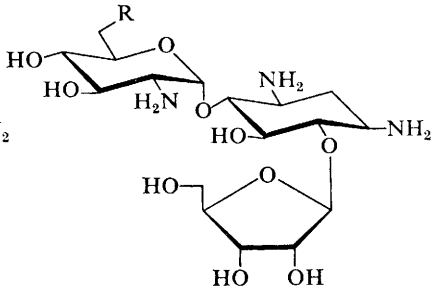
Hydrolysis of flavumycin A, an aromatic heptaenic antibiotic, yielded mycosamine (3-amino-3,6-dideoxy-D-mannose) and 4-acetylaniline.[540]

Risto-biose and -triose, obtained on mild acidic hydrolysis of the tetrasaccharide side-chain of ristomycin A (from *Proactinomyces fructiferi* var. *ristomycini*) have been shown to be 2-*O*-α-D-mannopyranosyl-D-glucose and *O*-α-L-rhamnopyranosyl-(1 → 6)-[*O*-α-D-mannopyranosyl-(1 → 2)]-D-glucose,[541] respectively, by synthesis.

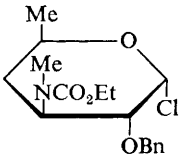
Another total synthesis of ribostamycin (363), a broad-spectrum antimicrobial antibiotic produced by *Streptomyces ribosidificus*, has been reported.[542] The synthetic route involved the condensation of a suitably protected 5-*O*-β-D-ribofuranosyl-2-deoxystreptamine derivative with 3,4-di-*O*-acetyl-2,6-dideoxy-2-(2,4-dinitroanilino)-6-phthalimido-α-D-glucopyranosyl bromide under modified Koenigs–Knorr conditions. A standard Koenigs–Knorr reaction has been used



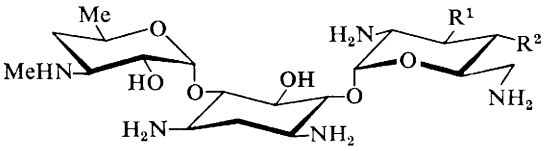
(363)



(364) R = OH or NH_2



(365)



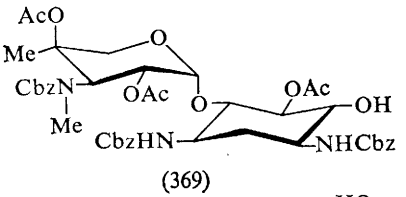
(366) $R^1 = R^2 = OH$
(367) $R^1 = H$; $R^2 = OH$
(368) $R^1 = R^2 = H$

[539] P. J. L. Daniels, R. S. Jaret, T. L. Nagabhushan, and W. N. Turner, *J. Antibiotics*, 1976, **29**, 488.
[539a] I. A. Spiridonova, M. S. Yurina, N. N. Lomakina, F. Sztaricskai, and R. Bognár, *Antibiotiki*, 1976, **21**, 304 (*Chem. Abs.*, 1976, **85**, 108 925z).
[540] L. F. Kruglikova and Yu. D. Shenin, *Antibiotiki*, 1976, **21**, 407 (*Chem. Abs.*, 1976, **85**, 108 914v).
[541] F. Sztaricskai, A. Lipták, I. F. Pelyvás, and R. Bognár, *J. Antibiotics*, 1976, **29**, 626.
[542] H. Fukami, K. Kitahara, and M. Nakajima, *Tetrahedron Letters*, 1976, 545.

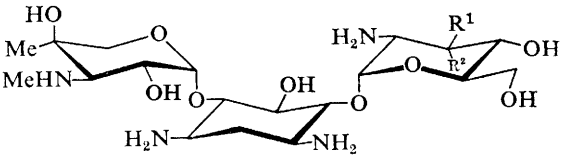
in the synthesis of 4-*O*-β-D-ribofuranosyl derivatives (364) of paromamine, which are analogues of paromomycin and ribostamycin (363), respectively.[543]

The amino-sugar derivative (365) obtained from desosamine (3,4,6-trideoxy-3-dimethylamino-D-*xylo*-hexose) has been condensed with neamine derivatives to yield, after removal of the protecting groups, analogues (366)—(368) of kanendomycin, tobramycin, and gentamicin C_1A, respectively, containing a modified amino-sugar residue.[544] The semisynthetic antibiotics (366)—(368) are active against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *etc.*, whereas the synthetic C-1″ epimer of (366) is inactive.

Mallams's group has reported the synthesis of selectively protected garamine derivatives [*e.g.* (369)].[545] A number of novel aminoglycoside antibiotics related to the gentamicins were then synthesized, *via* glycosylation of the garamine



(369)



(370)

derivatives, in the hope of improving the potency and antibacterial spectra, particularly against resistant organisms. This semisynthetic approach was used to obtain gentamicin X_2 (370; R^1 = OH; R^2 = H) (produced as a minor component by *Micromonospora purpurea*) and related compounds,[546] 3′-deoxygentamicin X_2 (370; R^1 = R^2 = H), a D-*allo* analogue (370; R^1 = H; R^2 = OH) of gentamicin X_2, and 3′-*O*-methylgentamicin X_2 (370; R^1 = OMe; R^2 = H),[547] antibiotic JI-20A (a minor component of the fermentation of a mutant strain of *M. purpurea*) and gentamicin B,[548] and novel 4-*O*-pento-furanosyl and -pyranosyl derivatives of garamine.[549] Selective acylation at N-1 of the gentamicin antibiotics has been achieved on treatment of acid addition salts with one molar equivalent of triethylamine and an acylating reagent.[550] In continuing their

[543] F. Arcamone, G. Cassinelli, P. B. Cuccia, and G. Dicolo, *Ann. Chim.* (*Italy*), 1974, **64**, 485 (*Chem. Abs.*, 1976, **84**, 5315a).

[544] J.-B. Chazan and J.-C. Gasc, *Tetrahedron Letters*, 1976, 3145.

[545] M. Kugelman, A. K. Mallams, H. F. Vernay, D. F. Crowe, and M. Tanabe, *J.C.S. Perkin I*, 1976, 1088.

[546] M. Kugelman, A. K. Mallams, H. F. Vernay, D. F. Crowe, G. Detre, M. Tanabe, and D. M. Yasuda, *J.C.S. Perkin I*, 1976, 1097.

[547] M. Kugelman, A. K. Mallams, and H. F. Vernay, *J.C.S. Perkin I*, 1976, 1113.

[548] M. Kugelman, A. K. Mallams, and H. F. Vernay, *J.C.S. Perkin I*, 1976, 1126.

[549] A. K. Mallams, S. S. Saluja, D. F. Crowe, G. Detre, M. Tanabe, and D. M. Yasuda, *J.C.S. Perkin I*, 1976, 1135.

[550] J. J. Wright, A. Cooper, P. J. L. Daniels, T. L. Nagabhushan, D. Rane, W. N. Turner, and J. Weinstein, *J. Antibiotics*, 1976, **29**, 714.

work on the synthesis of aminoglycoside antibiotics and their analogues, Umezawa and his co-workers have reported a synthesis of 3″-deoxydihydrostreptomycin, which first required a multi-stage synthesis of a protected 2-*O*-(2,3-dideoxy-2-methylamino-α-L-*ribo*-hexopyranosyl)-dihydrostreptose derivative from dihydrostreptobiosamine,[551] and an improved route to 3′,4′-dideoxykanamycin B from kanamycin B.[552]

The relative reactivity of the amino-groups of the gentamicin–sisomicin antibiotics towards reductive alkylation in the presence of an aldehyde and a hydride reducing agent (*e.g.* sodium cyanoborohydride) has been found to be pH-dependent.[553] The amino-group at C-1 is selectively alkylated at low pH, but this selectively is lost on raising the pH. For example, sisomicin sulphate gave 1-*N*-ethylsisomicin (25%) on treatment in aqueous solution with one molar equivalent of sodium cyanoborohydride and an excess of acetaldehyde, while similar treatment of gentamicin C_1A gave principally the 1-*N*-ethyl derivative and some of the 3-*N*-isomer. 1-*N*-Ethylsisomicin is active against many 2″-*O*-adeninylating and 3-*N*-acetylating bacteria and exhibits lower chronic toxicity than either gentamicin or sisomicin.

Periodate oxidation of suitably protected derivatives of paromomycin and neomycin B, followed by base-catalysed β-elimination, yielded 5-*O*-β-D-ribofuranosyl- and 5-*O*-β-neobiosaminyl-2-deoxystreptamine derivatives, respectively[554] (see also Vol. 8, p. 136). Acidic hydrolysis of penta-*N*-acetylparomomycin liberated 2-amino-2-deoxy-D-glucose, 2-deoxystreptamine, neosamine B (2,6-diamino-2,6-dideoxy-L-idose), and D-ribose, which were separated on a cation-exchange resin.[555] (2-Amino-2-deoxy- and 6-amino-6-deoxy-α-D-glucopyranosyl)-2,5-dideoxystreptamine have been synthesized and shown to possess antibacterial activities.[67]

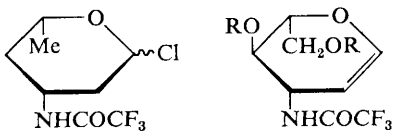
The biosynthesis of neomycin has been investigated using ^3H- and ^{14}C-labelled neamines, which were obtained by hydrolysis of the neomycins produced by a mutant of *Streptomyces fradiae* growing in the presence of D-[6-^3H]- and D-[U-^{14}C]-glucose.[556] Constant ^3H : ^{14}C ratios in the neamines used or recovered and in the neomycins produced from the labelled precursors by *S. rimosus* forma *paromomycinus* indicated that neamines are incorporated directly into the antibiotics and that they are probably formed in the first step of the biosynthesis. Both ^{13}C and ^{15}N n.m.r. studies on aminoglycoside antibiotics are referred to in Chapter 23, and the m.s. of several aminoglycoside antibiotics is mentioned in Chapter 24.

Anthracycline Antibiotics

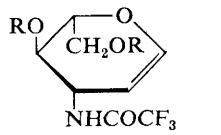
Several reports have described the glycosylation of suitably protected daunomycinones and related compounds. Methyl *N*-trifluoroacetyl-α-daunosaminide

[551] H. Sano, T. Tsuchiya, S. Kobayashi, M. Hamada, S. Umezawa, and H. Umezawa, *J. Antibiotics*, 1976, **29**, 978.
[552] T. Miyake, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Carbohydrate Res.*, 1976, **49**, 141.
[553] J. J. Wright, *J.C.S. Chem. Comm.*, 1976, 206.
[554] S. Hanessian, T. Takamoto, and R. Masse, *J. Antibiotics*, 1975, **28**, 835.
[555] B. Liebermann, H. Koester, and G. Reuter, *Pharmazie*, 1975, **30**, 796 (*Chem. Abs.*, 1976, **84**, 150 893s).
[556] C. J. Pearce, J. E. G. Barnett, C. Anthony, M. Akhtar, and S. D. Gero, *Biochem. J.*, 1976, **159**, 601.

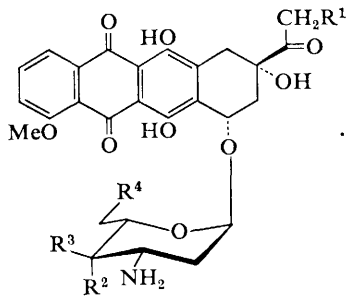
(methyl 2,3,6-trideoxy-3-trifluoroacetamido-α-L-*lyxo*-hexopyranoside) has been converted into the corresponding 4-deoxy-*N*-trifluoroacetylglycosyl chloride (371), which was used to prepare 4′-deoxydaunorubicin (372) and 4′-deoxyadriamycin (373).[557] *N*,*O*-Bis(trifluoroacetyl)-α-daunosaminyl chloride or related

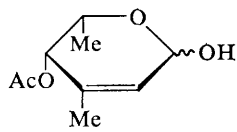


(371)



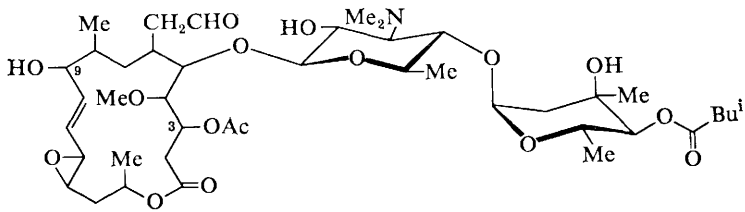
(376) R = *p*-$NO_2C_6H_4CO$





(379)

(372) $R^1 = R^2 = R^3 = R^4 = H$
(373) $R^1 = OH$; $R^2 = R^3 = R^4 = H$
(374) $R^1 = R^3 = R^4 = H$; $R^2 = OH$
(375) $R^1 = R^2 = OH$; $R^3 = R^4 = H$
(377) $R^1 = R^2 = H$; $R^3 = R^4 = OH$



(378)

glycosyl halides were similarly used to synthesize daunorubicin (374),[558] adriamycin (375),[558, 559] and the corresponding β-anomers,[558] and related 4′-[558] and 3′-epimers[302] of daunorubicin. A stereocontrolled synthesis of 7-*O*-(3-amino-2,3-dideoxy-α-L-*arabino*-hexopyranosyl)daunomycinone (377) was achieved *via* acid-catalysed addition of daunomycinone to the protected glycal (376).[560]

[557] F. Arcamone, S. Penco, S. Redaelli, and S. Hanessian, *J. Medicin. Chem.*, 1976, **19**, 1424.
[558] F. Arcamone, S. Penco, and A. Vigevani, *Cancer Chemother. Report, Part* 3, 1975, **6**, 123 (*Chem. Abs.*, 1976, **84**, 44 593j).
[559] T. H. Smith, A. N. Fujiwara, D. W. Henry, and W. W. Lee, *J. Amer. Chem. Soc.*, 1976, **98**, 1969.
[560] F. Arcamone, A. Bargiotti, G. Cassinelli, S. Redaelli, S. Hanessian, A. Di Marco, A. M. Casazza, T. Dasdia, A. Necco, P. Reggiani, and R. Supino, *J. Medicin. Chem.*, 1976, **19**, 733.

Macrolide Antibiotics

The complete structure (378) of a new antibiotic, maridomycin II, has been reported.[561] A related disaccharide composed of a 4-*O*-propionyl derivative of mycarose (2,6-dideoxy-3-*C*-methyl-L-*ribo*-hexose) and mycaminose (3,6-dideoxy-3-dimethylamino-D-glucose) is present in midecamycin (antibiotic SF837) (see Vol. 5, p. 135). The structures of the closely related antibiotics maridomycins I and III—VI have also been elucidated; they differ from maridomycin II (378) in the nature of the substituents either at O-4 of the mycarose residue or at C-3 of the macrolide ring.[562] Carbomycin A is the 9-oxo analogue of maridomycin II.[561] It was necessary to use an indirect route to prepare 3″,9-di-*O*-acetylmidecamycin, since acetylation of midecamycin itself resulted in migration of the propionyl residue from O-4″ to O-3″.[563]

The structure of rimocidin (from *S. rimosus*), which contains mycosamine attached to a polyene macrolide ring, has been reported.[564] 3-*O*-(2,6-Dideoxy-α-L-*ribo*- and -*arabino*-hexopyranosyl)erythronolide B, aberrant metabolites in the biosynthesis of erythromycin, have been isolated from the fermentation broth of *S. erythreus*, an erythromycin-producing organism.[565] A new lankamycin antibiotic, isolated from the fermentation broth of *S. violaceoniger*, has been shown to contain the unsaturated derivative 4-*O*-acetyl-2,3,6-trideoxy-3-*C*-methyl-L-*threo*-hex-2-enopyranose (379) in place of 4-*O*-acetylarcanose (4-*O*-acetyl-2,6-dideoxy-3-*C*,*O*-dimethyl-L-*xylo*-hexose).[566]

Methanolysis of the turimycin complex from a *Streptomyces* species yielded methyl α- and β-mycarosides and the corresponding 4-*O*-acetyl, 4-*O*-propionyl, and 4-*O*-butyryl derivatives.[566a]

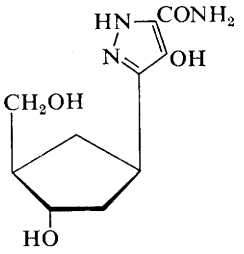
The use of m.s. in elucidation of the structures of polyene macrolide antibiotics is referred to in Chapter 24.

Nucleoside Antibiotics

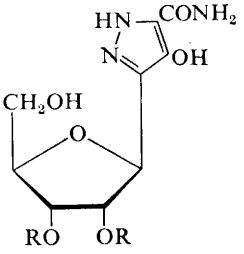
The 2′-deoxycarbocyclic analogue (380) of DL-pyrazomycin (DL-pyrazofurin A) has been synthesized[567] (see also p. 154). Pyrazomycin (381; R = H), an antiviral metabolite produced by *S. candidus*, has been synthesized from 2,3-*O*-isopropylidene-5-*O*-(4-nitrobenzoyl)-β-D-ribofuranosyl bromide, which reacted with diethyl 1,3-acetonedicarboxylate to give an α-*C*-glycoside (382) from which the pyrazole ring was elaborated.[568] Prolonged treatment of 3-(2,3-*O*-isopropylidene-α-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxylic acid ethyl ester (the penultimate intermediate in the synthesis) with methanolic ammonia at 100 °C furnished the 1′-epimeric amide (381; RR = CMe_2), which gave pyrazomycin (381; R = H) on mild hydrolysis with acid.

[561] M. Muroi, M. Izawa, and T. Kishi, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 450.
[562] M. Muroi, M. Izawa, and T. Kishi, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 463.
[563] S. Omoto, K. Iwamatsu, S. Inouye, and T. Niida, *J. Antibiotics*, 1976, **29**, 536.
[564] L. Falkowski, J. Golik, J. Zieliński, and E. Borowski, *J. Antibiotics*, 1976, **29**, 197.
[565] P. Collum, R. S. Egan, A. W. Goldstein, and J. R. Martin, *Tetrahedron*, 1976, **32**, 2375.
[566] J. R. Martin, R. S. Egan, A. W. Goldstein, S. L. Mueller, W. Keller-Schierlein, L. A. Mitscher, and R. L. Foltz, *Helv. Chim. Acta*, 1976, **59**, 1886.
[566a] R. Hüttner and G. Reuter, *Pharmazie*, 1976, **31**, 254.
[567] G. Just and S. Kim, *Canad. J. Chem.*, 1976, **54**, 2935.
[568] S. de Bernardo and M. Weigele, *J. Org. Chem.*, 1976, **41**, 287.

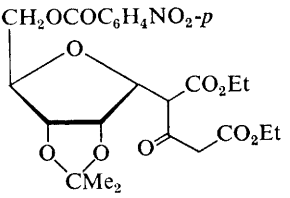
Lichtenthaler and Kulikowski have reported a synthesis of 1-(2,6-dideoxy-β-D-*arabino*-hexopyranosyl)cytosine (383), the nucleoside moiety of oxamicetin (a disaccharide-containing nucleoside antibiotic recently isolated from the fermentation broth of *Arthobacter oxamicetus*).[569] Lichtenthaler has also reviewed his synthesis of gougerotin (384; R^1 = H, R^2 = NHMe) (see Vol. 9, p. 136) and has



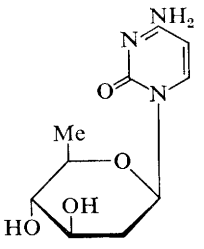
(380)



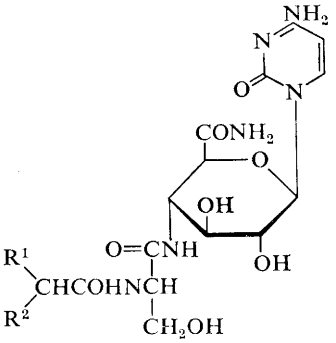
(381)



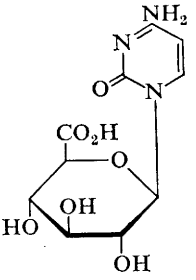
(382)



(383)



(384)



(385)

summarized the evidence establishing that aspiculamycin, an antibiotic produced by *S. toyocaensis aspiculamyceticus* and originally thought to have the structure (384; R^1 = CH_2OH, R^2 = $NHCOCH_2NHMe$) (see Vol. 8, p. 130), is identical to gougerotin.[570]

1-(β-D-Glucopyranosyluronic acid)cytosine (pentopyranic acid) (385) has been synthesized by way of condensation of methyl (1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranosyl)uronate with bis(trimethylsilyl)-*N*-acetylcytosine, followed by hydrolysis of the product with acid.[571]

Investigation of the biosynthesis of polyoxin antibiotics from labelled substrates by *S. cacaoi* has revealed that the 5-amino-5-deoxy-β-D-allofuranosyluronic acid residue of these antibiotics is not derived *via* oxidation of either D-glucose or D-allose at C-6, but is formed from triose precursors.[572]

The other nucleoside antibiotics are referred to in Chapters 21 and 24.

[569] F. W. Lichtenthaler and T. Kulikowski, *J. Org. Chem.*, 1976, **41**, 600.
[570] F. W. Lichtenthaler, T. Morino, and W. Winterfeldt, *Nucleic Acids Res., Special Publ.*, 1975, 1 (3rd Symposium Chem. Nucleic Acids Components, 1975), S33 (*Chem. Abs.*, 1976, **85**, 46 987k).
[571] K. A. Watanabe, D. H. Hollenberg, and J. J. Fox, *J. Antibiotics*, 1976, **29**, 597.
[572] K. Isono and R. J. Suhadolnik, *Arch. Biochem. Biophys.*, 1976, **173**, 141.

Miscellaneous Antibiotics

A new antibiotic metabolite isolated from *S. hygroscopicus* has been identified as 1-deoxy-D-*threo*-pentulose (386).[14] This is the first example of a naturally occurring 1-deoxypentulose. Hygromycin (387) has been shown to contain 6-deoxy-β-D-*arabino*-hexofuranos-5-ulose and 4,5-*O*-methylene-*neo*-inosamine-2.[573]

A full report on the structures of ezomycins A_1 and A_2 has appeared [574] (see Vol. 8, p. 130).

A new synthesis of prumycin (388; R = H) has been accomplished in nine steps from D-xylose,[575] and the D-galactose analogue (388; R = CH_2OH) of prumycin has been derived from 2-amino-2-deoxy-D-glucose by a route that entailed the novel hydrolysis of a benzamido-group and the selective reduction of an azido-group (see p. 65).[337] The D-galactose analogue (388) (R = CH_2OH) of prumycin does not appear to have any biological activity.

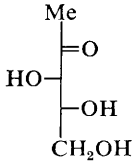
X-Ray analysis of a brominated derivative of coumermycin A_1 has confirmed that the coumerose residues are attached to the rest of the molecule by α-linkages.[448]

Following close on their disclosure of the structure of everninomicin D (see Vol. 9, p. 131), Ganguly and his co-workers have reported the structures of everninomicins B,[576] C,[577] and -2[578] produced by *Micromonospora carbonaceae*. A method for converting everninomicin D into everninomicin-2, which lacks the evernitrose (2,6-dideoxy-3-*C*,-4*O*-dimethyl-3-nitro-*arabino*-hexose*) residue, was also described.[578] Ollis's group has reported further studies of a closely related antibiotic flambamycin (see Vol. 8, p. 131) produced by a strain of *S. hygroscopicus*.[579, 580] Mild alkaline hydrolysis of flambamycin, followed by mild acidic hydrolysis, yielded flambeurekanose, a pentasaccharide composed of flambatetraose (see Vol. 8, p. 131) and eurekanic acid (see p. 98). Methylation analysis in conjunction with ^{13}C n.m.r. studies of flambamycin and its hydrolytic products suggested that flambamycin has the oligosaccharide structure (389); thus it is structurally related to the oligosaccharide antibiotics everninomicins A, C, and D, curamycin, and avilamycin.

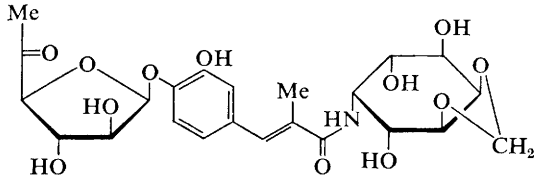
^{14}C-Labelled D-glucose was incorporated into the 2-amino-2-deoxy-D-gulose residue present in racemomycin A, a streptothricin antibiotic.[581] ^{13}C N.m.r. measurements have been used to investigate the incorporation of D-[6-^{13}C]glucose into spectinomycin hydrate (390) by growing cultures of *S. spectabilis*.[582] The

[573] K. Kakinuma, S. Kitahara, K. Watanabe, V. Sakagami, T. Fukuyasu, M. Shimura, M. Ueda, and Y. Sekizawa, *J. Antibiotics*, 1976, **29**, 771.
[574] K. Sakata, A. Sakurai, and S. Tamura, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1993.
[575] J. Yoshimura, H. Hashimoto, and T. Nishide, *Chem. Letters*, 1976, 201.
[576] A. K. Ganguly and A. K. Saksena, *J. Antibiotics*, 1975, **28**, 707.
[577] A. K. Ganguly and S. Szmulewicz, *J. Antibiotics*, 1975, **28**, 710.
[578] A. K. Ganguly, S. Szmulewicz, O. Z. Sarre, and V. M. Girijavallabhan, *J.C.S. Chem. Comm.*, 1976, 609.
[579] W. D. Ollis, C. Smith, and D. E. Wright, *J.C.S. Chem. Comm.*, 1976, 348.
[580] W. D. Ollis, C. Smith, I. O. Sutherland, and D. E. Wright, *J.C.S. Chem. Comm.*, 1976, 350.
[581] Y. Sawada, T. Kubo, and H. Taniyama, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 2163.
[582] R. M. Stroshane, M. Taniguchi, K. L. Rinehart, jun., J. P. Rolls, W. J. Haak, and B. A. Ruff, *J. Amer. Chem. Soc.*, 1976, **98**, 3025.

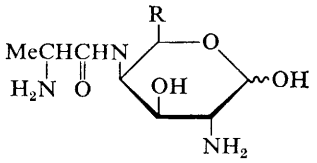
* *Editorial note*: evernitrose has the L-*arabino* configuration rather than the L-*ribo* configuration originally assigned (A. K. Ganguly, O. Z. Sarre, A. T. McPhail, and K. D. Onan, *J.C.S. Chem. Comm.*, 1977, 313).



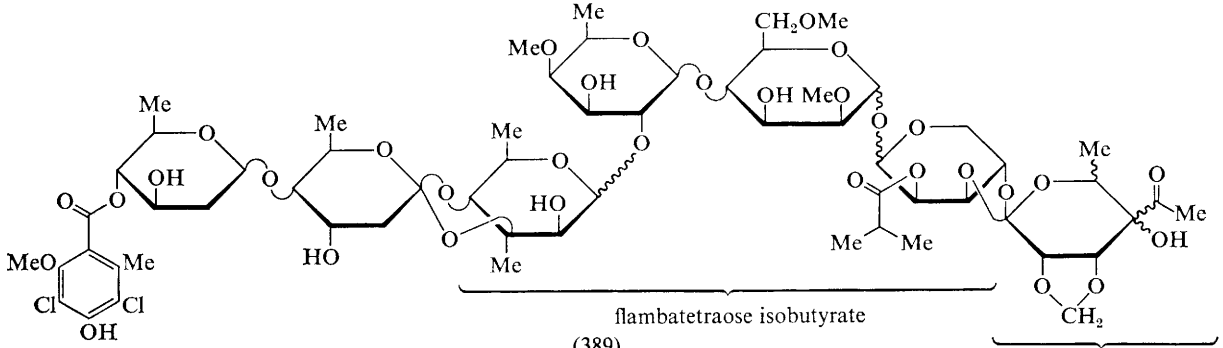
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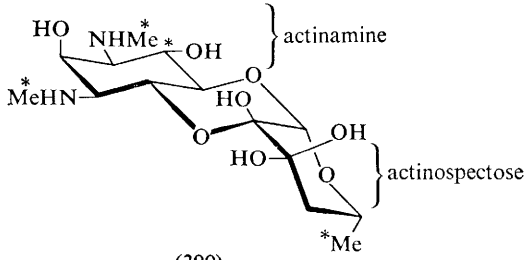
(387)



(388)



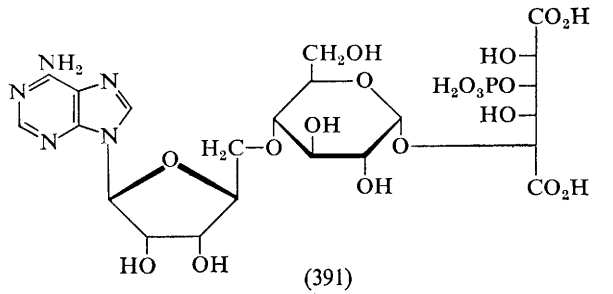
(389)



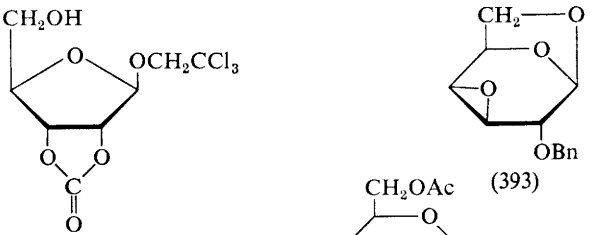
(390)

^{13}C-label [shown by asterisks in (390)] was found at C-6 of actinospectose, indicating that this neutral sugar is formed directly from D-glucose, while the label at C-6 of actinamine argues for a biosynthetic pathway to this aminocyclitol related to that for streptidine (see Vol. 9, p. 138), rather than that for deoxystreptamine (see Vol. 8, p. 137). Labelling of the *N*-methyl groups presumably occurs following the conversion of D-[6-^{13}C]glucose into [*methyl*-^{13}C]-methionine.

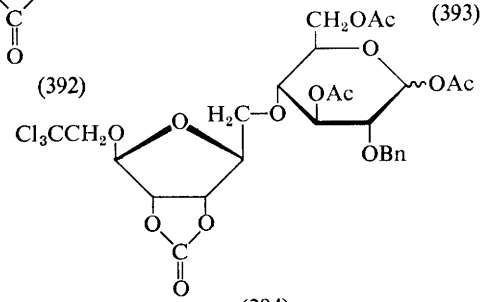
A series of papers from Šorm's group has given details of their approaches to the synthesis of the exotoxin (391) produced by *Bacillus thuringiensis*.[583–585] The first paper describes the synthesis of the protected disaccharide moiety (394),



(391)



(392) (393)



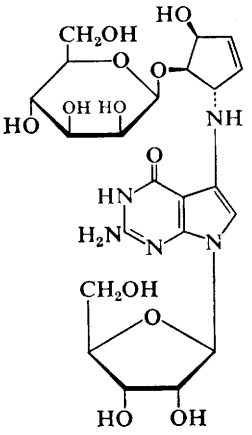
(394)

via coupling of (392) with (393), and subsequent papers report the attachment of the allaric acid, adenin-9-yl, and phosphate residues to (394) and related disaccharides. The 2,2,2-trichloroethyl group, which was used in this work to protect C-1 of the D-ribofuranosyl residue, is readily removed with zinc in acid solution, but is stable to acids, bases, oxidizing agents, and hydrogenolysis.

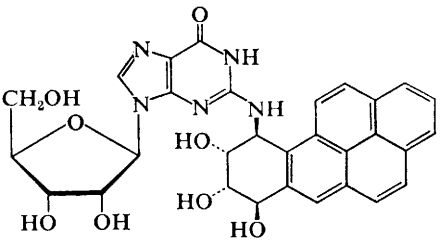
[583] L. Kalvoda, M. Prystaš, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1976, **41**, 788.
[584] L. Kalvoda, M. Prystaš, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1976, **41**, 800.
[585] M. Prystaš, L. Kalvoda, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1976, **41**, 1426.

21
Nucleosides

Groups in the United States, West Germany, and Japan have jointly announced the isolation and structure (395) of the most complex tRNA nucleoside known to date.[586] It consists of a β-D-mannopyranosyl residue linked to the side-chain



(395)



(396)

of nucleoside Q (see Vol. 9, p. 139), and was accompanied by a small amount of an isomeric nucleoside having a β-D-galactopyranosyl residue instead of a β-D-mannopyranosyl residue. The isolation of the nucleic acid component (396) has provided an important link in the understanding of the carcinogenicity of such polycyclic aromatic hydrocarbons as benzo[*a*]pyrene.[587] The benzo[*a*]-pyrene-guanosine derivative (396) is presumably formed by opening of the oxiran ring in 9,10-anhydro-7,8,9,10-tetrahydro-7,8,9,10-tetrahydroxybenzo[*a*]-pyrene, which has been implicated in the binding of the polycyclic aromatic hydrocarbon to cellular nucleic acids. 1-Methylpseudouridine has been identified as a metabolite produced by *Streptomyces platensis*,[588] and 5′-(3-aminoprop-1-yl)-5′-thioadenosine has been found in the eyes of the sea catfish (*Arius felis* L.).[589] A recent volume of *Ann. New York Acad. Sci.* is devoted to the chemistry,

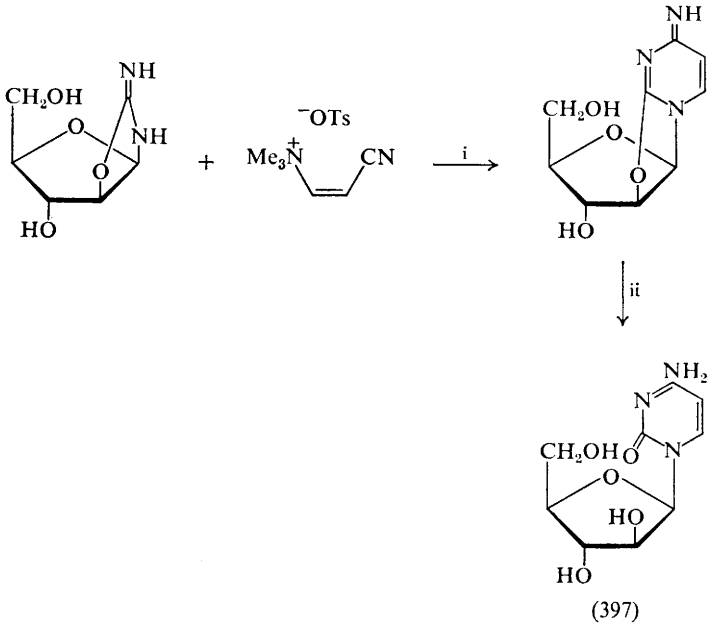
[586] H. Kasai, K. Nakanishi, R. D. MacFarlane, D. F. Torgerson, Z. Ohashi, J. A. McCloskey, H. J. Gross, and S. Nishimura, *J. Amer. Chem. Soc.*, 1976, **98**, 5044.
[587] A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, I. Miura, and K. Nakanishi, *J. Amer. Chem. Soc.*, 1976, **98**, 5714.
[588] A. D. Argoudelis and S. A. Mizsak, *J. Antibiotics*, 1976, **29**, 818.
[589] S. Ito and J. A. C. Nicol, *Biochem. J.*, 1976, **153**, 567.

biology, and clinical uses of nucleoside analogues, including the chemistry of 2,2′-anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine (an antileukaemic agent) [590] and the synthesis of acyclic sugar nucleosides from aldose dialkyl dithioacetals.[591]

Nucleoside antibiotics are covered in Chapter 20.

Synthesis

An improved synthesis of the antileukaemic agent 1-β-D-arabinofuranosylcytosine (397) is shown in Scheme 80.[592] Ethyl 3,5,6-tri-*O*-acetyl-2-*S*-ethyl-



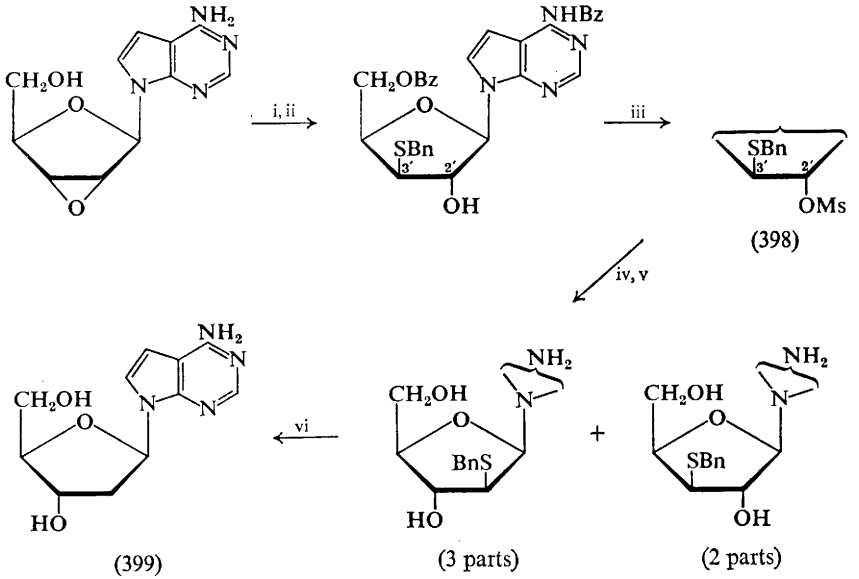
(397)

Reagents: i, DMF at 50 °C; ii, aqueous NH_3

Scheme 80

1,2-dithio-α-D-mannofuranoside has been used as the starting material for the synthesis of several purine and pyrimidine nucleosides [*e.g.* 9-(2-deoxy-β-D-*threo*-pentofuranosyl)-thymine and -uracil].[414] 2′-Deoxytubercidin (399) has been obtained in 27% overall yield from tubercidin by the route outlined in Scheme 81; the key step in the sequence involved migration of the 3′-*S*-benzyl group to C-2 when the 2′-methanesulphonate (398) was heated with sodium benzoate in DMF.[593] Syntheses of 2′,5′-dideoxy-5-iodouridine (from 2′-deoxyuridine),[594] 5′-deoxy- and 2′,5′-dideoxyuridine (from uridine 2′,3′-carbonate),[595] and

[590] J. J. Fox and B. A. Otter, *Ann. New York Acad. Sci.*, 1975, **255**, 59.
[591] D. Horton, D. C. Baker, and S. S. Kokrady, *Ann. New York Acad. Sci.*, 1975, **255**, 131.
[592] E. J. Hessler, *J. Org. Chem.*, 1976, **41**, 1828.
[593] M. J. Robins and W. H. Muhs, *J.C.S. Chem. Comm.*, 1976, 269.
[594] T.-S. Lin and W. H. Prusoff, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 309.
[595] L. Hein, P. Drašar, and J. Beránek, *Nucleic Acid Res.*, 1976, **3**, 1125.

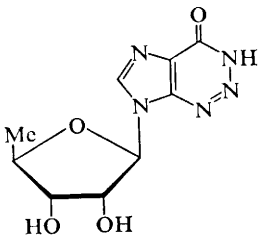


Reagents: i, BzCl–py; ii, NaSBn–THF; iii, MsCl–py; iv, NaOBz–DMF; v, MeONa–MeOH; vi, H_2–Ni

Scheme 81

5′-deoxy-2-azainosine (400) (from 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamine)[596] have also been reported.

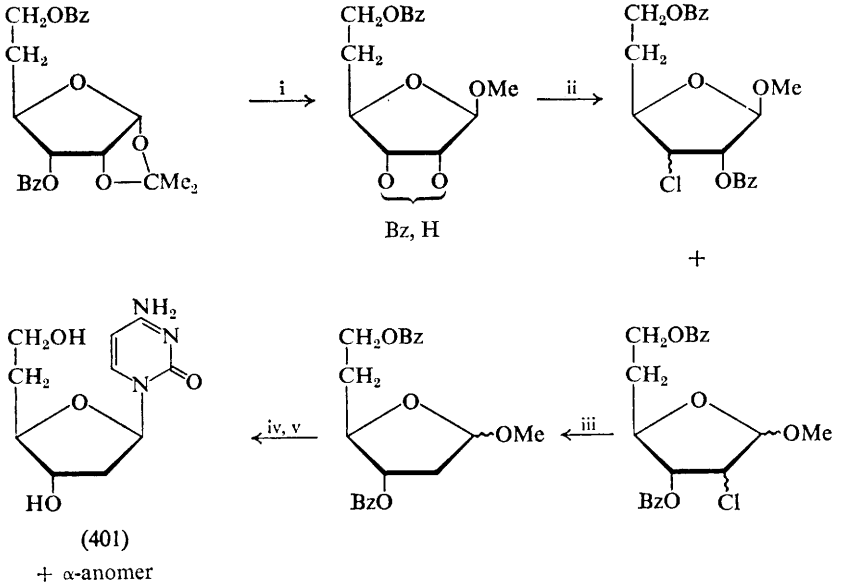
Both 1-α- and -β-D-glucopyranosyl-cytosine and -uracil derivatives have been obtained *via* the condensation of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl chloride with 2,4-diethoxypyrimidine under the original Hilbert–Johnson conditions.[597] 'Homodeoxycytidine' (401) has been synthesized by the route



(400)

outlined in Scheme 82,[598] and standard procedures have been employed to prepare adenin-9-yl nucleosides of 6-deoxy-β-D- and -L-gulofuranose and 6-deoxy-α-D- and -L-idofuranose.[599] *S*-Adenosylhomocysteine and related compounds,

[596] P. C. Srivastava, A. R. Newman, and R. K. Robins, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 327.
[597] C. L. Stevens, R. Radha Krishnan, and P. M. Pillai, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 71.
[598] S. David and G. de Sennyey, *Compt. rend.*, 1976, **283**, *C*, 21.
[599] L. M. Lerner, *J. Org. Chem.*, 1976, **41**, 306.



Reagents: i, MeOH–*aci*-resin; ii, Ph_3P–CCl_4; iii, Bu_3SnH; iv, bis(trimethylsilyl)-*N*-acetylcytosine–$SnCl_4$–$ClCH_2CH_2Cl$; v, MeONa–MeOH

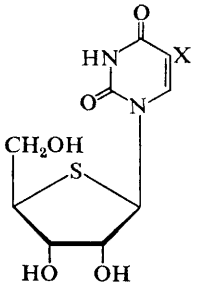
Scheme 82

including the 2′- and 3′-deoxy-analogues,[600] and the nucleosides (402), (403),[600a] and (404),[601] containing 4-thio-β-D-ribofuranose, have been synthesized. An improved synthesis of 1-β-D-xylopyranosyluracil involved the reaction of α-D-xylopyranose tetra-acetate with 2,4-bis(triethylsilyloxy)pyrimidine in the presence of stannic chloride.[602] Bromination of the unprotected nucleoside gave the 5-bromo derivative. Dihydrofuran and dihydropyran reacted with substituted imidazoles to give the corresponding tetrahydrofuranyl and tetrahydropyranyl nucleosides.[603] Syntheses of racemic carbocyclic analogues of adenosine (*i.e.* DL-aristeromycin) (405),[604, 605] uridine,[604–606] and 2′- and 3′-deoxyuridine [606] have been reported.

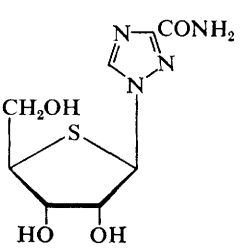
The influence of 5- and 6-substituents on silylated uracils in the stannic-chloride-catalysed reactions with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose and 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose has been investigated.[607] Electron-releasing substituents (*e.g.* OMe and NH_2) at C-5 of uracil promoted

[600] R. T. Borchardt, J. A. Huber, and Y. S. Wu, *J. Org. Chem.*, 1976, **41**, 565.
[600a] J. C. Kim, W.-J. C. Lee, M. Bobek, and R. L. Whistler, *Taehan Hwahak Hoechi*, 1975, **19**, 438 (*Chem. Abs.*, 1976, **84**, 150 879s).
[601] M. V. Pickering, J. T. Witkowski, and R. K. Robins, *J. Medicin. Chem.*, 1976, **19**, 841.
[602] V. Cheng and A. P. Ollapally, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 229.
[603] G. Alonso, C. Díez, G. García-Muñoz, F. G. de las Heras, and P. Navarro, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 157.
[604] A. Holý, *Nucleic Acids Res., Special Publ.*, 1975, 1 (3rd Symposium Chem. Nucleic Acids Components, 1975), S73 (*Chem. Abs.*, 1976, **85**, 63 277w).
[605] A. Holý, *Coll. Czech. Chem. Comm.*, 1976, **41**, 2096.
[606] Y. F. Shealy and C. A. O'Dell, *J. Heterocyclic Chem.*, 1976, **13**, 1015.
[607] U. Niedballa and H. Vorbrüggen, *J. Org. Chem.*, 1976, **41**, 2084.

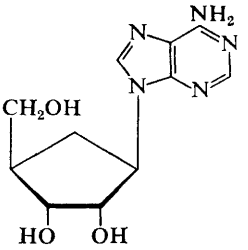
the formation of stable stannic chloride–base complexes, which were glycosylated only upon the addition of more catalyst. Ribonucleosides containing the uracil analogues (406) [608] and (407) [609] have been synthesized, and 5′-*O*-trityluridine and 5′-*O*-trityl-6-azauridine 2′,3′-carbonates have been converted into 2′-deoxyuridine and 2′-deoxy-6-aza-uridine and -cytidine, respectively, *via* the corresponding 2,2′-anhydro-β-D-arabinofuranosyl nucleosides.[610] Ribonucleosides containing 5-substituted 6-azauracils have also been derived from 1,2,4-triazines [611] and by base-catalysed cyclization of N^3-β-D-ribofuranosyl glyoxylic acid semicarbazone derivatives.[612]



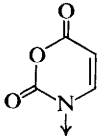
(402) X = F
(403) X = Cl



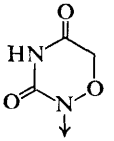
(404)



(405)



(406)



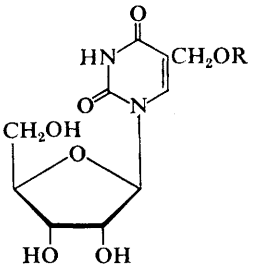
(407)

Standard procedures have been employed in the preparation of various 5-substituted 2′-deoxyuridines,[613, 614] including the 5-ethynyl [615] and 5-methyl [616] derivatives, 5-cyclopropyluridine,[617] the 5-(fluoroalkyloxymethyl)uridines (408),[618] and the pyridine nucleosides (409) [619] and (410).[620] The pyridine nucleosides

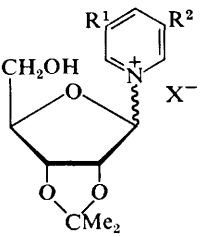
[608] T. L. Chwang, W. F. Wood, J. R. Parkhurst, S. Nesnow, P. V. Danenberg, and C. Heidelberger, *J. Medicin. Chem.*, 1976, **19**, 643.
[609] P. T. Berkowitz, R. K. Robins, P. Dea, and R. A. Long, *J. Org. Chem.*, 1976, **41**, 3128.
[610] P. Drašar, L. Hein, and J. Beránek, *Coll. Czech. Chem. Comm.*, 1976, **41**, 2110.
[611] C. Cristescu, *Rev. Roumaine Chim.*, 1975, **20**, 1287 (*Chem. Abs.*, 1976, **84**, 59 916r).
[612] H. Hrebabecky, P. Fiedler, and J. Beránek, *Nucleic Acids Res., Special Publ.*, 1975, 1 (3rd Symposium Chem. Nucleic Acids Components, 1975), S9 (*Chem. Abs.*, 1976, **85**, 124 262m).
[613] A. Szabolcs, J. Sagi, and L. Otvos, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 197.
[614] A. Kampt, C. J. Pillar, W. J. Woodford, and M. P. Mertes, *J. Medicin. Chem.*, 1976, **19**, 909.
[615] J. Perman, R. A. Sharma, and M. Bobek, *Tetrahedron Letters*, 1976, 2427.
[616] J. Cadet, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 459.
[617] I. Basnak and J. Farkas, *Nucleic Acids Res., Special Publ.*, 1975, 1 (3rd Symposium Chem., Nucleic Acids Components, 1975), S81 (*Chem. Abs.*, 1976, **85**, 46 989n).
[618] S. Ya. Mel'Nik, A. A. Bakhmedova, M. N. Preobrazhenskaya, G. N. Platonova, N. A. Lesnaya, and Z. P. Sof'ina, *Zhur. org. Khim.*, 1976, **12**, 652 (*Chem. Abs.*, 1976, **85**, 33 327v).
[619] E. J. Freyne, J. A. Lepoivre, F. C. Alderweireldt, M. J. O. Anteunis, and A. de Bruyn, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 113.
[620] E. L. Esmans, J. A. Lepoivre, F. C. Alderweireldt, and A. de Bruyn, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 93.

(410) were reduced to the corresponding 1,4-dihydropyridine nucleosides with sodium dithionate.

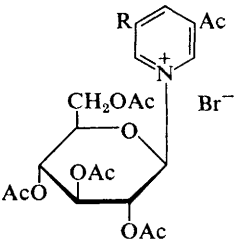
The 6-benzylamino-7-β-D-gluco-furanosyl- and -pyranosyl-purines [(411) and (412), respectively] have been synthesized *via* fusion of 4-bromo-5-nitroimidazole



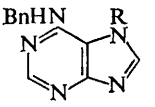
(408) R = CH_2CF_3 or $CH_2CF_2CF_3$



(409) R^1 = H, Me, or ^{i}Pr; R^2 = $CONH_2$, *etc.*; X = Cl or Br

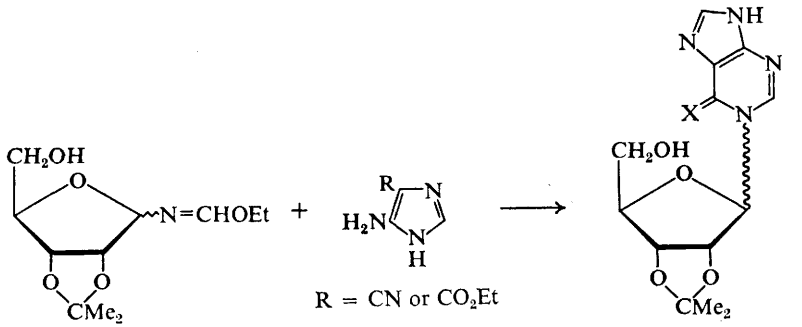


(410) R = Me, Et, Pr, *etc.*



(411) R = β-D-Glc*f*
(412) R = β-D-Glc*p*

with β-D-gluco-furanose or -pyranose penta-acetates.[621] The pyranose derivative (412) was shown to be identical with a cytokinin metabolite, previously thought to be the furanose derivative (411). N.m.r. examination of 5,6-trimethyleneuridine (prepared by a standard procedure) revealed that it adopted the *syn* conformation.[622] Derivatives of 1-D-ribofuranosylpurines have been synthesized by the



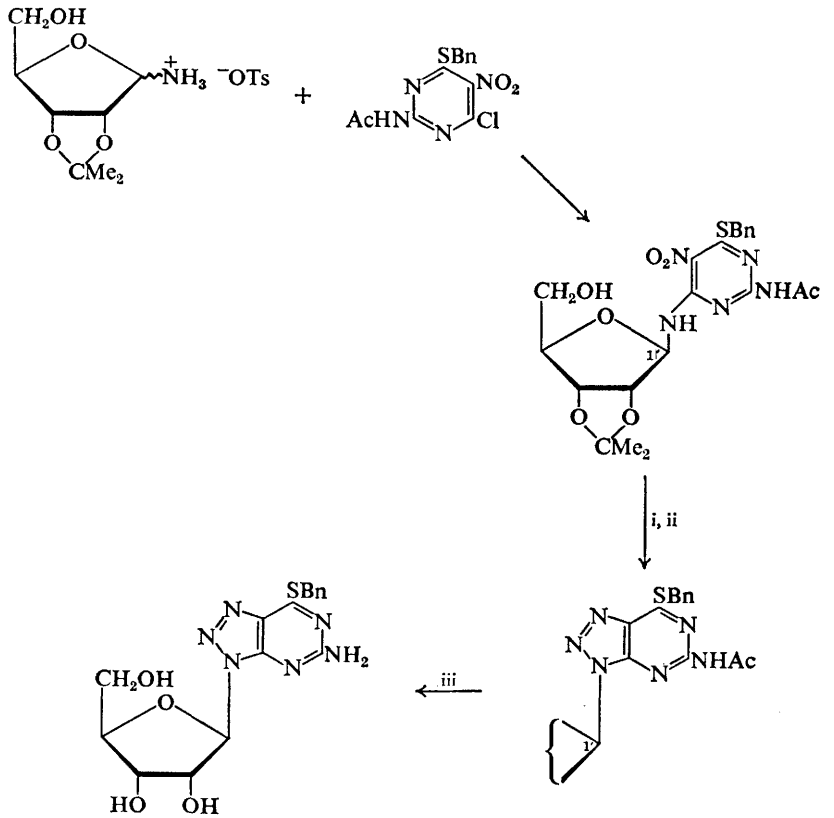
R = CN or CO_2Et

X = NH or O

Scheme 83

[621] J. D. McChesney and R. Buchman, *Heterocycles*, 1976, **4**, 1065 (*Chem. Abs.*, 1976, **85**, 124 277v).

[622] M. Draminski and E. Frass, *Bull. Acad. polon. Sci., Sér. Sci. chim.*, 1976, **24**, 37 (*Chem. Abs.*, 1976, **84**, 180 526s).

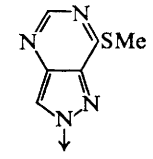


Reagents: i, H_2–cat.; ii, HNO_2; iii, H_3O^+

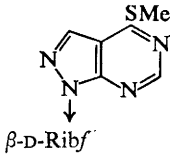
Scheme 84

condensation shown in Scheme 83.[623] Syntheses of 1-[624] and 3-deazaguanosines,[625] 3-deaza-6-thioguanosine and its derivatives,[626, 627] and 6-substituted 8-azaguanosines (Scheme 84) [628] have been described. 7-Methylthiopyrazolo[4,3-*d*]pyrimidine and 4-methylthiopyrazolo[3,4-*d*]pyrimidine reacted with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose to give, after deacetylation, the nucleosides (413) and (414), respectively.[629, 629a] 2-β-D-Ribo- and -xylo-furanosylbenzotriazoles have been obtained *via* treatment of the 2,3,5-tri-*O*-benzoylglycosyl chlorides with 2-chloro-

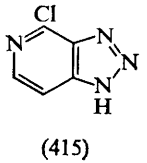
[623] A. Dhainaut, J. L. Montero, B. Rayner, C. Tapiero, and J. L. Imbach, *Tetrahedron Letters*, 1976, 45.
[624] J. E. Schelling and C. A. Salemink, *Rec. Trav. chim.*, 1975, **94**, 153 (*Chem. Abs.*, 1976, **84**, 17 646x).
[625] P. D. Cook, R. J. Rousseau, A. M. Mian, P. Dea, R. B. Meyer, and R. K. Robins, *J. Amer. Chem. Soc.*, 1976, **98**, 1492.
[626] J. A. May, jun. and L. B. Townsend, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 271.
[627] J. A. May, jun. and L. B. Townsend, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 371.
[628] R. D. Elliott and J. A. Montgomery, *J. Medicin. Chem.*, 1976, **19**, 1186.
[629] O. V. Budanova, I. A. Korbukh, and M. N. Preobrazhenskaya, *Zhur. org. Khim.*, 1976, **12**, 1131 (*Chem. Abs.*, 1976, **85**, 108 922w).
[629a] F. F. Blanko, I. A. Korbukh, and M. N. Preobrazhenskaya, *Zhur. org. Khim.*, 1976, **12**, 1132.



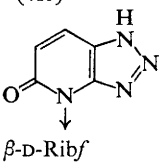
(413)



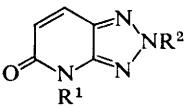
(414)



(415)



(416)



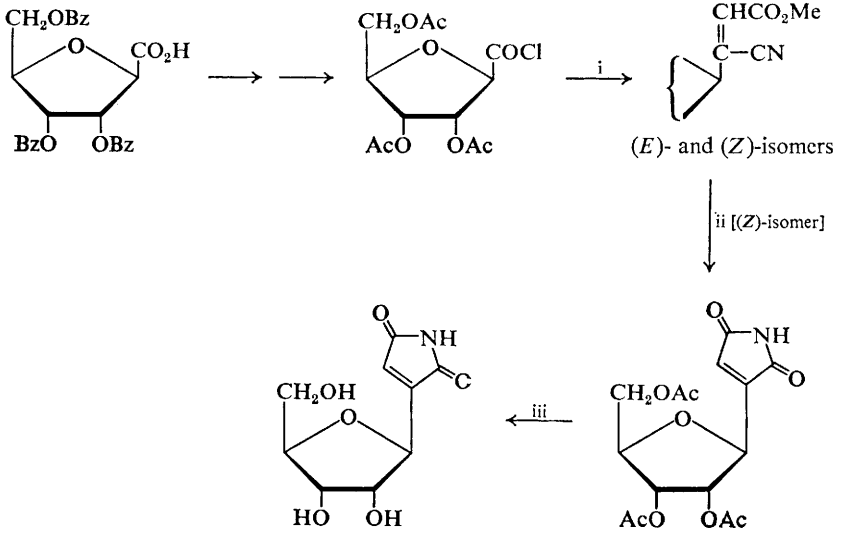
(417) $R^1 = R^2 = \beta$-D-Ribf
(418) R^1 = Me; $R^2 = \beta$-D-Ribf

mercuribenzotriazole.[630] Nucleosides having β-D-ribofuranosyl residues attached to different nitrogen atoms of the triazole ring have been derived from the *vic*-triazolo[4,5-*c*]pyridine (415).[631] Suitably protected 1-β-D-ribofuranosylpyrid-2-ones have been converted into 2-, 4-, and 2,4-substituted triazolo[4,5-*b*]pyridones [*e.g.* (416)—(418)].[632] An unexpected furanose-to-pyranose interconversion occurred when (418) was acetylated.

Imidazo[1,2-*c*]pyrimidine nucleosides containing β-D-ribofuranosyl[633] and β-D-arabinofuranosyl residues,[634] imidazo[1,2-*a*]pyrimidine ribonucleosides,[635] 4-amino-5-β-D-ribofuranosyl-5-azaindole,[636] and 1-(2-deoxy-α- and -β-D-*erythro*-pentofuranosyl)indoles[637] have been reported.

A new synthesis of showdomycin is outlined in Scheme 85.[638] 5-Amino-1-(3-deoxy-β-D-*erythro*-pentofuranosyl)imidazole-4-thiocarboxamide (419) has been synthesized (from a related 4-carboxamide) and shown to possess no biological activity, although the 3′-hydroxy-analogue is biologically active.[639] Among several analogues of pyrazomycin (see p. 135) to be synthesized, (420) and (421) were shown to possess antileukaemic and antitumour activities.[640, 641] Other nucleosides to be synthesized include ethyl 1-β-D-ribofuranosyl- and 1-β-D-gluco-pyranosyl-5-hydroxymethylpyrazole-3-carboxylates,[642, 642a] and the isomeric

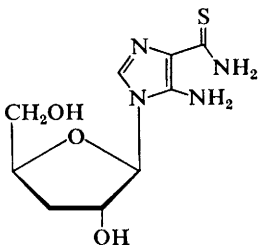
[630] E. E. Rengevich, V. P. Chernetskii, and N. G. Burlii, *Ukrain. khim. Zhur.* (*Russ. Edn.*), 1975, **41**, 1104 (*Chem. Abs.*, 1976, **84**, 74 555r).
[631] J. A. May, jun., and L. B. Townsend, *J. Org. Chem.*, 1976, **41**, 1449.
[632] B. M. Lynch and S. C. Sharma, *Canad. J. Chem.*, 1976, **54**, 1029.
[633] D. G. Bartholomew, P. Dea, R. K. Robins, and G. R. Revankar, *J. Org. Chem.*, 1975, **40**, 3708.
[634] D. G. Bartholomew, J. H. Huffman, T. R. Matthews, R. K. Robins, and G. R. Revankar, *J. Medicin. Chem.*, 1976, **19**, 814.
[635] G. R. Revankar and R. K. Robins, *Ann. New York Acad. Sci.*, 1975, **255**, 166.
[636] C. Ducrocq, E. Bisagni, J.-M. Lhoste, J. Mispelter, and J. Defaye, *Tetrahedron*, 1976, **32**, 773.
[637] T. H. Dinh, M.-J. Bayard, and J. Igolen, *Compt. rend.*, 1976, **283**, *C*, 227.
[638] L. Kalvoda, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 47.
[639] T. Miyoshi, S. Suzaki, and A. Yamazaki, *Chem. and Pharm. Bull.* (*Japan*), 1976, **24**, 2089.
[640] I. A. Korbukh, S. V. Kitaev, and M. N. Preobrazhenskaya, *Zhur. org. Khim.*, 1976, **12**, 682 (*Chem. Abs.*, 1976, **85**, 33 328w).
[641] O. Makabe, M. Nakamura, and S. Umezawa, *Bull. Chem. Soc. Japan*, 1975, **48**, 3210.
[642] A. A. Akhrem, N. I. Garbuz, E. I. Kvasyuk, and I. A. Mikhailopulo, *Zhur. obshchei Khim.*, 1976, **46**, 1394 (*Chem. Abs.*, 1976, **85**, 160 453h).
[642a] A. A. Akhrem, E. I. Kvasyuk, and I. A. Mikhailopulo, *Zhur. obshchei Khim.*, 1976, **46**, 1403 (*Chem. Abs.*, 1976, **85**, 143 390t).



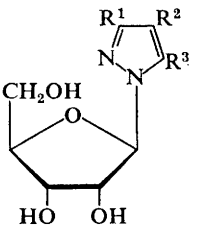
Reagents: i, $Ph_3P{=}CHCO_2Me$–HCN; ii, Ac_2O–H_2SO_4; iii, MeOH–H^+

Scheme 85

3-amino-1-, -2-, and -4-β-D-ribofuranosyl-1,2,4-triazoles.[643] The tetrazole ribonucleosides (422),[644] (423), (424),[645] and 1,2,4-thiadiazole ribonucleosides (425) [646] have been prepared by standard procedures. Fusion of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose with carbazole gave the corresponding anomeric nucleosides,[647] and the synthesis and biological activities of some *N*-β-D-glucopyranosyl-pyrazolosteroids and related model nucleosides [*e.g.* (426)] have been reported.[648] The nucleoside (426) (R^1 = H; R^2 = Ac) exhibited high biological

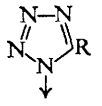


(419)



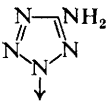
(420) R^1 = $CONH_2$; R^2 = R^3 = H
(421) R^1 = R^3 = $CONH_2$; R^2 = NO_2

[643] M. Fuertes, R. K. Robins, and J. T. Witkowski, *J. Carbohydrates, Nucleosides, Nucelotides*, 1976, **3**, 169.
[644] M. S. Poonian, E. F. Nowoswiat, J. F. Blount, T. H. Williams, R. G. Pitcher, and M. J. Kramer, *J. Medicin. Chem.*, 1976, **19**, 286.
[645] M. S. Poonian, E. F. Nowoswiat, J. F. Blount, and M. J. Kramer, *J. Medicin. Chem.*, 1976, **19**, 1017.
[646] G. R. Revankar and R. K. Robins, *J. Heterocyclic Chem.*, 1976, **13**, 169.
[647] C. Chavis, F. Dumont, G. Gosselin, and J. Imbach, *Carbohydrate Res.*, 1976, **46**, 43.
[648] M. M. Hashem, K. D. Berlin, R. W. Chesnut, and N. N. Durham, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 357.



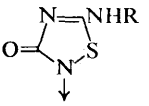
β-D-Ribf

(422) R = H, $CONH_2$, or CH_2CONH_2
(423) R = NH_2



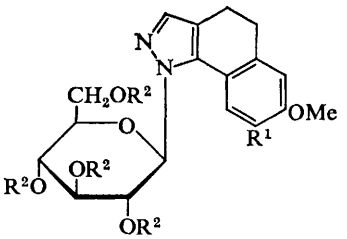
β-D-Ribf

(424)



β-D-Ribf

(425) R = H or Ac



(426) R^1 = H or OMe; R^2 = H or Ac

activity against several micro-organisms. D-Xylopyranosyl nucleosides of ellipticine and its 9-acetoxy-derivative have been reported,[649] and Pfleiderer's group has extended its investigations of D-ribosylations to the pteridine[650] and tetrahydropteridine[651] series.

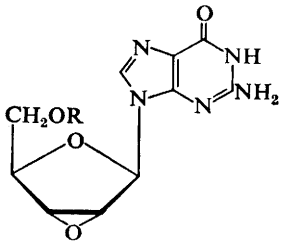
The synthesis of thiolumazine nucleosides has been reviewed.[652] Both 1-α- and 1-β- and 3-α-D-arabinofuranosyl-6,7-diphenyl-lumazines,[653] and 1- and 3-β-D-ribofuranosyl-lumazines[654] and -6,7-diphenyl-2- and -4-thiolumazines[655] have been obtained by way of the silyl procedure. Mercuric salts of thiolumazines gave the N^3-nucleosides preferentially, whereas *O*-ribosylation occurred when silver salts were used.[655]

Bridged Nucleosides and Isonucleosides

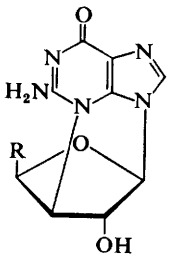
Treatment of 2′,3′-*O*-methoxyethylideneguanosine with pivalyl chloride and of the resulting chloro derivatives (see p. 158) with sodium methoxide furnished the 2,′3′-oxiran (427), which underwent ring-opening to give the bridged nucleoside (428).[656]

On heating in boiling water the lumazine nucleoside 5′-sulphonate (429) rearranged to 5′-deoxy-5′-(lumazin-8-yl)-D-ribofuranose (430), which could be converted into methyl furanoside, dialkyl dithioacetal, and osazone derivatives.[657]

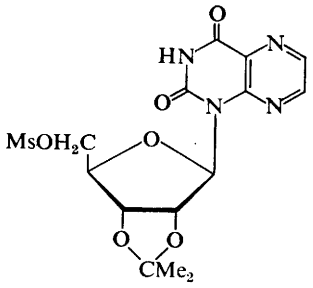
[649] M. Bessodes, N. Dat-Xuong, and K. Antonakis, *Compt. rend.*, 1976, **282**, *C*, 1001.
[650] K. Eistetter and W. Pfleiderer, *Chem. Ber.*, 1976, **109**, 3208.
[651] T. Itoh and W. Pfleiderer, *Chem. Ber.*, 1976, **109**, 3228.
[652] I. Southon and W. Pfleiderer, *Chem. Biol. Pteridines, Proc. 5th Internat. Symposium*, 1975, 783 (*Chem. Abs.*, 1976, **84**, 180 481y).
[653] W. Hutzenlaub, K. Kobayashi, and W. Pfleiderer, *Chem. Ber.*, 1976, **109**, 3217.
[654] K. Kobayashi and W. Pfleiderer, *Chem. Ber.*, 1976, **109**, 3194.
[655] I. Southon and W. Pfleiderer, *Nucleic Acids Res., Special Publ.*, 1975, 1 (3rd Symposium Chem. Nucleic Acids Components, 1975), S37 (*Chem. Abs.*, 1976, **85**, 46 988m).
[656] R. Mengel and W. Muhs, *Nucleic Acids Res., Special Publ.*, 1975, 1 (3rd Symposium Chem. Nucleic Acids Components, 1975), S41 (*Chem. Abs.*, 1976, **85**, 78 294y).
[657] K. Kobayashi and W. Pfleiderer, *Chem. Ber.*, 1976, **109**, 3175.



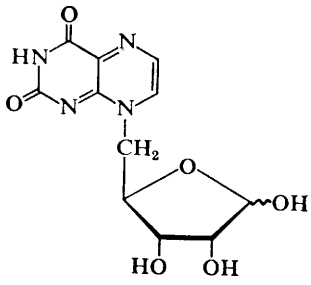
(427) R = CO·Bu^t



(428) R = $CH_2OCO{\cdot}Bu^t$



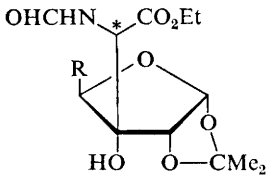
(429)

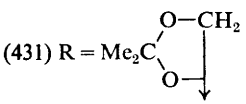


(430)

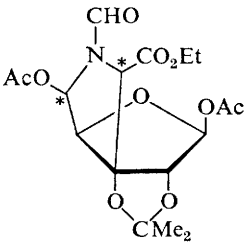
Nucleosides containing Branched-chain Components

The 3^1,5-*N*-formylepimine derivative (432) [derived from the diacetal (431)] has been converted into a protected analogue (433) of the polyoxin antibiotics.[658] The stereochemistry of the *N*-formylepimine derivative (432) was established by *X*-ray crystallographic analysis. A series of lipophilic 9-(3-alkyl-3-deoxy-β-D-ribofuranosyl)adenines (434) has been prepared by glycosylation of 6-benzamido-9-chloromercuripurine with the appropriate branched-chain sugar

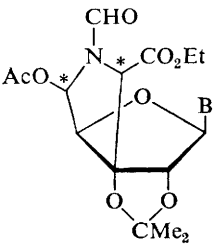




(431) R = Me_2C



(432)

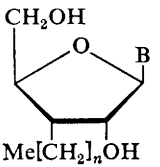


(433) B = uracil-1-yl

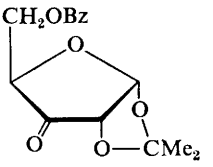
* denotes the (*R*)-configuration

[658] A. J. Brink, J. Coetzer, O. G. de Villiers, R. H. Hall, A. Jordaan, and G. J. Kruger, *Tetrahedron*, 1976, **32**, 965.

in the presence of titanium chloride.[659] The branched-chain sugars were prepared by Wittig reactions on 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranos-3-ulose (435) and hydrogenation of the resulting 3-*C*-alkylidene derivatives. The 3-butyl-3-deoxy (434; $n = 3$) and 3-deoxy-3-hexyl (434; $n = 5$)



(434) B = adenin-9-yl
n = 0, 1, 3, or 5



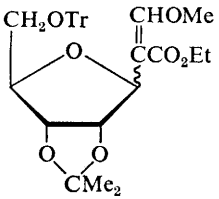
(435)

derivatives were more effective than 3′-deoxyadenosine (cordycepin) in inhibiting the synthesis of RNA and DNA.

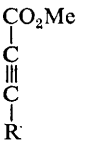
C-Nucleosides

Naturally occurring *C*-nucleosides and the synthesis of analogous *C*-nucleosides and functionalized *C*-glycosides have been reviewed.[660]

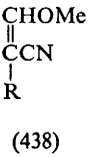
Fox's group has extended its work on the synthesis of pseudocytidine (ψ-cytidine) (Vol. 9, p. 148) to include other 5-D-ribofuranosylpyrimidines. Thus the 3-methoxyacrylate derivative (436) reacted with guanidine, urea, or thiourea to give derivatives of ψ-cytidine, ψ-uridine, and 2-thio-ψ-uridine and their α-anomers.[661] The D-ribofuranosyl-ethyne and -ethene derivatives (437) and



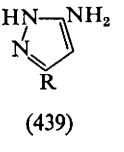
(436)



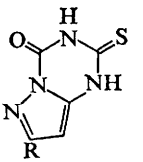
(437) β-anomer



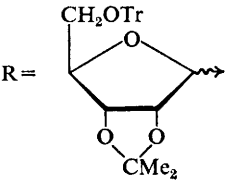
(438)



(439)

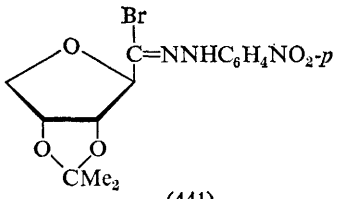


(440)

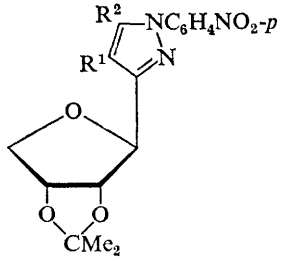


(438) have been used as starting materials for syntheses of 8-α- and 8-β-D-ribofuranosylpyrazolo[1,5-*a*]1,3,5-triazine derivatives (440), the triazine ring being elaborated by treatment of the intermediate pyrazole (439) with ethoxycarbonyl

[659] A. Rosowsky, H. Lazarus, and A. Yamashita, *J. Medicin. Chem.*, 1976, **19**, 1265.
[660] S. Hanessian and A. G. Pernet, *Adv. Carbohydrate Chem. Biochem.*, 1976, **33**, 111.
[661] C. K. Chu, I. Wempen, K. A. Watanabe, and J. J. Fox, *J. Org. Chem.*, 1976, **41**, 2793.

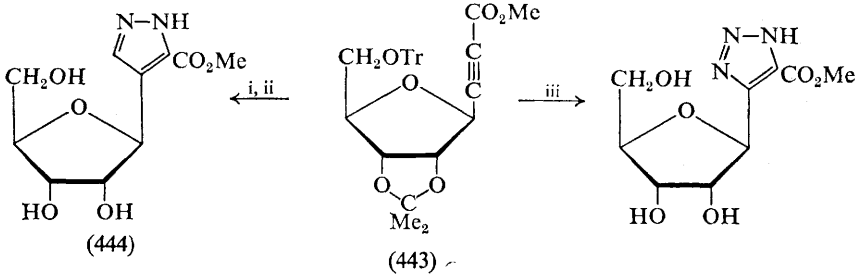


(441)



(442) R^1 = H; R^2 = CO_2Me
or R^1 = CO_2Me; R^2 = H

isothiocyanate and cyclization.[662] Cycloaddition of methyl acetylenecarboxylate to the nitrilimine derived from 2,5-anhydro-1-bromo-1-deoxy-3,4-*O*-isopropylidene-1-(4-nitrophenylhydrazono)-D-ribose (441) afforded the isomeric pyrazole carboxylic esters (442).[663] *C*-Nucleosides [*e.g.* (444)] related to formycin have been synthesized by 1,3-dipolar cycloadditions to the protected β-D-ribofuranosylpropiolate (443) (Scheme 86).[664] Similar cycloadditions of diazomethane and



(444) (443)

Reagents: i, CH_2N_2; ii, H^+; iii, Me_3SiN_3

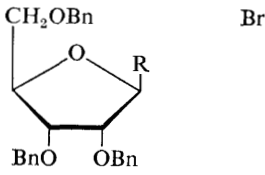
Scheme 86

phenylhydrazine to the β-D-ribofuranosylethyne derivatives (445) have been reported by Buchanan's [665] and Moffatt's groups.[666] Appreciably lower yields of 3- and 4-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)pyrazoles were obtained from the reaction of (445) (R = C≡CH) with diazomethane [665] than from the corresponding reaction with (445) (R = C≡CCO_2Me), which gave 4-(2,3,5-tri-*O*-benzyl-β-D-ribofuranosyl)-3(5)-carbomethoxypyrazole regioselectively.[665, 666] Alternative approaches to the synthesis of the latter compound have been investigated by Moffatt's group.[666] One route involved cycloaddition of diazomethane to the β-D-ribofuranosylacrylate derivative (446) and dehydrogenation of the inter-

[662] F. G. de las Heras, C. K. Chu, S. Y.-K. Tam, R. S. Klein, K. A. Watanabe, and J. J. Fox, *J. Heterocyclic Chem.*, 1976, **13**, 175.
[663] J. M. J. Tronchet, F. Perret, F. Barbalat-Rey, and T. Nguyen-Xuan, *Carbohydrate Res.*, 1976, **46**, 19.
[664] F. G. de las Heras, S. Y.-K. Tam, R. S. Klein, and J. J. Fox, *J. Org. Chem.*, 1976, **41**, 84.
[665] J. G. Buchanan, A. R. Edgar, M. J. Power, and G. C. Williams, *Nucleic Acids Res., Special Publ.*, 1975, 1 (3rd Symposium Chem. Nucleic Acids Components, 1975), S69 (*Chem. Abs.*, 1976, **85**, 63 276v).
[666] C. M. Gupta, G. H. Jones, and J. G. Moffatt, *J. Org. Chem.*, 1976, **41**, 3000.

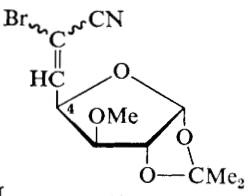
mediate pyrazoline with chlorine, while another depended on the addition of diazomethane to methyl 5,6,8-tri-*O*-benzyl-2,3-dideoxy-D-*altro*-oct-2-ynonate (prepared by the reaction of 2,3,5-tri-*O*-benzyl-D-ribofuranose with the Grignard reagent derived from methyl propiolate), followed by acid-catalysed cyclization.

Several heterocyclic derivatives have been elaborated from the 6-bromo-6-cyanohex-5-enose (447). For example, the (*E*)- and (*Z*)-isomers of (447) reacted with benzylamine to give the *cis*- and *trans*-aziridines (448), respectively, and with guanidine in an alkaline medium to give the diaminopyrimidine derivative (449).[667]

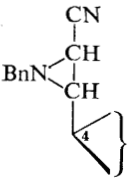


(445) R = $C{\equiv}CCO_2Me$ or $C{\equiv}CH$

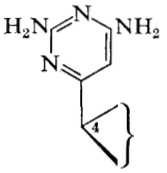
(446) R = $CH{=}^{t}CHCO_2Me$



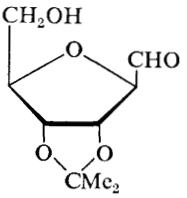
(447)



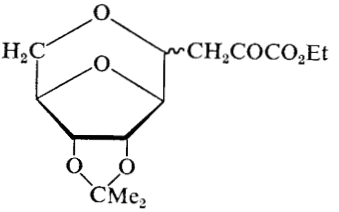
(448)



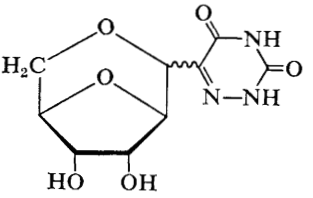
(449)



(450)



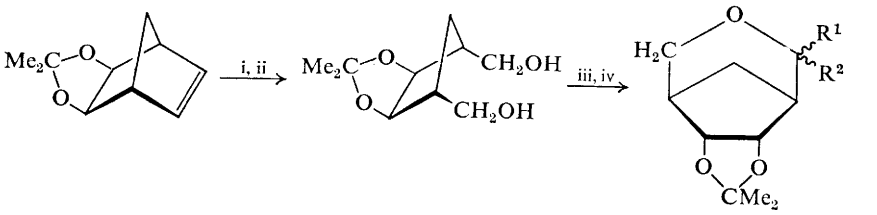
(451)



(452)

The reaction of 2,5-anhydro-3,4-*O*-isopropylidene-DL-allose (450) (see Vol. 9, p. 22) with ethyl triphenylphosphoranylidene pyruvate at 90 °C gave the internal Michael-addition product (451) *via* the αβ-unsaturated ketoester formed in the Wittig reaction.[668] Homo-*C*-nucleosides (452) containing 6-azauracil or 4-hydroxy-5-carboxamidopyrazole were elaborated from (451). Details of an earlier synthesis of the racemic hemiacetal (454), a precursor of carbocyclic *C*-nucleosides, have been published, together with an improved synthesis of (454) *via* the lactone (453); part of this route is outlined in Scheme 87.[669] Condensation of the lactone

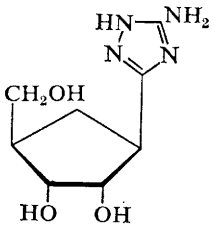
[667] J. M. J. Tronchet and O. R. Martin, *Helv. Chim. Acta*, 1976, **59**, 945.
[668] G. Just, M. Ramjeesingh, and T. J. Liak, *Canad. J. Chem.*, 1976, **54**, 2940.
[669] G. Just, G. Reader, and B. Chalard-Faure, *Canad. J. Chem.*, 1976, **54**, 849.



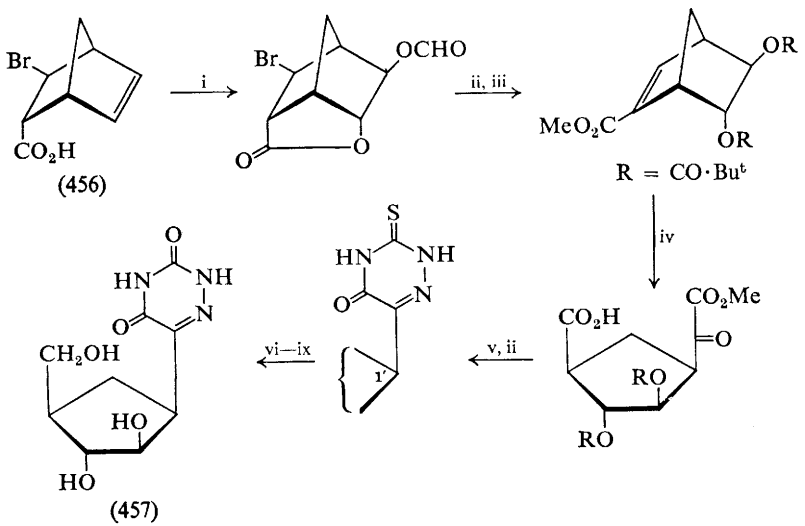
(453) $R^1,R^2 = O$
(454) $R^1 = H$; $R^2 = OH$

Reagents: i, O_3; ii, $NaBH_4$; iii, Ag_2CO_3–Celite; iv, Bu^i_2AlH

Scheme 87



(455)



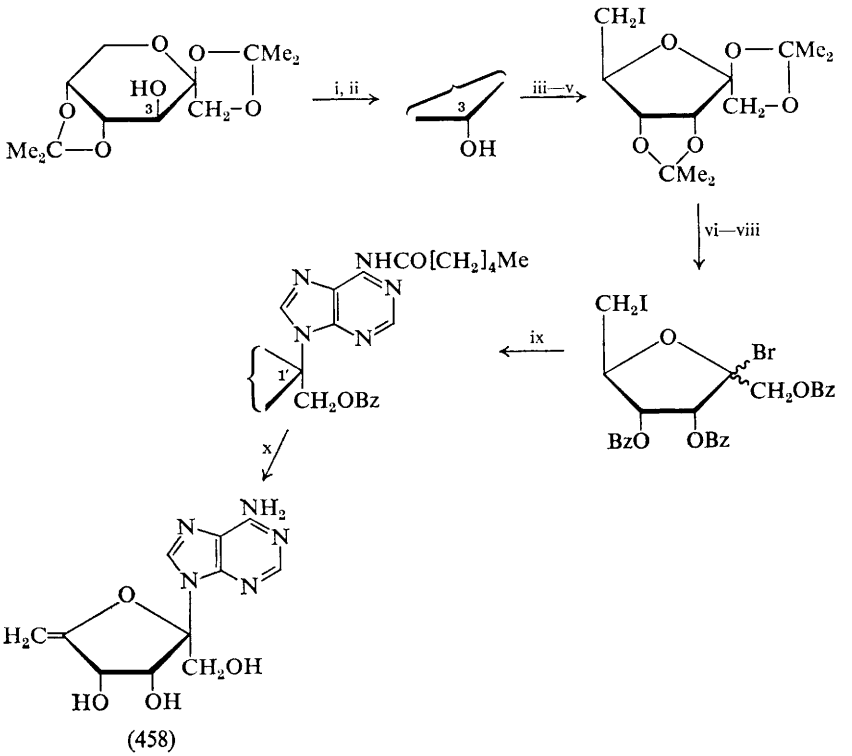
(456) (457)

R = CO·Bu^t

Reagents: i, H_2O_2–HCO_2H; ii, MeONa; iii, Bu^tCOCl–py; iv, IO_4^-–$KMnO_4$; v, $H_2NCSNHNH_2$; vi, B_2H_6; vii, NaOH; viii, MeI; ix, H_3O^+

Scheme 88

(453) with aminoguanidine in pyridine gave, after removal of the protecting group, the 3-amino-1,2,4-triazole carbocyclic *C*-nucleoside (455).[670] The Diels–Alder adduct (456) has been used as the starting material in a synthesis of the carbocyclic analogue (457) of 5-β-arabinofuranosyl-6-azauracil (Scheme 88).[671] Carbocyclic analogues of the *C*-nucleosides DL-pyrazomycin (see p. 135) and DL-showdomycin (see p. 147) have also been derived from a Diels–Alder adduct, but they did not show any antibacterial, antiviral, or antifungal activities.[672] D-Pentofuranosyl *S*-benzylthioformimidates have been converted into 2-α-D-arabinofuranosylimidazoles, 8-α-D-arabinofuranosylpurines,[673] 3-α- and 3-β-D-ribofuranosyl-*s*-triazolo[4,3-*a*]pyridine, and 8-amino-2-β-D-ribofuranosyl-*s*-triazolo[1,5-*a*]pyridine.[674] Some thiazole *C*-nucleosides containing either cyclic or acrylic sugar residues have been prepared by reaction of the thioamide derived



Reagents: i, DMSO–DCC; ii, $NaBH_4$; iii, Me_2CO–H^+; iv, TsCl–py; v, NaI–DMF; vi, H^+; vii, BzCl–py; viii, HBr–CH_2Cl_2; ix, N^6-hexanoyladenine–$SnCl_4$; x, MeONa

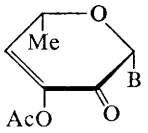
Scheme 89

[670] G. Just and B. Chalard-Faure, *Canad. J. Chem.*, 1976, **54**, 861.
[671] G. Just and R. Ouellet, *Canad. J. Chem.*, 1976, **54**, 2925.
[672] G. Just and S. Kim, *Tetrahedron Letters*, 1976, 1063.
[673] G. Barnathan, Huynh Dinh Tam, A. Kolb, and J. Igolen, *European J. Medicin. Chem. – Chim. Ther.*, 1976, **11**, 67 (*Chem. Abs.*, 1976, **85**, 33 341v).
[674] J.-P. Marquet, E. Bisagni, and J.-M. Lhoste, *J. Org. Chem.*, 1976, **41**, 3124.

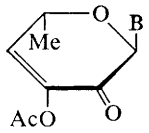
from 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allononitrile with monochloroacetone or of 3,4,5,6-tetra-*O*-acetyl-1-chloro-1-deoxy-D-*ribo*-hexulose and homologous α-chloroketose derivatives with thiourea or thioacetamide.[675]

Unsaturated Nucleosides

Efficient syntheses of angustamycin A (458) (Scheme 89), a nucleoside antibiotic exhibiting modest antimicrobial and antitumour activities, and some base analogues of angustamycin A have been described.[676] Reduction of the unsaturated keto-nucleosides (459) and (460) with sodium borohydride has provided



(459) B = theophyllin-9-yl or 6-chloropurin-9-yl



(460) B = theophyllin-9-yl

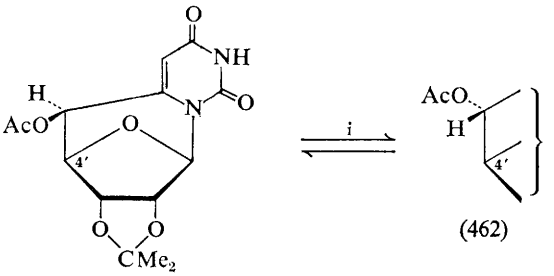
a route to nucleosides containing rare dideoxy-sugars.[677] Reduction of (459) gave 7-(2-*O*-acetyl-4,6-dideoxy-β-L-*xylo*-hexopyranosyl)-theophylline or -6-chloropurine, whereas that of the α-anomer (460) afforded 7-(3-*O*-acetyl-4,6-dideoxy-α-L-*ribo*-hexopyranosyl)theophylline. The mechanisms of these reductions were examined using sodium borodeuteride in deuteriated solvents.

3′-Deoxy-3′-iodo-nucleoside derivatives (prepared as described on p. 159) have been converted into 9-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)adenine, *via* the corresponding 2′-methanesulphonates, and related unsaturated nucleosides.[678, 679] Details of the preparation of 9-(5-hydroxymethylfuran-2-yl)adenine (see p. 159) and 9-(2-deoxy-D-*erythro*-pent-1-enofuranosyl)adenine (see Vol. 8, p. 145) have also been published.[679]

Cyclonucleosides

Base-catalysed epimerization of (461) to give an equilibrium mixture containing (461) and (462) (Scheme 90) has been used to prepare conformationally restricted 5′,6(*S*)- and 5′,6(*R*)-cyclouridines.[680] 2,5′-Anhydronucleosides have been synthesized from 1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)lumazine and its 6,7-dimethyl and 6,7-diphenyl derivatives.[681] The reactivity of these 2,5′-anhydronucleosides towards various nucleophiles was also investigated. 2′,8-Anhydro-9-β-D-arabinofuranosyl-8-hydroxyadenine and its benzoylated derivative were transformed into 2′,3′-anhydro-8-hydroxyadenosine and its N^6-benzoyl derivative on treatment with alkali.[682] 2′,3′-Anhydro-8-hydroxyadenosine was converted

[675] M. Fuertes, M. T. García-López, G. García-Muñoz, and R. Madroñero, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 277.
[676] E. J. Prisbe, J. Smejkal, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, 1976, **41**, 1836.
[677] J. Herscovici, M. Bessodes, and K. Antonakis, *J. Org. Chem.*, 1976, **41**, 3827.
[678] R. Mengel and H. Wiedner, *Chem. Ber.*, 1976, **109**, 433.
[679] M. J. Robins, R. A. Jones, and R. Mengel, *J. Amer. Chem. Soc.*, 1976, **98**, 8213.
[680] B. A. Otter, E. A. Falco, and J. J. Fox, *J. Org. Chem.*, 1976, **41**, 3133.
[681] K. Kobayashi and W. Pfleiderer, *Chem. Ber.*, 1976, **109**, 3159.
[682] J. B. Chattopadhyaya and C. B. Reese, *J.C.S. Chem. Comm.*, 1976, 860.



(461) (462)

Reagent: i, py at 115 °C

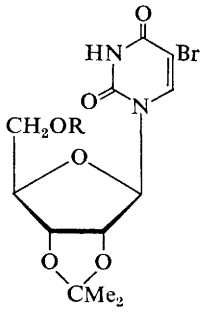
Scheme 90

back into 2′,8-anhydro-9-β-D-arabinofuranosyl-8-hydroxyadenine on heating with an excess of morpholine in DMSO. A 2′,3′-*O*-dibutylstannylene derivative has been used in the synthesis of 2′,8-anhydro-9-β-D-arabinofuranosyl-8-hydroxy-N^6-dimethyladenine [683] (see Vol. 9, p. 153), and 8-bromo- and 8-hydroxyadenosine 2′,3′-sulphites gave 2′,8-*S*-anhydro-9-β-D-arabinofuranosyl-8-mercaptoadenine and 2′,8-anhydro-9-β-D-arabinofuranosyl-8-hydroxyadenine on heating with thiourea in n-butanol or with sodium acetate in DMF, respectively.[684] Several 2′,8- and 3′,8-anhydro-8-hydroxy-nucleosides have been prepared by cyclization of 8-hydroxy-2′- or -3′-*O*-(2,4,6-tri-isopropylbenzenesulphonyl)-hypoxanthine, -6-mercaptopurine, and -6-methylmercaptopurine.[685] The oxidation of 2′,8-*S*-anhydro-9-β-D-arabinofuranosyl- and 3′,8-*S*-anhydro-9-β-D-xylofuranosyl-8-mercaptoadenine and 5′,8-*S*-anhydro-8-mercaptoadenosine to the corresponding 8-sulphoxides has been reported.[686] The 8(*S*)-sulphoxides were obtained in the former two cases and both 8(*S*)- and 8(*R*)-sulphoxides from the 5′,8-*S*-anhydronucleoside.

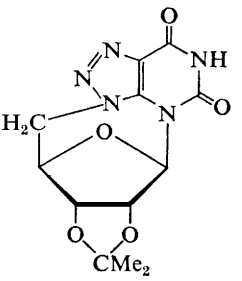
Pyrimidine nucleosides have been converted directly into 2,2′-anhydronucleosides by the action of 2-acetoxybenzoyl chloride in refluxing acetonitrile.[687]

5′,9-Cyclo-3-(5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-8-azaxanthine (464) was formed when the 5-bromouridine derivatives (463) were heated with sodium azide in DMF, whereas similar treatment of 5-bromo-2′,3′-*O*-isopropylidene-5′-*O*-methanesulphonylcytidine gave a mixture of 5′-azido-5-bromo-5′-deoxy-2′,3′-*O*-isopropylidenecytidine and 5′,9-cyclo-3-(5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-8-azaguanine.[688] The 5′,9-cyclo-derivatives were also obtained on heating 5′-azido-5-bromo-5′-deoxy-2′,3′-*O*-isopropylidene-uridine and -cytidine, suggesting that they are formed by an intramolecular addition-elimination mechanism in the reactions of the 5-bromo-nucleoside derivatives [*e.g.* (463)] with azide ion.

[683] M. Ikehara and T. Maruyama, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 335.
[684] T. Sowa and K. Tsunoda, *Bull. Chem. Soc. Japan*, 1975, **48**, 3243.
[685] M. Ikehara and M. Muraoka, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 672.
[686] M. Ikehara, Y. Ogiso, and T. Morii, *Tetrahedron*, 1976, **32**, 43.
[687] U. Reichman, C. K. Chu, D. H. Hollenbery, K. A. Watanabe, and J. J. Fox, *Synthesis*, 1976, 533.
[688] T. Sasaki, K. Minamoto, M. Kino, and T. Mizuno, *J. Org. Chem.*, 1976, **41**, 1100.

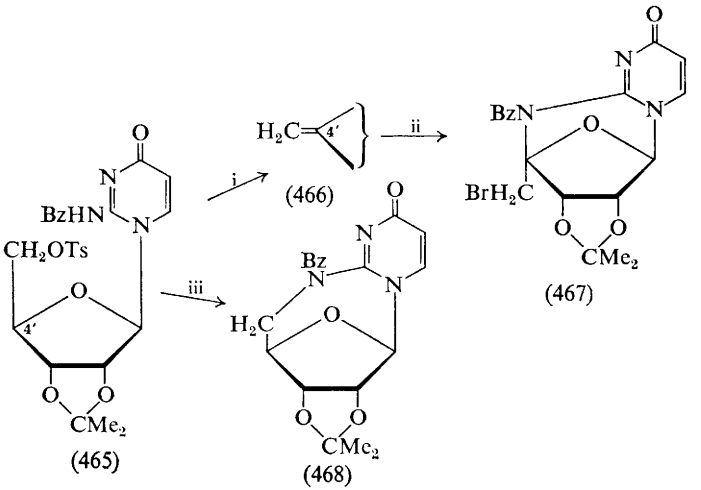


(463) R = Bz or Ts



(464)

Syntheses of the 4′,5′-unsaturated derivative (466) and the *N*-bridged compounds (467) and (468) from the 5′-toluene-*p*-sulphonate (465) (Scheme 91) have been described in a preliminary communication, although the structure (467) needs to be established conclusively.[689]



Reagents: i, Bu^tOK–Bu^tOH; ii, Br_2–$CHCl_3$; iii, Bu^tOK–Bu^tOH–py

Scheme 91

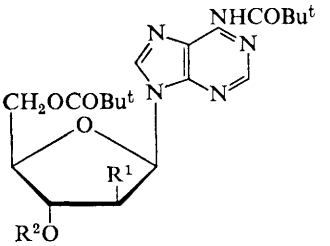
Halogeno-sugar Nucleosides *

2′,3′-*O*-Methoxyethylideneadenosine reacted with pivalyl chloride in refluxing pyridine to give a mixture containing mainly (469) and (470), as well as the *O*-(4,4-dimethyl-3-pivaloxypent-2-enoyl)(Dmpp) derivatives (471; R^1 = Cl) and (472; R^1 = Cl) formed by acylation of an intermediate 2′,3′-*O*-keten acetal that is in equilibrium with the initially formed 2′,3′-acetoxonium ion.[690] In the

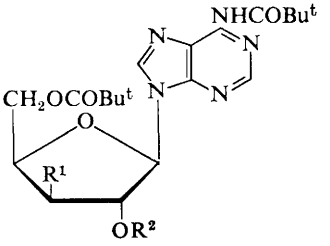
[689] V. Skaric and J. Matulic, *Croat. Chem. Acta*, 1975, **47**, 159 (*Chem. Abs.*, 1976, **84**, 90 477x).
[690] M. J. Robins, R. Mengel, R. A. Jones, and Y. Fouron, *J. Amer. Chem. Soc.*, 1976, **98**, 8204.

* See also p. 156.

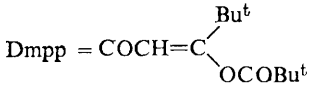
presence of an excess of sodium iodide, 2′,3′-*O*-methoxyethylideneadenosine reacted with pivalyl chloride in refluxing pyridine to give the iodo derivatives (471; R^1 = I) and (472; R^1 = I), together with the unsaturated products (473) and (474).[679,690] A pivalylketen_acetal (475) was isolated, *inter alia*, when

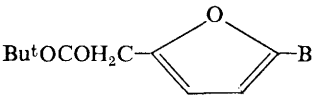


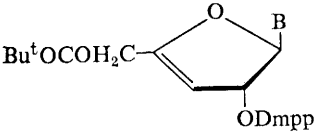
(469) R^1 = Cl; R^2 = Ac
(471) R^1 = Cl or I; R^2 = Dmpp



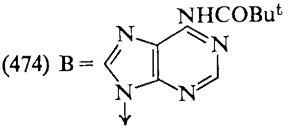
(470) R^1 = Cl; R^2 = Ac
(472) R^1 = Cl or I; R^2 = Dmpp

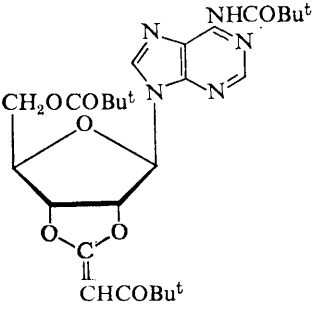






(473)

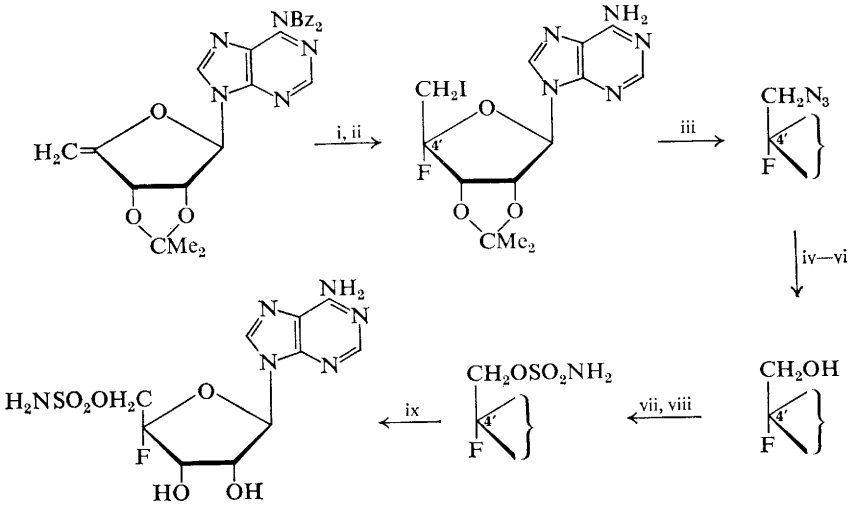




(475)

2′,3′-*O*-methoxyethylideneadenosine was treated with pivalyl chloride in pyridine at 50 °C, and it was rapidly converted into (471; R^1 = I) and (472; R^1 = I) with pivalyl chloride–sodium iodide in refluxing pyridine.[690] The 4,4-dimethyl-3-pivaloxypent-2-enoyl (Dmpp) groups could be readily removed with potassium permanganate in cold aqueous pyridine.[679] Treatment of (469) and (470) with tributyltin hydride or hydrogenolysis of (471; R^1 = I) and (472; R^1 = I) over palladized charcoal gave, after deblocking, 2′-deoxyadenosine and 3′-deoxyadenosine (cordycepin).

Moffatt and his co-workers have reviewed the procedures for preparing 4′-fluoro- and 4′-methoxy-nucleosides,[691] which culminated in their elegant syntheses of nucleocidin (476) (Scheme 92),[692] 4′-fluoro-5′-*O*-sulphamoyluridine,



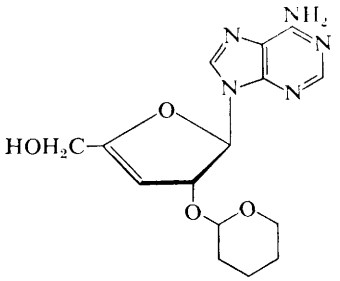
(476)

Reagents: i, I_2–AgF; ii, NH_3–MeOH; iii, LiN_3–DMF; iv, *hν*; v, H^+; vi, $NaBH_4$; vii, $(Bu_3Sn)_2O$; viii, NH_2SO_2Cl; ix, CF_3CO_2H

Scheme 92

an analogue of nucleocidin, and 4′-fluorouridine 5′-phosphate.[693] Ring-opening of 9-(2,3-anhydro-β-D-ribofuranosyl)adenine with hydrogen chloride or hydrogen bromide yielded 9-(3-chloro- or 3-bromo-3-deoxy-β-D-xylofuranosyl)adenine, while the corresponding 3-deoxy-3-iodo derivative was obtained by ring-opening of the oxiran with sodium iodide in the presence of boron trifluoride etherate.[694] 9-(2,3-Anhydro-β-D-lyxofuranosyl)adenine reacted with alkali-metal halides in the presence of boron trifluoride etherate to give 2′- and 3′-halogeno-nucleosides, which were converted into 2′- and 3′-deoxyadenosine with tributyltin hydride. Direct displacements on N^6-pivalyl-9-(3-deoxy-3-iodo-5-*O*-pivalyl-β-D-xylofuranosyl)- or N^6-pivalyl-9-(2-deoxy-2-iodo-5-*O*-pivalyl-β-D-arabinofuranosyl)-adenine (see p. 159) by chloride ions in DMF yielded, after removal of the protecting groups, 3′-chloro-3′-deoxy- and 2′-chloro-2′-deoxy-adenosine, respectively, whereas the unsaturated nucleoside (477) resulted from the action of azide ion on 9-[3-deoxy-3-iodo-2-*O*-(tetrahydropyran-2-yl)-β-D-xylofuranosyl]-adenine.[678]

[691] J. P. H. Verheyden, I. D. Jenkins, G. R. Owen, S. D. Dimitrijevich, C. M. Richards, P. C. Srivastava, Le Hong Nghiep, and J. G. Moffatt, *Ann. New York Acad. Sci.*, 1975, **255**, 151.
[692] I. D. Jenkins, J. P. H. Verheyden, and J. G. Moffatt, *J. Amer. Chem. Soc.*, 1976, **98**, 3346.
[693] G. R. Owen, J. P. H. Verheyden, and J. G. Moffatt, *J. Org. Chem.*, 1976, **41**, 3010.
[694] R. Mengel and H. Wiedner, *Chem. Ber.*, 1976, **109**, 1395.



(477)

3′-Deoxy-3′-fluorouridine has been synthesized in four steps from 5-*O*-benzyl-3-deoxy-3-fluoro-D-arabinofuranose, but it did not inhibit RNA synthesis *in vitro*.[695] 1-(6-Deoxy-6-fluoro-β-D-glucopyranosyl)thymine has been prepared by a direct displacement on a corresponding nucleoside 6′-sulphonate with fluoride ion, whereas 1-(6-deoxy-6-fluoro-β-D-galactopyranosyl)thymine was obtained *via* condensation of 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-fluoro-β-D-galactopyranose with 5-methyl-2,4-bis(trimethylsilyloxy)pyrimidine.[695a]

Keto-nucleosides and Nucleosides containing Uronic Acid Components

1-(6-Deoxy-2,3-*O*-isopropylidene-α-L-*lyxo*-hexopyranosyl-4-ulose)thymine (478) has been synthesized by a route similar (see Vol. 8, p. 150) to that used to obtain a related purine 4′-keto-nucleoside.[696]

Adenosine-5′-carboxylic acid and its 2′,3′-*O*-isopropylidene derivative have been converted into a variety of alkyl esters,[697] and pentofuranuronic acid nucleosides derived by condensation of the methyl penturonate (479) with purines and pyrimidines have been reported.[698] The reaction of 2′,3′-*O*-isopropylidene-5′-*O*-toluene-*p*-sulphonyl-adenosine or -uridine with potassium cyanide in *p*-dioxan or acetonitrile in the presence of the complexing agent [18]crown-6 gave the corresponding 5′-cyano-5′-deoxy-nucleosides, although some 2,5′-anhydro-2′,3′-*O*-isopropylideneuridine was also formed.[699] Hydrolysis of 5′-cyano-5′-deoxyadenosine with alkaline hydrogen peroxide gave both 5′-deoxyadenosine-5′-carboxamide and -5′-carboxylic acid. 1,2,5-Tri-*O*-acetyl-D-glucofuranurono-6,3-lactone has been used to prepare purine and pyrimidine nucleosides, which were also converted into the corresponding carboxamides.[700]

1-(4-Amino-3,4-dideoxy-β-D-*ribo*-hexopyranosyluronic acid)cytosine (480) has been synthesized from (481) and shown to be identical with pentopyranamine D,

[695] G. Kowollik, K. Gaertner, and P. Langen, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 191.

[695a] G. Etzold, M. von Janta-Lipinski, and P. Langen, *J. prakt. Chem.*, 1976, **318**, 79 (*Chem. Abs.*, 1976, **84**, 165 145y).

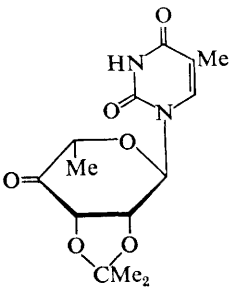
[696] J. Herscovici, A. Ollapally, and K. Antonakis, *Compt. rend.*, 1976, **282**, *C*, 757.

[697] R. N. Prasad, A. Fung, K. Tietje, H. H. Stein, and H. D. Brondyk, *J. Medicin. Chem.*, 1976, **19**, 1180.

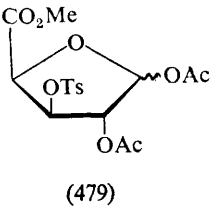
[698] A. A. Akhrem, L. N. Kulinkovich, A. G. Lapko, I. A. Mikhailopulo, and V. A. Timoshchuk, *Bioorg. Khim.*, 1976, **2**, 621 (*Chem. Abs.*, 1976, **85**, 177 850h).

[699] W. Meyer, E. Böhnke, and H. Follmann, *Angew. Chem. Internat. Edn.*, 1976, **15**, 499.

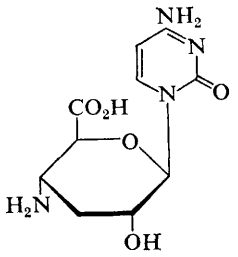
[700] A. A. Akhrem, V. A. Timoshchuk, L. N. Kulinkovich, and I. A. Mikhailopulo, *Bioorg. Khim.*, 1976, **2**, 513 (*Chem. Abs.*, 1976, **85**, 177 846m).



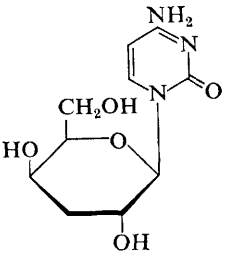
(478)



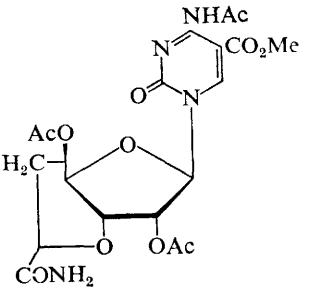
(479)



(480)



(481)



(482)

the nucleoside moiety of blasticidin H (isolated from fermentation broths of *S. griseochromogenes*).[701] The uracil base of the fully protected derivative (482) from octosyl acid A (see Vol. 9, p. 158) has been replaced by adenine by transglycosylation with N^6-benzoyl-N^6N^9-bis(trimethylsilyl)adenine in the presence of trimethylsilyl perchlorate, followed by removal of the protecting groups with aqueous alkali.[702]

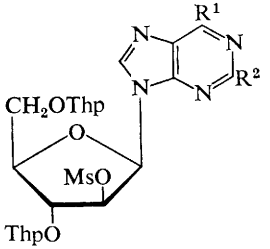
The synthesis of gougerotin is mentioned in Chapter 20.[570]

Nucleosides containing Amino-sugar Components

Treatment of 9-(2,3-anhydro-β-D-lyxofuranosyl)adenine with azide ion, followed by inversion of the configuration of the neighbouring *trans*-hydroxy-group in the products, has been used to obtain 2′-azido-2′-deoxy- and 3′-azido-3′-deoxyadenosine.[678]

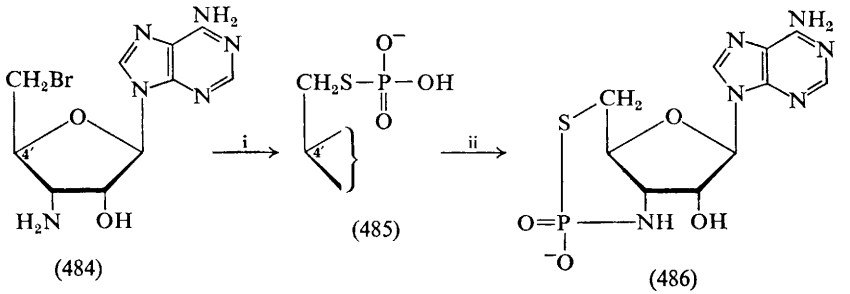
1-(3,5-Di-*O*-benzoyl-2-*O*-methanesulphonyl-β-D-arabinofuranosyl)uracil reacted with azide ion to give 2′-azido-3′,5′-di-*O*-benzoyl-2′-deoxyuridine and the 3′-*O*-benzoyl derivative, while a similar reaction with 1-(3-azido-5-*O*-benzoyl-3-deoxy-2-*O*-methanesulphonyl-β-D-arabinofuranosyl)uracil gave 2′,3′-diazido-5′-*O*-benzoyl-2′,3′-dideoxyuridine and deglycosylated by-products.[703] The mono- and di-azido derivatives were converted into 2′-amino-2′-deoxy- and 2′,3′-

[701] K. A. Watanabe, T. M. K. Chiu, U. Reichman, C. K. Chu, and J. J. Fox, *Tetrahedron*, 1976, **32**, 1493.
[702] T. Azuma and K. Isono, *Tetrahedron Letters*, 1976, 1687.
[703] T. Sasaki, K. Minamoto, T. Sugiura, and M. Niwa, *J. Org. Chem.*, 1976, **41**, 3138.



(483) R^1 = OH; R^2 = NHBz
or R^1 = NH_2; R^2 = H

diamino-2′,3′-dideoxy-uridine, respectively. 2′,8-Anhydro-8-hydroxypurine nucleosides have been converted into the nucleoside 2′-sulphonates (483), which gave 2′-amino-2′-deoxy-adenosine and -guanosine following treatment in turn with sodium azide in DMF, acetic acid, and hydrogen over palladized charcoal.[704] The 5′-bromo-5′-deoxy derivative (484), obtained by the action of thionyl bromide in triethyl phosphate on 3′-amino-3′-deoxyadenosine,[705] has been transformed *via* the 5′-*S*-thiophosphate (485) into 3′-amino-3′-deoxy-5′-thio-adenosine 3,5-phosphate (486) (Scheme 93), an analogue of cAMP.[706] An



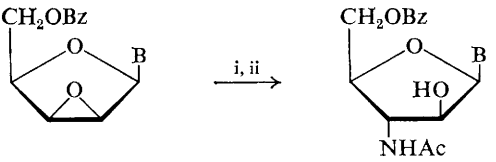
Reagents: i, Li_3O_3PS; ii, 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide

Scheme 93

acetamido group has been introduced directly into pyrimidine nucleosides by ring-opening of 2′,3′-oxiran derivatives with acetonitrile in the presence of boron trifluoride etherate (Scheme 94).[707] A direct procedure for replacing the hydroxy-group at C-5′ of nucleosides with an amino-group is based on the reaction with phthalimide, triphenylphosphine, and diethyl azodicarboxylate.[708]

Syntheses of 5′-amino-2′,5′-dideoxy-5-trifluoromethyluridine [709] (*via* a standard displacement with azide ion) and 5′-amino-5′-deoxy-nucleosides (involving a

[704] M. Ikehara, T. Maruyama, and H. Miki, *Tetrahedron Letters*, 1976, 4485.
[705] M. Morr, *Tetrahedron Letters*, 1976, 2125.
[706] M. Morr, *Tetrahedron Letters*, 1976, 2127.
[707] U. Reichman, D. H. Hollenberg, C. K. Chu, K. A. Watanabe, and J. J. Fox, *J. Org. Chem.*, 1976, **54**, 2042.
[708] O. Mitsunobu, S. Takizawa, and H. Morimato, *J. Amer. Chem. Soc.*, 1976, **98**, 7858.
[709] T.-S. Lin, C. Chai, and W. H. Prusoff, *J. Medicin. Chem.*, 1976, **19**, 915.



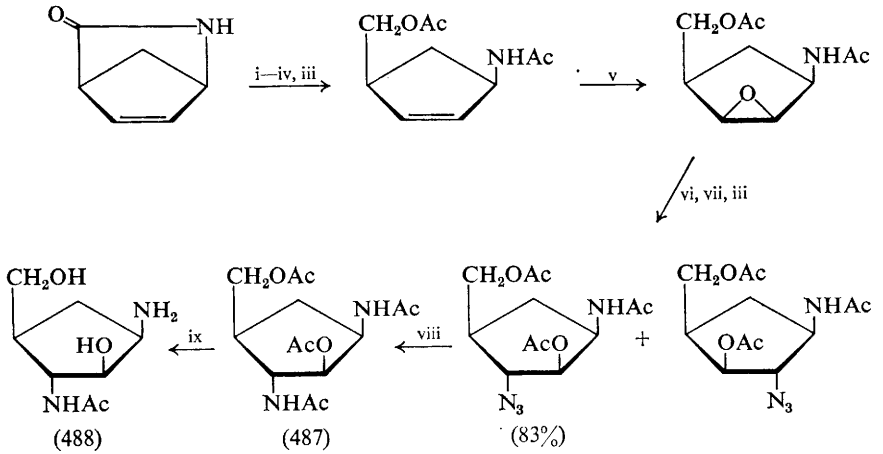
B = uracil-1-yl or N^4-benzoylcytosin-1-yl

Reagent: i, MeCN–BF_3; ii, H_2O

Scheme 94

novel reduction of the corresponding 5′-azido-5′-deoxy-nucleosides with trimethylsilyl phosphites)[710] have been reported.

Part of a route used to synthesize a carbocyclic analogue of puromycin is shown in Scheme 95, the purine base being elaborated from the intermediate



Reagents: i, 2M-HCl; ii, MeOH–H^+; iii, Ac_2O–py; iv, $Ca(BH_4)_2$; v, *m*-$ClC_6H_4CO_3H$; vi, NH_3–MeOH; vii, NaN_3–DMF; viii, H_2–Pt–Ac_2O; ix, 2M-HCl at 70 °C

Scheme 95

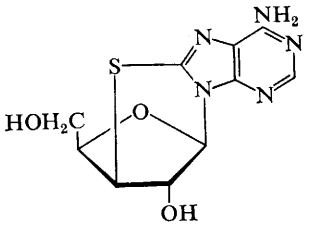
(488).[711] Selective acidic hydrolysis of the diacetamido derivative (487) to the monoacetamido derivative (488) depended on an *N*-1 → *O*-2 acyl migration. Carbocyclic puromycin was synthesized from (488) by inversion of the configuration at C-2′ after the purine base had been formed.

Phosphate and Related Esters of Nucleosides

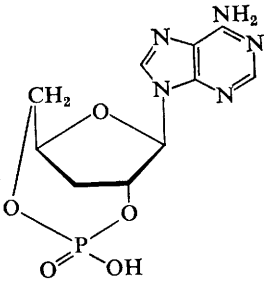
The 3′,8-*S*-anhydronucleoside (489) has been converted into the corresponding 2′,5′-phosphate, which gave 9-(3-deoxy-β-D-*erythro*-pentofuranosyl)adenine (cordycepin) 2′,5′-phosphate (490) on desulphurization with Raney nickel.[712] An improved synthesis of β-hydroxyethyl esters of nucleoside 5′-phosphates was

[710] T. Hata, I. Yamamoto, and M. Sekine, *Chem. Letters*, 1976, 601.
[711] S. Daluge and R. Vince, *Tetrahedron Letters*, 1976, 3005.
[712] M. Ikehara and J. Yano, *Nucleic Acid Res.*, 1974, **1**, 1783 (*Chem. Abs.*, 1976, **85**, 143 391u).

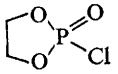
based on the reaction of 2′,3′-*O*-isopropylidene-nucleosides with chlorodioxaphospholane (491), followed by hydrolysis of the isopropylidene group with aqueous formic acid.[713] A new approach to the synthesis of phosphorothioates has been reported.[714] Thus phosphorylation of 5′-methoxytritylthymidine with 2-chlorophenyl *N*-phenylphosphoramidochloridate gave the diastereoisomeric



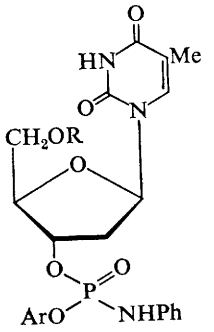
(489)



(490)



(491)



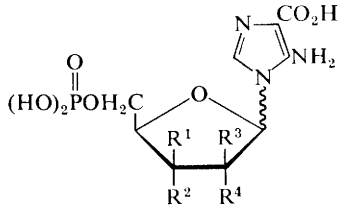
(492) R = *p*-$MeOC_6H_4Ph_2C$; Ar = *o*-ClC_6H_4

anilidates (492) (ratio 62 : 38), which are enantiomeric at the phosphorus atom. Removal of the *P*-anilino-group (with NaH) from (492) and treatment with carbon disulphide gave the corresponding 3′-*O*-arylphosphorothioates, which gave the *S*-methylthioates on reaction with methyl iodide.

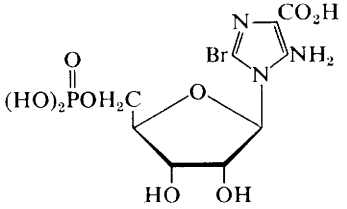
2′,3′-(Adamant-1-yl)phosphonates have been prepared from 5′-deoxy-5′-halogenouridines and 6-azauridine (see Vol. 9, p. 162).[237]

The α- and β-D-arabinofuranosyl and α- and β-D-xylofuranosyl nucleotide analogues [(493) and (494), respectively] and the 2-bromo derivative (495) of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid 5′-phosphate (a central intermediate in the *de novo* synthesis of purine nucleotides) and its α-anomer have been synthesized from 2,3,5-tri-*O*-benzoyl-αβ-D-arabinofuranosyl azide, 3,5-*O*-isopropylidene-αβ-D-xylofuranosylamine, and ethyl 5-amino-1-α- and -β-D-ribofuranosylimidazole-4-carboxylates, respectively.[715] The β-anomers of (493)—

[713] W. S. Zieliński, *Nucleic Acid Res.*, 1976, **3**, 1769 (*Chem. Abs.*, 1976, **85**, 193 002p).
[714] W. S. Zieliński, Z. J. Leśnikowski, and W. J. Stec, *J.C.S. Chem. Comm.*, 1976, 772.
[715] G. MacKenzie, G. Shaw, and S. E. Thomas, *J.C.S. Chem. Comm.*, 1976, 453.



(493) $R^1 = R^4 = H$; $R^2 = R^3 = OH$
(494) $R^1 = R^4 = OH$; $R^2 = R^3 = H$



(495)

(495), but not the α-anomers, inhibited the enzymes phosphoribosylaminoimidazole-carboxylase (E.C. 4.1.1.21) and -succinocarboxyamide synthase (E.C. 6.3.2.6). Chiral 2′(*R*),3′(*S*),5′-trihydroxypentyl-adenine and -cytosine have been synthesized from 2-deoxy-D-*erythro*-pentose.[716] These non-glycosidic analogues of nucleosides, which have opposite configurations at C-2′ and C-3′ to those of natural nucleosides, were converted into 3′-phosphates and dinucleotide analogues with 3′ → 5′-phosphodiester linkages.

A powerful phosphorylating reagent, generated *in situ* from cyanoethyl phosphate, DCC, and mesitylenesulphonyl chloride, has been used to prepare nucleoside 3′,5′-di(pyrophosphates) from the corresponding 3′,5′-diphosphates.[242] Adenosine 5′-(1-thiotriphosphate) and 5′-(2-thiotriphosphate) have been synthesized.[717] Both exist as a pair of diastereoisomers; the isomers of adenosine 5′-(1-thiotriphosphate) could be distinguished on the basis of their reactivity with myokinase or nucleoside diphosphate kinase and those of adenosine 5′-(2-thiotriphosphate) on the basis of their reactivity with myosin or nucleoside diphosphate kinase.

The metal-ion(Eu^{3+}, Pr^{3+}, and Mn^{2+})-catalysed degradation of nucleoside diphosphate derivatives of D-glucose, D-galactose, D-mannose, L-fucose, and 2-acetamido-2-deoxy-D-glucose has been studied as a function of pH.[718] UDP-D-glucose and -galactose were degraded smoothly to the hexose 1,2-phosphates and uridine 5′-phosphate in the presence of Mn^{2+} and other cations, whereas GDP-L-fucose was slowly degraded to L-fucose. The other nucleoside diphosphate derivatives were unaffected. It appears that high Mn^{2+}-ion concentrations can catalyse appreciable decomposition that could lead to difficulties in kinetic and incorporation experiments.

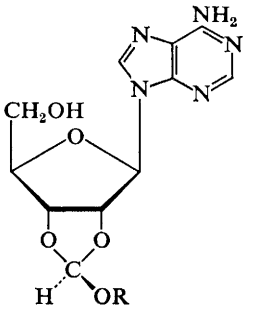
Other Derivatives

Adenosine reacted with trimethyl or triethyl orthoformate in the presence of trichloroacetic acid to give only *exo*-2′,3′-*O*-alkoxymethylene derivatives (496), which were isolated in yields of 55% and 82%, respectively.[224] Carbonyldiimidazole has been used in the synthesis of uridine and 6-azauridine 2′,3′-carbonates.[719] Phosphorylation and alkaline hydrolysis of the acetals (497) (pre-

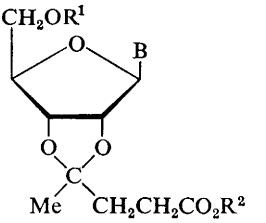
[716] S. N. Mikhailov, L. I. Kolobushkina, A. M. Kritzyn, and V. L. Florentiev, *Tetrahedron*, 1976, **32**, 2409.
[717] F. Eckstein and R. S. Goody, *Biochemistry*, 1976, **15**, 1685.
[718] H. A. Nunez and R. Barker, *Biochemistry*, 1976, **15**, 3843.
[719] P. Dražar, L. Hein, and J. Beránek, *Nucleic Acids Res., Special Publ.*, 1975, **1** (3rd Symposium Chem. Nucleic Acids Components, 1975), S61 (*Chem. Abs.*, 1976, **85**, 63 275u).

pared by treatment of adenosine or guanosine with ethyl laevulinate) converted them into derivatives (498) that could be attached, *via* the carboxy-group, to a polymeric support.[720]

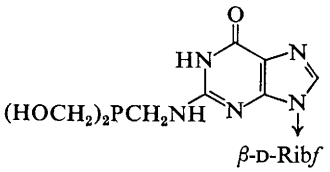
Partial methylation of 9-β-D-arabinofuranosyladenine with dimethyl sulphate yielded a mixture of mono-, di-, and tri-*O*-methyl derivatives.[721] 5′-*O*-Methyladenosine was found to resist enzymic deamination. Syntheses of 5′-*O*-acyl and



(496) R = Me or Et



(497) R^1 = H; R^2 = Et
(498) R^1 = PO_3H_2; R^2 = H
B = adenin-9-yl or guanin-9-yl



(499)

N-alkyl derivatives of tubercidin [722] and of 9-(6-deoxy-6-nitro-β-D-glucopyranosyl)adenine [379] have been reported. Guanosine reacted with tetrakis(hydroxymethyl)phosphonium chloride to give the N^2-substituted phosphine (499).[723]

The preparation and properties of osmium-ligand complexes of nucleosides are mentioned in Chapter 18.[516]

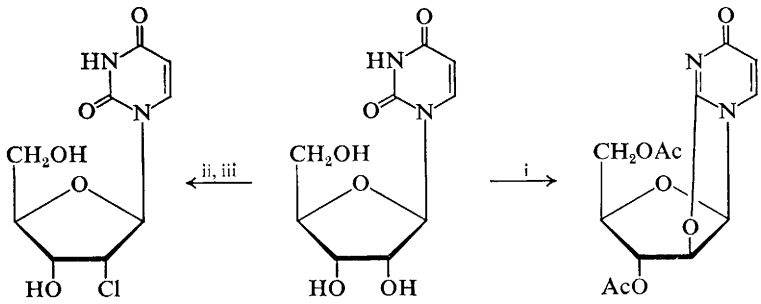
Reactions

Chemical transformations on pyrimidine nucleosides have been reviewed.[724]

Selective acetylation of cytidine using a mixture of acetic anhydride and acetyl chloride gave the 2′,3′,5′-triacetate, but prolonged treatment of purine nucleosides with acetyl chloride or bromide resulted in cleavage of the glycosidic linkage and the formation of acetylated D-ribosyl halides.[725] Benzoyl tetrazole has been recommended as a benzoylating reagent for the hydroxy-groups of most common

[720] F. Seela and S. Waldek, *Nucleic Acid Res.*, 1975, **2**, 2343 (*Chem. Abs.*, 1976, **84**, 90 496c).
[721] E. Darzynkiewicz, Z. Kazimierczuk, and D. Shugar, *Cancer Biochem. Biophys.*, 1976, **1**, 203 (*Chem. Abs.*, 1976, **85**, 160 456n).
[722] P. F. Wiley, J. H. Johnson, and A. R. Hanze, *J. Antibiotics*, 1976, **29**, 720.
[723] G. Loenwengart and B. L. van Duuren, *Tetrahedron Letters*, 1976, 3473.
[724] J. J. Fox, B. A. Otter, J. A. Rabi, and R. S. Klein, *Lect. Heterocyclic Chem.*, 1974, **2**, 1 (*Chem. Abs.*, 1976, **85**, 124 245h).
[725] J. Beránek and H. Hrebabecky, *Nucleic Acids Res., Special Publ.*, 1975, 1 (3rd Symposium Chem. Nucleic Acids Components, 1975), S57 (*Chem. Abs.*, 1976, **85**, 63 274t).

nucleosides.[214] As well as the hydroxy-groups, the primary amino-group of 2′-deoxycytidine, but not of 2′-deoxyguanosine or 2′-deoxyadenosine, was benzoylated when an excess of the reagent was used, and there were also indications that the reagent can distinguish between primary and secondary hydroxy-groups. Tetra-acetoxysilane reacted with 5′-*O*-acetyluridine to give principally 3′,5′-di-*O*-acetyluridine, whereas 2,2′-anhydro-1-(3,5-di-*O*-acetyl-β-D-arabino-furanosyl)uracil was obtained when the reaction was conducted with uridine or its 5′-acetate in the presence of zinc chloride (Scheme 96).[726] Uridine reacted



Reagents: i, $Si(OAc)_4$–$ZnCl_2$; ii, $SiCl_4$–AcOH; iii, 2M-NaOH

Scheme 96

with tetrachlorosilane in acetic acid to give, after alkaline hydrolysis, 2′-chloro-2′-deoxyuridine, while similar treatment of 5-bromouridine furnished 5-bromo-2′-chloro-2′-deoxyuridine (34%) and 5-bromo-1-(3-chloro-3-deoxy-β-D-xylo-furanosyl)uracil (2%). These reagents were also used to prepare 2,2′-anhydro-1-β-D-arabinofuranosylcytosine and its 5-bromo-derivative.

Selective 2′-*O*-deacylation of fully benzoylated purine and pyrimidine nucleosides (*e.g.* N^6N^6,2′,3′,5′-pentabenzoyladenosine and N^2,2′,3′,5′-tetrabenzoylguanosine) has been achieved by hydrazinolysis (hydrazine hydrate in acetic acid–pyridine), although the procedure was less selective with fully benzoylated uridine and cytidine.[212] The 4-oxopentanoyl(laevulinyl) group has been shown to be 10^2 times more labile than the β-benzoylpropionyl group towards hydrazine hydrate in pyridine–acetic acid, and to be stable under the conditions of the modified triester procedure used to synthesize oligonucleotides.[727]

A series of three papers has described the reactions of 2-acyloxyisobutyryl chlorides with nucleosides (particularly cytidine) to give 3′- and 3′,5′-acylated 2,2′-anhydro-β-D-arabinofuranosyl-nucleosides, which can be converted into the corresponding 1-β-D-arabinofuranosyl-nucleotides with either aqueous pyridine or sodium carbonate–sodium hydrogen carbonate in aqueous *p*-dioxan.[728] 2,2′-Anhydro-5-chloro-1-(5-chloro-5-deoxy-β-D-arabinofuranosyl)cytosine was obtained on treatment of cytidine with sulphuryl chloride in acetonitrile; similarly, 1-β-D-arabinofuranosylcytosine gave 2,2′-anhydro-5-chloro-1-(5-chloro-5-deoxy-β-D-

[726] K. Kondo, T. Adachi, and I. Inoue, *J. Org. Chem.*, 1976, **41**, 2995.
[727] J. H. van Boom and P. M. J. Burgers, *Tetrahedron Letters*, 1976, 4875.
[728] E. K. Hamamura, M. Prystasz, J. P. H. Verheyden, J. G. Moffatt, K. Yamaguchi, N. Uchida, K. Sato, A. Nomura, O. Shiratori, S. Takase, and K. Katagiri, *J. Medicin. Chem.*, 1976, **19**, 654, 663, 667.

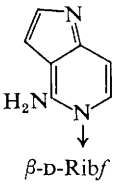
lyxofuranosyl)cytosine.[729] Uridine reacted with this reagent to give 2,2′-anhydro-5-chloro-1-(5-chloro-5-deoxy-β-D-arabinofuranosyl 3-chlorosulphate)uracil.

A simple procedure has been reported for the isolation of the dialdehydes obtained on oxidation of adenosine, guanosine, cytidine, *etc.* with periodate.[730] The 'ribonucleoside dialdehydes', which are more stable than earlier work suggests, appear to be mixtures of monomeric, hydrated forms.

The reactions of several silylating reagents with ribonucleosides have been reported [731] and a mixture of a dialkyl disulphide (or diselenide) and tributylphosphine in DMF has been shown to be a useful *S*-(or *Se*-)alkylating reagent for seleno- and thio-substituted nucleosides (*e.g.* 9-β-D-ribofuranosyl-6-selenopurine).[415] Several 2′-deoxy- and 2,2′-anhydro-nucleosides reacted with tributylammonium monothiophosphate (in the monoanionic form) in DMF at 70 °C to give a mixture of the corresponding 5′- and 3′-*O*-phosphorothioates in the ratio of *ca.* 2 : 1, and with bis(triethylammonium) dithiophosphate at room temperature to give a mixture of the corresponding 5′- and 3′-*O*-phosphorothioates in the ratio of *ca.* 3 : 2.[732]

Physical Measurements

The existence of an intramolecular hydrogen bond between HO-3′ and O-5′ has been demonstrated in the crystal structure of 2,2′-anhydro-1-α-D-xylofuranosyluracil, and reasonably accounts for the high acidity of HO-3′.[733] Polish workers have also presented detailed analyses of the ^{1}H n.m.r. spectra of 1-β-D-arabinofuranosyl-cytosine and -uracil and some of their *O*-methyl derivatives in strongly alkaline media.[734] They concluded that ionization of HO-2′ gives rise to a HO-5′···O$^-$-2′ hydrogen bond, which requires the furanose ring to adopt the $^2T_3$ conformation and the exocyclic 5′-hydroxymethyl group to adopt the *gauche–gauche* conformation. The crystal structures of the product (500) obtained on D-ribosylation of 4-amino-5-azaindole [735] and of adenosine 5′-(barium phosphate) heptahydrate, in which the furanose ring adopts the unusual ^4E conformation,[736] have been determined.



(500)

[729] K. Kikugawa, I. Kawada, and M. Ichino, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 3154.
[730] A. S. Jones, A. F. Markham, and R. T. Walker, *J.C.S. Perkin I*, 1976, 1567.
[731] K. K. Ogilvie, S. L. Beaucage, D. W. Entwistle, E. A. Thompson, M. A. Quilliam, and J. B. Westmore, *J. Carbohydrates, Nucleosides, Nucleotides*, 1976, **3**, 197.
[732] D. Dunaway-Mariano, *Tetrahedron*, 1976, **32**, 2991.
[733] G. I. Birnbaum, J. Giziewicz, C. P. Huber, and D. Shugar, *J. Amer. Chem. Soc.*, 1976, **98**, 4640.
[734] M. Remin, E. Darzynkiewicz, A. Dworak, and D. Shugar, *J. Amer. Chem. Soc.*, 1976, **98**, 367.
[735] A. Ducruix, C. Riche, and C. Pascard, *Tetrahedron Letters*, 1976, 51.
[736] H. Sternglanz, E. Subramanian, J. C. Lacey, jun. and C. E. Bugg, *Biochemistry*, 1976, **15**, 4797.

A computer program has been developed to enable the pseudo-rotational itinerary of furanoid nucleosides to be calculated[737] and a simple graphical method has been used to determine the conformations of the furanoid ring in ribonucleosides from the vicinal coupling constants.[738] A study of the conformations of cytidine nucleotides in aqueous solution – by measurement of nuclear Overhauser effects using an INDOR method – has indicated that the 5′-phosphate prefers an *anti* conformation, whereas the 2′-, 3′-, and 2′,3′-phosphates prefer *syn* conformations.[738a] The nuclear Overhauser effect was independent of temperature over the range 34—80 °C, confirming that free-energy changes in *syn–anti* transitions are due to entropy contributions.

The ^{13}C n.m.r. spectra of formycin, formycin B, and certain pyrazolo[4,3-*d*]-pyrimidines have been examined in DMSO and HMPT, and as a function of temperature, in order to probe the prototropic tautomerism of the pyrazole portion of the heterocyclic system.[739] Only the 1-NH ⇋ 2-NH prototropic process was observed, and the proportion of the 2-NH tautomer (a higher energy species than the 1-NH tautomer) was shown to depend on the substituent present at C-7 of the pyrimidine portion of the heterocyclic system.

Aspects of the conformations of nucleoside diphosphohexoses (*e.g.* UDP-D-Glc) and their components in aqueous solution have been examined using ^{1}H n.m.r. measurements.[740] The hexose and nucleoside components were each shown to prefer identical conformations both in free and combined forms.

Reference to the use of n.m.r. measurements in studies of the conformations of nucleosides is made in Chapter 23, and other physical measurements on nucleosides are covered in Chapter 24.

[737] D. R. Petzold and D. Schwarz, *Studies Biophys.*, 1976, **55**, 93 (*Chem. Abs.*, 1976, **85**, 124 281s).
[738] W. Guschlbauer and Tran Dinh Son, *Nucleic Acids Res., Special Publ.* 1975, 1 (3rd Symposium Chem. Nucleic Acids Components, 1975), S85 (*Chem. Abs.*, 1976, **85**, 46 990f).
[738a] M. Ya. Karpeiskii and G. I. Yakovlev, *Bioorg. Khim.*, 1975, **1**, 749 (*Chem. Abs.*, 1976, **84**, 31 346f).
[739] M.-T. Chenon, R. P. Panzica, J. C. Smith, R. J. Pugmire, D. M. Grant, and L. B. Townsend, *J. Amer. Chem. Soc.*, 1976, **98**, 4736.
[740] Che-Hung Lee and R. H. Sarma, *Biochemistry*, 1976, **15**, 679.

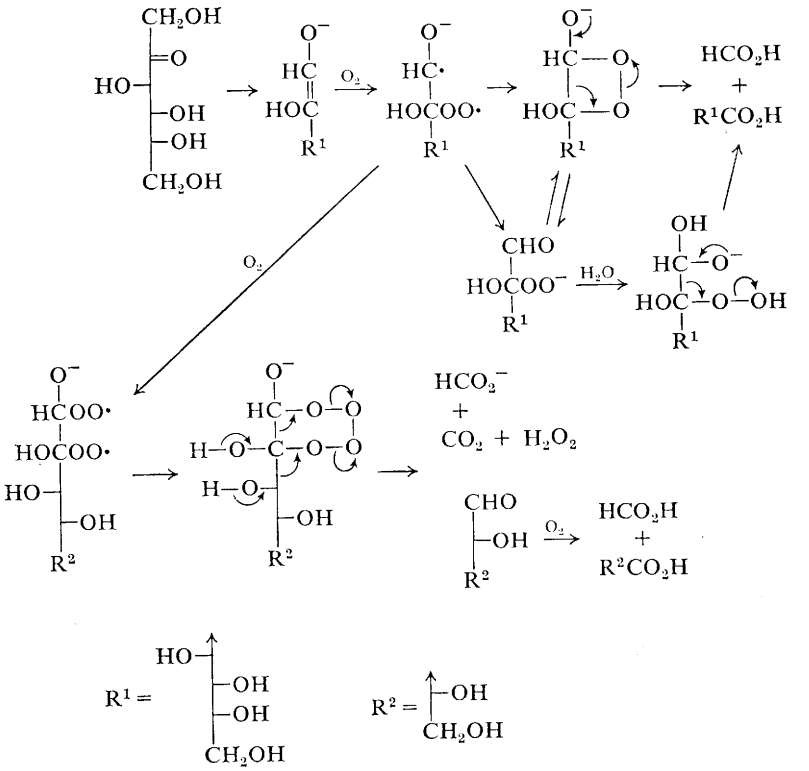
22
Oxidation

A mixture of a ferrous salt and an excess of alkaline hydrogen peroxide degraded alditols almost entirely into formic acid, and aldonic acids into formic acid, oxalic acid, and carbon dioxide.[741] The degradation of these non-reducing sugars is assumed to involve the oxidation of a primary hydroxy-group to an aldehydo-group, which initiates stepwise degradation of the carbon skeleton (see Vol. 7, p. 9). Isbell has proposed a diradical mechanism, involving only 1,2- and 2,3-enediols, to account for the degradation of reducing sugars by oxygen in alkaline solution.[53] The degradation of D-fructose *via* the 1,2-enediol is illustrated in Scheme 97.

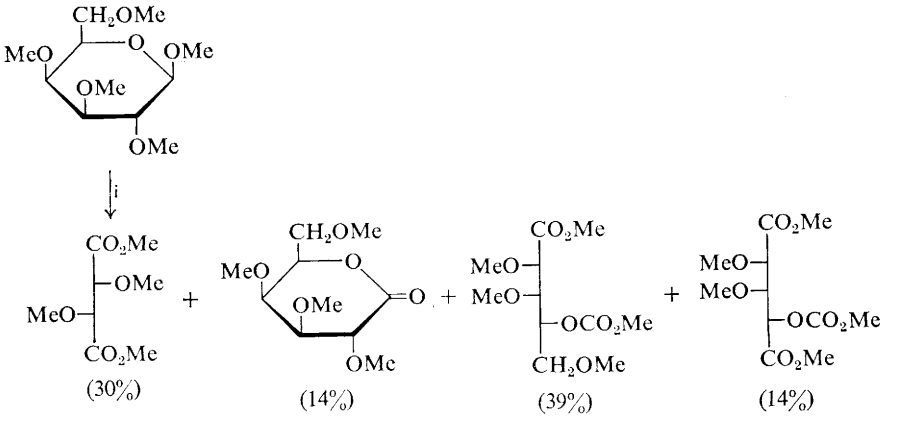
A new procedure for oxidizing primary and secondary hydroxy-groups in suitably protected sugars (*e.g.* 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose) involved photolysis of the corresponding pyruvate esters in benzene at room temperature.[742] Trifluoroacetic anhydride—DMSO in methylene chloride at *ca.* −50 °C converted simple primary and secondary alcohols into the corresponding alkoxydimethylsulphonium trifluoroacetates, which were converted into aldehydes or ketones upon the addition of triethylamine.[743] The yields of carbonyl compounds increased in the order primary < secondary < allylic and benzylic alcohols, although, under appropriate conditions, a primary or secondary hydroxy-group could be oxidized in the presence of an allylic or a benzylic hydroxy-group. The ruthenium-catalysed oxidation of alcohols to aldehydes or ketones by amine-*N*-oxides might also be used in carbohydrate chemistry.[744]

The oxidation of methyl α- and β-pyranosides of D-galactose, D-glucose, and D-mannose with bromine in aqueous solution has been examined at various pH values.[745] The resulting methyl hexopyranosiduloses were converted into *O*-methyloxime derivatives, which were characterized by ^{1}H n.m.r. spectroscopy and g.l.c.–m.s. of their TMS derivatives. It appears that bromine oxidation at a ring-carbon atom with an axial hydrogen atom is hindered by bulky substituents in a 1,3-diaxial relationship; thus the aglycone in the α-anomers protects HO-3 from oxidation, and the axial HO-4 in D-galactopyranosides protects HO-2, *etc.* The degradation of methyl β-D-glucopyranoside with alkaline hydrogen peroxide appears to involve the formation of an α-hydroxyhydroperoxide, which yielded a product tentatively identified as methyl β-D-*arabino*-hexopyranosidulose on subsequent treatment with sodium hydrogensulphite.[746]

[741] H. S. Isbell, H. L. Frush, and E. W. Parks, *Carbohydrate Res.*, 1976, **51**, C5.
[742] R. W. Binkley, *Carbohydrate Res.*, 1976, **48**, C1.
[743] K. Omura, A. K. Sharma, and D. Swern, *J. Org. Chem.*, 1976, **41**, 957.
[744] K. B. Sharpless, K. Akashi, and K. Oshima, *Tetrahedron Letters*, 1976, 2503.
[745] O. Larm, E. Scholander, and O. Theander, *Carbohydrate Res.*, 1976, **49**, 69.
[746] J. W. Weaver, L. R. Schroeder, and N. S. Thompson, *Carbohydrate Res.*, 1976, **48**, C5.



Scheme 97



Reagent: i, CrO_3–AcOH

Scheme 98

An unsuccessful attempt has been made to increase the yield of methyl α-D-hexopyranosiduloses – relative to *O*-acetyl and *O*-(methylthio)methyl derivatives – in the partial oxidation of methyl α-D-glucopyranoside with acetic anhydride–DMSO.[747]

The positions of the glycosidic linkages in micromolar amounts of various D-glucobioses, oligosaccharides from senega, and synthetic β-D-gluco-oligosaccharides have been determined by analysis of the component aldehydes in dialdehyde fragments obtained on periodate oxidation.[748] The aldehydes were converted into (2,4-dinitrophenyl)hydrazones – with concurrent hydrolysis of acetal and hemiacetal linkages – which were separated by chromatography on silica gel prior to spectrophotometric determination. Kochetkov's group has investigated the oxidation of permethylated glycopyranosides with chromium trioxide in acetic acid; the products obtained on oxidation of methyl 2,3,4,6-tetra-*O*-methyl-β-D-galactopyranoside are shown in Scheme 98.[749] Differences in the reactivities of α- and β-glycopyranosides were not particularly marked, and the prospects of using this technique in the linkage analysis of polysaccharides were not encouraging.

Kinetic studies of the oxidation of D-glucitol, D-mannitol,[750] and D-glucose [751] with alkaline potassium ferricyanide, and of D-xylose with Cu^{2+},[752] Ce^{4+},[752a] and other metal cations [753] have been reported.

Arabinitol, xylitol, and ribitol have been determined by oxidation with periodate, the excess of oxidant being measured by potentiometric titration with hydrazine sulphate.[754] Periodate ions have also been determined by spectrophotometric measurement of the absorbance at 555 nm following the addition of phenolphthalein at pH 11.6.[755]

A simple procedure for isolating 'ribonucleoside dialdehydes' is noted in Chapter 21.[730]

[747] E. Wirén, L. Ahrgren, and A. N. de Belder, *Carbohydrate Res.*, 1976, **49**, 201.
[748] S. Honda, K. Kakehi, and K. Takiura, *Carbohydrate Res.*, 1976, **47**, 213.
[749] N. K. Kochetkov, O. S. Chizhov, A. F. Sviridov, I. Szent-Kiralyi, and V. I. Kadentsev, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 635 (*Chem. Abs.*, 1976, **85**, 94 639v).
[750] M. P. Singh, M. S. Singh, M. C. Gangwar, P. Thakur, and A. K. Singh, *Proc. Indian Nat. Sci. Acad., Part A*, 1975, **41**, 178 (*Chem. Abs.*, 1976, **84**, 44 564a).
[751] A. M. El Wakil and D. M. Wagnerová, *Coll. Czech. Chem. Comm.*, 1976, **41**, 14.
[752] V. I. Krupenskii, I. I. Korol'kov, and N. P. Mikush, *Izvest. Vyssh. Uchebn. Zaved., Lesn. Zhur.*, 1976, **19**, 111 (*Chem. Abs.*, 1976, **85**, 192 981v).
[752a] V. I. Krupenskii, I. I. Korol'kov, and N. P. Mikush, *Izvest. Vyssh. Uchebn. Zaved., Lesn. Zhur.*, 1975, **18**, 163 (*Chem. Abs.*, 1976, **84**, 31 322v).
[753] V. I. Krupenskii, N. P. Mikush, and I. I. Korol'kov, *Zhur. obshchei Khim.*, 1976, **46**, 936 (*Chem. Abs.*, 1976, **85**, 177 805x).
[754] Z. Dusic, *Ark. Farm.*, 1975, **25**, 233 (*Chem. Abs.*, 1976, **84**, 159 358d).
[755] S. Honda, K. Adachi, K. Kakehi, H. Yuki, and K. Takiura, *Analyt. Chim. Acta*, 1975, **78**, 492.

23

N.M.R. Spectroscopy and Conformational Features of Carbohydrates

Reviews have appeared on the conformations of mono- and poly-saccharides (in Russian) [756] and the use of ^1H n.m.r. spectroscopy for determining the anomeric configuration of monosaccharides and their derivatives.[757]

Angyal's group has reported a detailed ^{13}C n.m.r. study of the tautomeric equilibria of the 2-hexuloses and some of their derivatives in aqueous solution – all the resonances in the ^{13}C n.m.r. spectra were assigned with the aid of specifically deuteriated compounds.[758] The proportions of the anomeric furanoses and pyranoses present in solutions of the 2-hexuloses at equilibrium in deuterium oxide at 27 °C are shown in the Table (*cf.* Volume 8, p. 172), and the results were discussed in terms of conformational analysis.

Table *Equilibrium compositions (%) of solutions of the 2-hexuloses in deuterium oxide at 27 °C*

2-Hexulose	α-Pyranose	β-Pyranose	α-Furanose	β-Furanose
D-Fructose	trace	75	4	21
D-Psicose	22	24	39	15
L-Sorbose	98	0	2	0
D-Tagatose	79	16	1	4

The use of ^{13}C n.m.r. spectroscopy in an automated procedure for the quantitative or qualitative analysis of mixtures of monosaccharides and cyclitols is referred to in Chapter 2.[19] The technique was applied to the analysis of components extracted from various tissues of *Pinus radiata* and gave results comparable with those obtained by other analytical techniques.

Medium effects (corrected for the bulk-susceptibility effect) have been reported for four commonly used solvent–internal reference systems in a paper by Stephen and his co-workers, who point out that the difference in chemical shifts between internal and external reference signals may be as great as 0.54 p.p.m.[759] A novel type of noise-modulated, heteronuclear decoupling has been used by Evelyn and Hall to simplify the ^1H n.m.r. spectra of sugar derivatives containing the $-CH_2F$ group.[283] By suitable adjustment of the modulation bandwidth and the radio-frequency intensity, the resonances of the coupled nuclei are broadened sufficiently

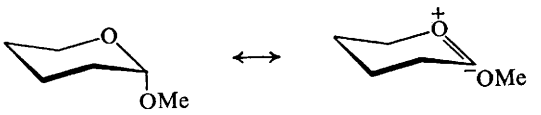
[756] V. G. Dashevskii, *Itogi Nauki Tech., org. Khim.*, 1975, **1**, 99 (*Chem. Abs.*, 1976, **85**, 160 425a).
[757] D. R. Bundle and R. U. Lemieux, *Methods in Carbohydrate Chem.*, 1976, **7**, 79.
[758] S. J. Angyal and G. S. Bethell, *Austral. J. Chem.*, 1976, **29**, 1249.
[759] K. G. R. Pachler, E. B. Rathbone, and A. M. Stephen, *Carbohydrate Res.*, 1976, **47**, 155.

to remove them effectively from the spectrum. The amount of broadening depends on the spectral density and the magnitude of the spin-coupling – the larger the coupling constant, the larger is the broadening. For example, the resonances of H-5, H-6, and H-6′ of 6-deoxy-6-fluoro-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose effectively disappeared from the ^{1}H n.m.r. spectrum on irradiation at the ^{19}F-resonance frequency with a substantially increased noise-bandwidth and the radiofrequency power-level decreased, so that transitions of protons having no coupling to the fluorine substituent were clearly resolved.

The ^{15}N n.m.r. spectra of some of the components of the nebramycin complex have been reported.[760] Resonances of the individual nitrogen nuclei were assigned by comparison of the spectra with those of structurally similar compounds. Substituent effects and the effects of pH on the ^{15}N chemical shifts were also examined.

A study of the complexes formed between Na^{+} cations and some monosaccharides using ^{23}Na n.m.r. measurements is noted in Chapter 18.[519] Studies on *myo*-inositol hexaphosphate using ^{31}P n.m.r. spectroscopy [227] and of the complexation of borax with D-glucose and D-glucobioses [272] are referred to in Chapter 6.

Computation of the conformational energies of 2-methoxytetrahydropyran, a model for the methyl aldopyranosides, gave results in agreement with experimental observations.[761] A preference for the axial conformer was apparent, but the nature of the stabilizing factor (anomeric effect) was not resolved. Force-field calculations have been extended to include alcohols, ethers, simple acetals, and 2-methoxytetrahydropyran.[762] The preference of 2-methoxytetrahydropyran for the axial conformer was attributed to interaction of the axial lone-pair orbital on the ring-oxygen atom with the axial C—O bond of the methoxy-group, which can be represented by the resonance structure (501) (see also, Vol. 6, p. 163).



(501)

The potential and free energies of the 2-acetamido-2-deoxyaldopyranoses have been computed using semi-empirical potential functions.[763] Comparison of the experimental and calculated values for the conformational equilibria for D-glucose, D-galactose, D-mannose, 2-acetamido-2-deoxy-D-glucose, -galactose, and -mannose suggested that the anomeric effect is small (0.55 kcal mol^{-1}) and does not depend significantly on the nature and configuration of the substituent at C-2 – this conclusion is contrary to the current view that such a dependence exists.

[760] D. E. Dorman, J. W. Paschal, and K. E. Merkel, *J. Amer. Chem. Soc.*, 1976, **98**, 6885.
[761] A. Abe, *J. Amer. Chem. Soc.*, 1976, **98**, 6477.
[762] N. L. Allinger and D. Y. Chung, *J. Amer. Chem. Soc.*, 1976, **98**, 6798.
[763] R. Virudachalam and V. S. R. Rao, *Carbohydrate Res.*, 1976, **51**, 135.

Acyclic Systems

Accurate ^1H n.m.r. parameters for four acetylated thiazolidines [*e.g.* (165)] derived from L-arabinose and L-cysteine have been obtained by computer-assisted analysis of the spectra.[384] The polyacetoxyalkyl side-chains of (165; R^1 = Me, R^2 = Ac) and its (*S*)-diastereoisomer adopt a planar zig-zag conformation, which tends to become somewhat distorted in certain derivatives [*e.g.* 3-acetyl-(*R*)-(L-*arabino*-2,3,4-triacetoxy-1-hydroxybutyl)thiazolidine-4(*R*)-carboxylic acid 4,1′-lactone].

Pyranoid Systems

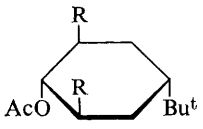
High-resolution (300 MHz) spectrometers have permitted complete analyses of the ^1H n.m.r. spectra of solutions of L-rhamnose, methyl α-L-rhamnopyranoside, 4-*O*-β-D-galactopyranosyl-L-rhamnose,[764] L-fucose and 2,6-dideoxy-L-*lyxo*-hexose and their methyl pyranosides,[765] 2-deoxy-D-*arabino*- and -*lyxo*-hexose and 2′-deoxylactose,[766] L-arabinose,[767] and some D-aldohexopyranosyl-D-fructos(id)es [13, 768] in deuterium oxide. Homo-INDOR measurements were sometimes used to verify the assignments to the ring protons, and coupling constants were extracted from the homo-INDOR spectra and refined by computer-aided simulation procedures. Increment rules were proposed for the L-rhamnose derivatives and the rules for D-glucobioses were extended to 4-*O*-β-D-galactopyranosyl-L-rhamnose.[764] The values of the coupling constants obtained from the 360 MHz ^1H n.m.r. spectrum of methyl 2,3-anhydro-4,6-*O*-(4-bromobenzylidene)-α-D-mannopyranoside are fully consistent with a $^0H_5$ conformation ($J_{4,5}$ = 10 Hz; *cf.* $J_{4,5} \sim$ 5—6 Hz obtained from the 100 MHz spectrum, for which some of the assignments were revised).[769]

Computer-refined parameters for a number of *N*-hexopyranosylimidazoles and their tetra-*O*-acetyl derivatives revealed that some of these compounds exist as mixtures of $^4C_1$ and $^1C_4$ conformers in solution; the possible contributions of steric and reverse anomeric (polar) effects were discussed.[769a] Mixtures of conformers – including boat conformers – were also indicated for several D-glucopyranosylpyridinium salts from the size of the proton–proton coupling constants.[620]

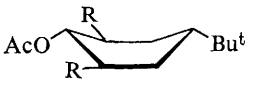
Paulsen has discussed the influence of *syn*-1,3-diaxial interactions and the anomeric effect on the conformations of various pyranoid derivatives.[770] High proportions of the $^1C_4$ forms, indicating that there are strong *syn*-diaxial interactions in the $^4C_1$ forms, were observed for 2,4-diazido-2,4,6-trideoxy-α-D-idopyranoside and the corresponding 2,4-diamino dihydrochloride derivative.[328] Strong *syn*-diaxial interactions in the usual chair forms of 1,6-anhydro-2,4-

[764] A. de Bruyn, M. Anteunis, R. de Gussem, and G. G. S. Dutton, *Carbohydrate Res.*, 1976, **47**, 158.
[765] A. de Bruyn, M. Anteunis, P. J. Garegg, and T. Norberg, *Acta Chem. Scand.*, 1976, **B30**, 820.
[766] A. de Bruyn and M. Anteunis, *Bull. Soc. chim. belges*, 1975, **84**, 1201.
[767] A. de Bruyn and M. Anteunis, *Bull. Soc. chim. belges*, 1975, **84**, 831.
[768] A. de Bruyn, J. van Beeumen, M. Anteunis, and G. Verhegge, *Bull. Soc. chim. belges*, 1975, **84**, 799.
[769] O. Achmatowicz, jun., and B. Szechner, *Carbohydrate Res.*, 1976, **48**, 125.
[769a] P. Finch and A. G. Nagpurkar, *Carbohydrate Res.*, 1976, **49**, 275.
[770] H. Paulsen, *Stärke*, 1975, **27**, 397.

diazido-2,4-dideoxy-β-D-glucopyranose and 2,4-diamino-1,6-anhydro-2,4-dideoxy-β-D-glucopyranose dihydrochloride forced the pyranoid ring to adopt $^1S_0$ and $^{1,4}B$ forms, respectively.[329] A closely related study of the t-butylcyclohexane derivatives (502) showed that the dication (502; R = $\overset{+}{N}H_3$) adopts the boat



(502) R = OAc, N_3, OMe, NHAc, or $\overset{+}{N}H_3$



(503) R = NHAc or $\overset{+}{N}H_3$

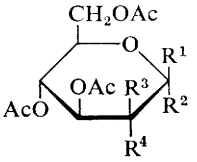
conformation (503) exclusively, while the corresponding 2,6-diacetamido derivative (502; R = NHAc) also shows a strong preference for the boat conformation (503).[771] *syn*-1,3-Diaxial interactions between the two R substituents in (502) increase in the order OAc < N_3 < OMe < NHAc < $\overset{+}{N}H_3$.

Computer-assisted analyses of the ^{1}H n.m.r. spectra of six fully acetylated glycals and 2,3,4,6-tetra-*O*-acetyl-2-hydroxy-D-glucal have been reported.[772] The coupling constants suggest rapid interconversion between the $^4H_5$ and $^5H_4$ forms, rather than ring-flattening (*cf.* A. A. Chalmers and R. H. Hall, *J.C.S. Perkin II*, 1974, 728). The 250 MHz ^{1}H n.m.r. spectra of specifically trideuterioacetylated α- and β-D-xylopyranose tetra-acetates have been fully analysed,[773] and in the conformational equilibria of derivatives of methyl 3,4-dideoxy-DL-glyc-3-enopyranoside have been studied using ^{1}H n.m.r. spectroscopy.[774]

The chemical shifts and coupling constants of the protons in the C-5–C-6 fragments in both anomers of D-gluco-, D-manno-, and D-galacto-pyranoses and in the corresponding methyl pyranosides have allowed qualitative conclusions to be drawn about the rotamer populations, particularly the influence of the axial HO-4 group in the D-galactose derivatives on the rotamer populations.[775] Differences between the vicinal ^{19}F–^{1}H couplings of 6-deoxy-6-fluoro-D-glucopyranose and -D-galactopyranose derivatives also demonstrated the influence of the C-4 substituent on the populations of the C-5–C-6 rotamers.[283] The magnitude of *gauche* proton–proton couplings in pyranoid derivatives has been rationalized in terms of the presence, or the absence, of antiperiplanar electronegative substituents; thus ^3J_g is 0.8—2.0, 2.0—3.5, and 3.5—5.5 Hz depending on whether both protons, one proton, or neither of the protons, respectively, are in an antiperiplanar arrangement with an electronegative substituent.[776]

Angular dependences have been found for the ^{1}H–^{199}Hg and ^{13}C–^{199}Hg coupling constants in methyl 3,4,6-tri-*O*-acetyl-2-chloromercuri-2-deoxy-β-D-glucopyranoside (504) and the corresponding α-D-mannopyranoside derivative (505).[777] The ^{1}H–^{199}Hg couplings appear to conform to a Karplus relationship,

[771] H. Paulsen and H. Koebernick, *Chem. Ber.*, 1976, **109**, 112.
[772] M. Rico and J. Santoro, *Org. Magn. Resonance*, 1976, **8**, 49.
[773] J. P. Utille and P. Votero, *Bull. Soc. chim. France*, 1976, 387.
[774] O. Achmatowicz, jun., A. Banaszak, M. Chimelewski, A. Zamojski, and W. Lobodzinski, *Uniw. Adama Mickiewicza Poznaniv, Mat., Fiz. Chem., Ser. Chem.*, 1975, **18**, 61 (*Chem. Abs.*, 1976, **84**, 135 994g).
[775] A. de Bruyn and M. Anteunis, *Carbohydrate Res.*, 1976, **47**, 311.
[776] A. de Bruyn and M. Anteunis, *Org. Magn. Resonance*, 1976, **8**, 228.
[777] V. G. Gibb and L. D. Hall, *Carbohydrate Res.*, 1976, **50**, C3.



(504) R^1 = OMe; R^2 = R^3 = H; R^4 = HgCl
(505) R^1 = R^4 = H; R^2 = OMe; R^3 = HgCl

and the vicinal ^{13}C–^{199}Hg couplings are quite different for dihedral angles of ~180° ($^3J_{^{13}C\text{-}4,\ ^{199}Hg}$ = 225.2 Hz) and ~60° ($^3J_{^{13}C\text{-}4,\ ^{199}Hg}$ = 53.3 Hz). ^{13}C–^{199}Hg couplings over five bonds were also observed.

Furanoid Systems

A study of the conformations of 1,2:5,6-di-*O*-isopropylidene-α-D-gluco-, -galacto-, and -allo-furanoses by 300 MHz ^1H n.m.r. spectroscopy has indicated that, because of pseudorotation, the relative chemical shifts are of more diagnostic value than the coupling constants in assigning configurations on the furanoid ring.[778] Specific trideuterioacetylation has aided the assignment of signals in the 250 MHz ^1H n.m.r. spectra of α- and β-D-xylofuranose tetra-acetates.[779]

High-resolution ^1H n.m.r. spectroscopy has been used to probe the conformations of a number of D-ribofuranosylamine derivatives[619] and such 'rigid' molecules as 2,2′-cyclonucleosides and nucleoside 3′,5′-phosphates in aqueous solution.[780] ^1H N.m.r. spectroscopy has also been used to study details of the intramolecular association and conformations of α- and β-linked pyridine ribonucleosides and their 5′-phosphates.[781] The results were analysed in terms of base–D-ribose, D-ribose–side-chain, and base–side-chain interactions and the conformational restraints imposed by the *cis* HO-2′–HO-3′ interaction in β-nucleotides and the additional *cis* HO-2′–base interaction in α-nucleotides. ^1H N.m.r. measurements – including measurements of nuclear Overhauser effects and paramagnetic relaxations effected by Mn^{2+} cations – have been used to investigate the preferred conformation about the *N*-glycosidic bond of 8-amino-, 8-methylamino-, and 8-dimethylamino-adenylic acid, all of which competitively inhibit the coenzyme NADH in the reaction with chicken-muscle lactate dehydrogenase.[782] The primary and secondary amines were shown to prefer *anti* conformations, whereas the tertiary amine prefers a *syn* conformation.

The origin of the criterion for determining the anomeric configuration of D-ribofuranosyl-nucleosides, based on differences in the chemical shifts between the methyl signals of the corresponding 2′,3′-*O*-isopropylidene derivatives, has been discussed.[783] Comparison of the chemical shifts of anomeric pairs of nucleosides having a reduced heterocyclic base with those of the non-reduced base showed that the chemical shift of the *endo*-methyl group in the α-anomer is

[778] A. de Bruyn, D. Danneels, M. Anteunis, and E. Saman, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 227.
[779] J. P. Utille and P. Vottero, *Bull. Soc. chim. France*, 1976, 1101.
[780] C.-H. Lee and R. H. Sarma, *J. Amer. Chem. Soc.*, 1976, **98**, 3541.
[781] N. J. Oppenheimer and N. O. Kaplan, *Biochemistry*, 1976, **15**, 3981.
[782] F. E. Evans and N. O. Kaplan, *J. Biol. Chem.*, 1976, **251**, 6791.
[783] B. Rayner, C. Tapiero, and J. Imbach, *Carbohydrate Res.*, 1976, **47**, 195.

influenced by the anisotropy of the aglycone. The method is therefore applicable only to D-ribofuranosyl compounds having an unsaturated base as the aglycone.

Studies of mono- and di-nucleotides using ^{31}P n.m.r. spectroscopy have reported chemical shifts [784] and coupling constants.[784a] Both ^{31}P and ^{1}H n.m.r. spectroscopy were used to examine the stereoisomeric spirophosphoranes obtained by the joint action of tris(dimethylamino)phosphine and carbon tetrachloride on 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-gluco- and -allo-furanose [513] (see Vol. 9, p. 47), and, in conjunction with ^{13}C n.m.r. spectroscopy, the stereochemistries of 1,2-substituted 6-deoxy-6-halogeno-D-glucofuranose 3,5-phosphates.[234]

Di-, Oligo-, and Poly-saccharides

The anomeric configurations of the glycosidic linkages in the capsular polysaccharide from *Klebsiella* strain 6412 have been assigned by examination of the ^{1}H n.m.r. spectra of the original and degraded materials.[785] The charge distributions and torsional potentials associated with β-D-(1 → 3)-glycosidic linkages in six glycosaminoglycans have been investigated using CNDO and PCILO molecular-orbital techniques on model compounds [786] (see also Vol. 9, p. 180). In each case, the glycosidic valence-bond angle of minimum energy was found at ~111°, and a secondary energy minimum was found at ~116°, which is the value observed experimentally for saccharides. It was concluded that long-range intra- or inter-molecular interactions or both are responsible for the overall preference for the valence-bond angle of ~116°.

^{1}H n.m.r. measurements on a number of disaccharides are referred to in the section on pyranoid systems, while the application of ^{13}C n.m.r. spectroscopy to disaccharides and their derivatives is dealt with in the following section.

^{13}C N.M.R. Spectroscopy

The use of ^{13}C n.m.r. spectroscopy in elucidating the structures of monosaccharides, polysaccharides, nucleosides, and nucleotides has been reviewed.[787] A study of the tautomerism of formycin and related compounds using ^{13}C n.m.r. spectroscopy is noted in Chapter 21.[739]

Earlier assignments of the ^{13}C resonances in the natural abundance ^{13}C n.m.r. spectra of monosaccharides (*e.g.* D-glucose, D-galactose, and D-mannose, *etc.*) have been revised in the light of coupling data obtained from the spectra of the corresponding [1-^{13}C]labelled monosaccharides.[788] In addition to a large (~46 Hz) one-bond coupling between C-1 and C-2, C-1 couples to C-3 (^{2}J ~ 4 Hz) only in the β-anomers, to C-5 (^{2}J ~ 2 Hz) only in the α-anomers, and to C-6 in both anomers, whereas it showed no evidence of coupling to C-4 in any of the monosaccharides examined. The geminal $^{2}J_{CCC}$ and $^{2}J_{COC}$ couplings were discussed in terms of a dihedral-angle dependence, where the angle is defined by

[784] P. J. Cozzone and O. Jardetzky, *Biochemistry*, 1976, **15**, 4853.
[784a] P. J. Cozzone and O. Jardetzky, *Biochemistry*, 1976, **15**, 4860.
[785] B. Lindberg and W. Nimmich, *Carbohydrate Res.*, 1976, **48**, 81.
[786] R. Potenzone, jun. and A. J. Hopfinger, *Carbohydrate Res.*, 1976, **46**, 67.
[787] A. S. Shashov and O. S. Chizhov, *Bioorg. Khim.*, 1976, **2**, 437 (*Chem. Abs.*, 1976, **85**, 1954f).
[788] T. E. Walker, R. E. London, T. W. Whaley, R. Barker, and N. A. Matwiyoff, *J. Amer. Chem. Soc.*, 1976, **98**, 5807.

the relative orientations of C-3 or C-5 and the electronegative oxygen substituents on C-1.

The use of ^{13}C n.m.r. spectroscopy to determine the equilibrium compositions of solutions of 2-hexuloses in deuterium oxide is mentioned on p. 174.[758] The equilibrium compositions of solutions of 1-deoxy-D-fructose, 1-deoxy-L-sorbose, 1-deoxy-L-psicose, D-*threo*-pentulose, D-*arabino*- and L-*xylo*-3-hexulose, and coriose in deuterium oxide have also been determined by Angyal's group using ^{13}C n.m.r. spectroscopy.[789] With the exception of coriose, aqueous solutions of the 1-deoxyhexuloses and 3-hexuloses contained significant proportions of the acyclic *keto*-form at equilibrium, particularly at elevated temperatures. The hydrated *keto*-form was not detected in aqueous solutions of any of the hexuloses or 1-deoxy-3,4,5,6-tetra-*O*-methyl-D-fructose (which cannot cyclize). The equilibrium compositions of D-fructose and some related Amadori-rearrangement products (143) in [$^2H_5$]pyridine have been studied qualitatively and quantitatively by ^{13}C n.m.r. spectroscopy, which showed that there is generally 46% of β-pyranose, 7% of α-pyranose, 30% of β-furanose, 12% of α-furanose, and 5% of the *keto*-form at equilibrium.[352]

Acetylation of simple acyclic and cyclic alcohols produced only a relatively small chemical shift of the ^{13}C resonance of the appended carbon nucleus, whereas appreciably larger shifts were observed on methoxycarbonylation or methanesulphonylation of the alcohols, although the resonances of the neighbouring carbon nuclei were also shifted by almost as much.[790] 3-*O*-Acetyl, 3-*O*-monochloroacetyl, 3-*O*-trichloroacetyl, and 3-*O*-methanesulphonyl derivatives of D-glucose have also been used in assigning the resonances in the ^{13}C n.m.r. spectrum of D-glucopyranose; the effects of esterification on the chemical shifts of the ^{13}C resonances of the appended and neighbouring carbon nuclei were more pronounced as the electronegativity of the substituent increased.[791]

The ^{13}C and ^1H resonances of each of the methyl groups have been assigned in studies of 2,3,4-tri-*O*-methyl derivatives of methyl β-L-arabinopyranoside, methyl α-D-lyxopyranoside, methyl β-D-ribopyranoside, and methyl α-D-xylopyranoside, in which some of the methyl groups were replaced by trideuteriomethyl groups.[204] The deshielding experienced on six-membered rings by ^{13}C nuclei in equatorial methoxy-groups that are flanked by two equatorial methoxy-(hydroxy)-groups was rationalized in terms of *syn*-axial δ-effects and rotamer populations. The effect of *O*-alkylation on the ^{13}C n.m.r. spectra of methyl pentofuranosides has also been examined.[792] *O*-Alkylation of a hydroxy-group displaced the signal of the appended ^{13}C nucleus downfield, whereas those of the adjacent ^{13}C nuclei were generally shifted upfield to a lesser extent. The effect of *O*-methylation on the ^{13}C chemical shifts was appreciably larger than that of either *O*-isopropylation or *O*-glycosylation, but *O*-methyl and *O*-isopropyl derivatives can be used as models for interpreting the spectra of oligo- and poly-saccharides containing furanoid components. The effect of *O*-methylation on the ^{13}C chemical shifts of the neighbouring carbon nuclei has been used in assigning the ^{13}C resonances in the spectrum of 1,4-di-*O*-methyl-*chiro*-inositol.[793]

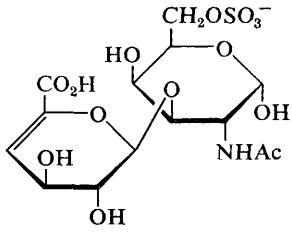
[789] S. J. Angyal, G. S. Bethell, D. E. Cowley, and V. A. Pickles, *Austral. J. Chem.*, 1976, **29**, 1239.
[790] Y. Terui, K. Tori, and N. Tsuji, *Tetrahedron Letters*, 1976, 621.
[791] M. R. Vignon and P. J. A. Vottero, *Tetrahedron Letters*, 1976, 2445.
[792] P. A. J. Gorin and M. Mazurek, *Carbohydrate Res.*, 1976, **48**, 171.
[793] J. W. Blunt, M. H. G. Munro, and A. J. Paterson, *Austral. J. Chem.*, 1976, **29**, 1115.

The signals in the ^{13}C n.m.r. spectra of the eight isomeric 1,6-anhydro-β-D-hexopyranoses having the $^1C_4$ conformation have been assigned by comparison with the spectra of selectively deuteriated derivatives and by observation of the effects produced by *O*-isopropylidenation – the signal for the equatorially substituted carbon atom was shifted to a lower field more strongly than that of the axially substituted carbon atom in the *O*-isopropylidene derivatives.[794] The chemical shifts, and their calculation using empirical parameters, were discussed. Perlin's group has also presented an analysis of configurational effects on the ^{13}C chemical shifts of 1,6-anhydro-β-D-hexopyranoses, 2,7-anhydro-β-D-heptulopyranoses, and their *O*-acetyl and *O*-isopropylidene derivatives.[795] Some characteristics of the ^{13}C–^1H couplings in these bicyclic compounds were described, and examples were given of how $^2J_{^{13}C,^1H}$ and $^3J_{^{13}C,^1H}$ couplings can be used to identify the ^{13}C signals.

^{13}C N.m.r. studies of β-D-glucopyranosyl fluoride and of anomeric pairs of 2-deoxy-2-fluoro-, 3-deoxy-3-fluoro-, 4-deoxy-4-fluoro-, 6-deoxy-6-fluoro-D-glucopyranose, 2-deoxy-2-fluoro-D-mannopyranose, and 4-deoxy-4-fluoro-D-galactopyranose have demonstrated the orientational dependence of substituent effects upon $^nJ_{^{19}F,^{13}C}$, which can be observed and rationalized over a large number of bonds.[796] INDO molecular-orbital calculations reproduced the trends in the $^3J_{^{19}F,^{13}C}$ and $^4J_{^{19}F,^{13}C}$ values, both of which show a marked dependence on the orientation of the coupled nuclei, and rationalized the long-range $J_{^{19}F,^1H}$ data obtained previously for these systems. ^{13}C-Substituent chemical shifts were observed for all carbon nuclei, but the complexity of the factors affecting these shifts precluded any detailed discussion.

Selective double irradiation permitted most of the resonances in the ^{13}C n.m.r. spectra of a series of peracetylated D-glucobioses to be assigned.[797] The factors that most affect the chemical shifts were discussed, and the spectra of β-cellobiose and β-maltose octa-acetates were compared with those of cellulose and amylose triacetates, respectively, to show the value and limitations of a disaccharide model for interpreting the ^{13}C n.m.r. spectrum of a polysaccharide. Complete assignments have been presented for the ^{13}C n.m.r. spectra of β-linked disaccharides containing various combinations of D-glucopyranosyl, D-galactopyranosyl, L-rhamnopyranosyl, and 2-acetamido-2-deoxy-D-gluco- and -galacto-pyranosyl residues, which are model compounds for ^{13}C n.m.r. studies of immunological polysaccharides.[798] The data showed that changing the nature of the reducing residue (*e.g.* D-glucose to L-rhamnose) has no significant effect on the chemical shifts of the carbon nuclei of the non-reducing residue (*e.g.* D-glucose). The converse is also true; *i.e.* the chemical shifts of the carbon nuclei of the reducing residue are not noticeably affected by a change of the non-reducing residue (*e.g.* D-glucose to D-galactose or 2-acetamido-2-deoxy-D-glucose). The ^{13}C n.m.r. spectrum of the unsaturated disaccharide (506) has been measured as part of a study of the heterogeneity of the chondroitin sulphates.[799] ^{13}C N.m.r. measure-

[794] H. Paulsen, V. Sinnwell, and W. Greve, *Carbohydrate Res.*, 1976, **49**, 27.
[795] R. G. S. Ritchie, N. Cyr, and A. S. Perlin, *Canad. J. Chem.*, 1976, **54**, 2301.
[796] V. Wray, *J.C.S. Perkin II*, 1976, 1598.
[797] D. Y. Gagnaire, F. R. Taravel, and M. R. Vignon, *Carbohydrate Res.*, 1976, **51**, 157.
[798] P. Colson and R. R. King, *Carbohydrate Res.*, 1976, **47**, 1.
[799] G. K. Hamer and A. S. Perlin, *Carbohydrate Res.*, 1976, **49**, 37.



(506)

ments on two sialic acid-containing disaccharides, *viz.* 4-*O*-α-D-glucopyranosyl- and 4-*O*-α-D-galactopyranosyl-β-D-*N*-acetylneuraminic acid, were used in structural studies of meningococcal polysaccharides.[800]

^{13}C Chemical shifts have been reported for a number of compounds derived from sucrose, including D-*galacto*-sucrose and 6,6′-dichloro-6,6′-dideoxy- and 1′,6,6′-trichloro-1′,6,6′-trideoxy-sucrose.[801]

Resonances in the ^{13}C n.m.r. spectrum of methyl α-daunosaminide hydrochloride have been assigned and used in the analysis of those in the spectra of daunorubicin and adriamycin (see p. 134).[802] ^{13}C N.m.r. spectroscopy promises to be an excellent tool for elucidating the structures of naturally occurring glycosides, *etc.*, without resorting to chemical degradation. ^{13}C Chemical shifts have been reported for saikosaponins A, C, D, and F (from *Bupleurum falcatum* L.), which contain side-chains of D-glucose and L-rhamnose or D-fucose,[803] aloenin (a bitter β-D-glucoside isolated from *Aloe arborescens* Mill.),[804] flavonoid glycosides,[805] and diterpenoid glycosides from *Stevia paniculata* (Compositae) and *S. rebaudiana*.[806] In the latter study it was shown that C-1 of a β-D-glucopyranosyl residue linked to a tertiary hydroxy-group resonates at an abnormally high field compared to that of C-1 of a β-D-glucopyranosyl residue linked to primary or secondary hydroxy-groups. The 67.7 MHz ^{13}C n.m.r. spectrum of vancomycin (a glycopeptide antibiotic) has confirmed the presence of 66 ± 1 carbon atoms, of which 8 are carbonyl carbons.[807]

A single seed from *Aucuba japonica* gave a surprisingly well-resolved ^{13}C n.m.r. spectrum, which consisted of 26 resonances – due to the presence of sucrose ($C_{12}H_{22}O_{11}$) and aucubin ($C_{15}H_{22}O_9$) – with only two coincident resonances.[808] However, all 27 resonances could be observed and assigned to the respective carbon nuclei by using a series of single-frequency, proton-decoupling experiments. Since the ^{13}C and ^{1}H chemical shifts for aucubin and

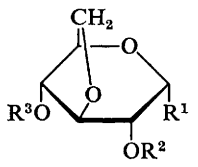
[800] A. K. Bhattacharjee, H. J. Jennings, C. P. Kenny, A. Martin, and I. C. P. Smith, *Canad. J. Biochem.*, 1976, **54**, 1.
[801] L. Hough, S. P. Phadnis, E. Tarelli, and R. Price, *Carbohydrate Res.*, 1976, **47**, 151.
[802] A. Arnone, G. Fronza, R. Mondelli, and A. Vigevani, *Tetrahedron Letters*, 1976, 3349.
[803] K. Tori, S. Seo, Y. Yoshimura, M. Nakamura, Y. Tomita, and H. Ishii, *Tetrahedron Letters*, 1976, 4167.
[804] K. Tori, T. Hirata, O. Koshitani, and T. Suga, *Tetrahedron Letters*, 1976, 1311.
[805] K. R. Markham and B. Ternai, *Tetrahedron*, 1976, **32**, 2607.
[806] K. Yamasaki, H. Kohda, T. Kobayashi, R. Kasai, and O. Tanaka, *Tetrahedron Letters*, 1976, 1005.
[807] D. H. Williams and J. R. Kalman, *Tetrahedron Letters*, 1976, 4829.
[808] M. Kainosho, *Tetrahedron Letters*, 1976, 4279.

sucrose differ little from those found in aqueous solutions, it appears that each of these compounds has an identical structure and conformation in the seed and in solution.

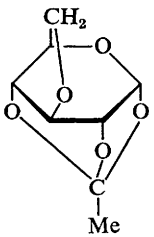
Lanthanide Shift Reagents

The complexes formed between lanthanide shift reagents and permethylated aldohexopyranosides and their 6-deoxy analogues having *gluco*, *galacto*, and *manno* configurations have been studied.[809] The lanthanide shift reagent was shown to bind preferentially to the oxygen atoms of two adjacent methoxy-groups having an axial–equatorial relationship. The following sequence of preference for binding can be given for $Eu(fod)_3$: $O\text{-}1_{eq.}\text{–}O\text{-}2_{ax.} \sim O\text{-}2_{ax.}\text{–}O\text{-}3_{eq.} \sim O\text{-}1_{ax.}\text{–}O\text{-}2_{eq.} > O\text{-}6_{ax.}\text{–}O\text{-}5 > O\text{-}1_{eq.} \sim O\text{-}2_{eq.} \sim O\text{-}3_{eq.} \sim O\text{-}6_{eq.} > O\text{-}4_{eq.\text{ or }ax.}$. The sequence is much the same for $Pr(fod)_3$ and $Gd(fod)_3$, except that $O\text{-}2_{ax.}\text{–}O\text{-}3_{eq.} > O\text{-}1_{ax.}\text{–}O\text{-}2_{eq.} \sim O\text{-}6_{ax.}\text{–}O\text{-}5$.

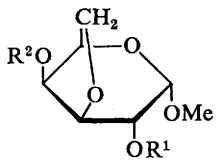
The configuration of the branch point in branched-chain sugar derivatives (*e.g.* methyl 2-benzamido-4,6-*O*-benzylidene-2-deoxy-3-*C*-nitromethyl-α-D-allo-pyranoside) has been assigned on the basis of the downfield chemical shifts obtained with $Eu(fod)_3$, as compared with those of model compounds of known configuration.[810] ^1H Chemical shifts and J values have been recorded for the 3,6-anhydro-sugar derivatives (507)—(509) in the presence, and in the absence,



(507) R^1 = OMe or H;
R^2, R^3 = H, Me, or Ac



(508)



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

of $Eu(dpm)_3$.[811] The work of Angyal's group on the complexation of lanthanide cations with *epi*-inositol and related compounds in aqueous solution is noted in Chapter 18.[514]

Spin-lattice Relaxation Times

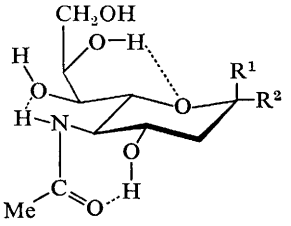
A Fourier-transform method has been used to measure the spin-lattice relaxation times (T_1-values) of the anomeric protons of a selection of di-, oligo-, and poly-saccharide derivatives (*e.g.* cellobiose, maltose, gentiobiose, maltotriose, and α- and β-Schardinger dextrins).[812] Differences in the T_1-values of the anomeric protons were found for each of the disaccharides examined, the anomeric proton of the non-reducing residue having a smaller T_1-value in each case – for cellobiose,

[809] D. G. Streefkerk and A. M. Stephen, *Carbohydrate Res.*, 1976, **49**, 13.
[810] J. J. Nieuwenhuis and J. H. Jordaan, *Carbohydrate Res.*, 1976, **51**, 207.
[811] O. S. Chizhov, A. S. Shashkov, A. I. Usov, and A. I. Shienok, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 2591 (*Chem. Abs.*, 1976, **84**, 90 506f).
[812] L. D. Hall and C. M. Preston, *Carbohydrate Res.*, 1976, **49**, 3.

for example, H-1β at the reducing end has a T_1-value of 0.84 s, whereas H-1′β at the non-reducing end has a T_1-value of 0.36 s (ratio H-1 : H-1′ = 2 : 3). It appears that the anomeric proton of the non-reducing residue of disaccharides receives relaxation contributions from the protons on *both* sugar rings, thereby offering a promising new method for studying the conformations of oligosaccharides in solution. However, the situation for higher polymers is less clear. Proton T_1-values have also been used to probe the conformations in aqueous solution of purine and pyrimidine nucleoside 5′-phosphates, all of which showed a preference for the *syn* arrangement about the *N*-glycosidic linkage.[813] The use of ^{7}Li and ^{35}Cl spin-lattice relaxation times to study the binding of Li^+ and Cl^- ions to nucleosides has already been mentioned in Chapter 18.[518] Lithium chloride appears to bind – probably as an ion-pair – to the D-ribofuranosyl residue.

The T_1-values of the ^{13}C nuclei have proved extremely helpful in elucidating the structures of monoterpenoid β-D-glucosides[814] and in analysing the ^{13}C n.m.r. spectra of macrolide antibiotics (*e.g.* leucomycin, spiramycin, and chalcomycin).[815]

Measurement of the ^{13}C spin-lattice relaxation times of *N*-acetylneuraminic acid and its methyl α- and β-glycosides and methyl ester in deuterium oxide has indicated that (i) the pyranose ring is rigid and tumbles isotropically in solution (*i.e.* it has no preferred axis of rotation), (ii) the glycosidic and ester methyl groups are free to rotate, (iii) the hydroxymethyl group is relatively mobile, and (iv) the motions of C-7 and C-8 are isotropic with that of the ring.[816] Based on these results, the hydrogen-bonded model (510) was proposed for the methyl glycosides of *N*-acetylneuraminic acid in aqueous solution.



(510) $R^1 = CO_2^-$; R^2 = OMe
R^1 = OMe; $R^2 = CO_2^-$

The use of ^{1}H, ^{2}H, and ^{13}C nuclear relaxation measurements to define the molecular motions of some mono- and di-saccharides in aqueous solution is referred to briefly in Chapter 2.[29] There is, however, serious disagreement between the n.m.r. rotational correlation time and that expected from dielectric-relaxation studies.[28] A strategy has been developed for the complementary use of dielectric and nuclear magnetic relaxation measurements in elucidating the molecular dynamics of small molecules in aqueous solution and for determining the extent of hydration.[30]

[813] C. Chachaty, T. Zemb, G. Langlet, Tran Dinh Son, H. Buc, and M. Morange, *European J. Biochem.*, 1976, **62**, 45.
[814] K. Yamasaki, M. Kaneda, and O. Tanaka, *Tetrahedron Letters*, 1976, 3965.
[815] A. Neszmelyi, S. Omura, and G. Lukacs, *J.C.S. Chem. Comm.*, 1976, 97.
[816] M. F. Czarniecki and E. R. Thornton, *J. Amer. Chem. Soc.*, 1976, **98**, 1023.

24
Other Physical Methods

I.R. Spectroscopy

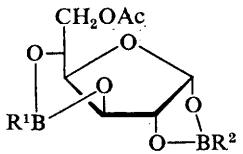
The i.r. spectra of disaccharides differing in monosaccharide composition and in the position and configuration of the glycosidic linkage, and also those of raffinose and model saccharides, have been recorded in the region 40—1000 cm^{-1}.[817] Absorption bands in the region 800—1000 cm^{-1} can be assigned to the vibrations of equatorial (α-anomer) and axial (β-anomer) C-1—H groups, but no useful correlations could be made in regions of higher wavelength. The i.r. spectra of D-glucose and cello-oligosaccharides have been examined and compared with that of cellulose over a wide range of temperatures.[36]

U.V. Spectroscopy

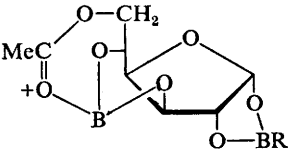
The carbonyl absorption of several *O*-methyl ketoses (*e.g.* 5- and 6-*O*-methyl-L-sorbose and 5-*O*-methyl-D-fructose) has been used to determine the proportion of the acyclic forms present in the tautomeric equilibria in methanol.[148]

Mass Spectrometry

Correlations have been made between the structures and mass-spectral behaviour of cyclic boronic esters of monoacetylated hexoses.[269] The interaction of an acetyl group with the boron atom, accompanied by loss of the alkyl substituent from the boron atom, occurs following electron-beam ionization in the mass spectrometer and is restricted to the adjacent cyclic boronate in the case of D-glucose and D-galactose alkaneboronate acetates. The isomeric 1,2:3,5-bis(alkaneboronates) (511) and (512), for example, gave fragment ions (513) at



(511) R^1 = Bu; R^2 = Me
(512) R^1 = Me; R^2 = Bu



(513) R = Bu or Me

m/e 255 $[M—Bu]^+$ and 297 $[M—Me]^+$, respectively, from which the geometry and substitution pattern could be deduced.

[817] V. M. Tul'chinsky, S. E. Zurabyan, K. A. Asankozhoev, G. A. Kogan, and A. Ya. Khorlin, *Carbohydrate Res.*, 1976, **51**, 1.

The mass spectra of the trifluoroacetates of methyl hexopyranosides and their partially deuteriated derivatives have been measured and used in the identification of some stereoisomeric hexoses.[818] The positions of the substituents in methyl di- and tri-*O*-methyl-D-glucopyranosides can be readily distinguished by m.s.,[818] which was also used to examine the stereochemical effects in some 2,6-dideoxy-3-*C*-methylhexopyranose derivatives.[819] The 4,7,8-, 4,7,9-, and 7,8,9-tri-*O*-methyl derivatives of methyl *N*-acetyl-*N*-methyl-β-D-neuraminate methyl glycoside could be identified on the basis of their characteristic fragmentation-patterns, indicating that m.s. should be applicable to determining the positions of linkages in the sialic acid residues of biopolymers.[820] Other groups of compounds to be examined by m.s. include *O*-acetyl and *O*-methyl derivatives of steroidal glycosides [821] and naturally occurring glycosides (using field-desorption m.s.).[822]

High-resolution mass-spectral studies of 1,6-anhydro-3,4-*O*-isopropylidene-β-D-talopyranose and some specifically deuteriated derivatives thereof,[823] and the acetates of a number of *C*-glycosides derived from D-gluco- and D-galactopyranose [824] have been reported.

Acyclic carbohydrate derivatives to be examined by m.s. include *O*-acetyl-*O*-ethyl- [825] and *O*-acetyl-*O*-methyl-alditols [826] (obtained during structural investigations of polysaccharides), permethylated 2-, 3-, 4-, and 6-*O*-D-glucopyranosyl-D-glucitols and 1-*O*-α-L-arabinopyranosyl-DL-xylitol, and two permethylated D-glucobiosylalditols (using chemical ionization m.s. with isobutane–ammonia as reagent gases),[827] and 5-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-, 2,5-di-*O*-acetyl-3,4,6-tri-*O*-methyl-, and 3,5-di-*O*-acetyl-2,4,6-tri-*O*-methyl-D-mannononitrile.[149]

A new soft ionization method in field-desorption m.s., which involves the attachment of Li^+ ions, has been applied to sucrose and the D-glucoside loroglossin, both of which show quasi-molecular ions at $[M + Li]^+$.[828] The method facilitates the generation of highly stable $[M + Li]^+$ ions with substances whose normal field-desorption spectrum contains no signal for the molecular ion.

The sequence of the trisaccharide moiety of the plant glycoside colubrinoside has been determined by m.s. of the methylated and the methylated and reduced trisaccharide or the acetylated or trimethylsilylated trisaccharide.[829] A g.l.c.–m.s.

[818] T. Matsubara and A. Hayashi, *Biomed. Mass Spectrometry*, 1974, **1**, 62 (*Chem. Abs.*, 1976, **85**, 6001c).
[819] B. Giola and A. Vigevani, *Org. Mass Spectrometry*, 1976, **11**, 71.
[820] A. K. Bhattacharjee and H. J. Jennings, *Carbohydrate Res.*, 1976, **51**, 253.
[821] T. Komori, Y. Ida, Y. Mutou, K. Miyahara, T. Nohara, and T. Kawasaki, *Biomed. Mass Spectrometry*, 1975, **2**, 65 (*Chem. Abs.*, 1976, **85**, 94 638u).
[822] H. R. Schulten and D. E. Games, *Biomed. Mass Spectrometry*, 1974, **1**, 120 (*Chem. Abs.*, 1976, **85**, 6002d).
[823] D. Horton, J. S. Jewell, E. K. Just, J. D. Wander, and R. L. Foltz, *Biomed. Mass Spectrometry*, 1974, **1**, 145 (*Chem. Abs.*, 1976, **85**, 21 738s).
[824] B. V. Rozynov, I. A. Bogdanova, S. D. Shiyan, M. L. Shul'man, L. R. Usubalieva, and A. Ya. Khorlin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1976, 1157 (*Chem. Abs.*, 1976, **85**, 193 023w).
[825] D. P. Sweet and R. H. Shapiro, *Biomed. Mass Spectrometry*, 1974, **1**, 263 (*Chem. Abs.*, 1976, **85**, 33 297k).
[826] R. A. Hancock, K. Marshall, and H. Weigel, *Carbohydrate Res.*, 1976, **49**, 351.
[827] O. S. Chizhov, V. I. Kadentsev, A. A. Solov'yov, P. F. Levonowich, and R. C. Dougherty, *J. Org. Chem.*, 1976, **41**, 3425.
[828] H. J. Veith, *Angew. Chem. Internat. Edn.*, 1976, **15**, 696.
[829] M. J. M. Colard, P. A. Dumont, and F. Compernolle, *Biomed. Mass Spectrometry*, 1975, **2**, 156 (*Chem. Abs.*, 1976, **85**, 143 395y).

procedure has been developed for the concurrent determination of the reducing-end and other residues of oligosaccharides on a microgram scale; it involves reduction of the oligosaccharide with sodium borodeuteride followed in turn by acidic hydrolysis, reduction, trifluoroacetylation, and analysis of the resulting trifluoroacetylated alditols by g.l.c.–m.s.[830]

The mass spectra of the TMS and permethylated derivatives of isomeric D-glucosides of the cytokinins zeatin and 6-benzylaminopurine have been recorded by combined g.l.c.–m.s.[831] Detailed analysis of the fragmentation-patterns indicated that it should be possible to determine the size of the sugar ring in these phytohormones. The mass-spectral fragmentation-patterns of cytosine nucleosides specifically labelled with deuterium and ^{18}O are compatible with bond-scissions, *inter alia*, between C-2′–C-3′ and between C-4′–O-4′, which give rise to fragments containing the base and C-1′, C-2′, H-2′, and O-4′.[832] The field-ionization mass spectra of multiple-labelled thymine and thymidine have been reported.[832a]

Reports of conformational control in the mass spectra of alkylated 2- and 5-methylcyclohexane-1,3-diols are of interest to carbohydrate chemists – for example, the configuration of the *C*-methyl group leads to secondary stereochemical effects in eliminations specific for the *cis*- and *trans*-diols.[833]

The electron-impact mass spectra of a series of underivatized aminoglycoside antibiotics (*e.g.* gentamicin B and kanamycin A) have been reported and discussed.[834] The information available from the mass spectra of the underivatized compounds (up to pseudotrisaccharides) is easier to analyse and is diagnostically more useful than that obtained from the mass spectra of derivatives of higher molecular weight. The chemical-ionization mass spectra of representative aminoglycoside antibiotics were also reported. Electron-impact m.s. of the corresponding TMS derivatives has been used to determine the structures of polyene macrolide antibiotics (*e.g.* nystatin, amphotericin B, and pimaricin), and the proposed fragmentation pathways were corroborated by deuterium labelling, accurate mass measurements, and the identification of metastable transitions.[835]

X-Ray Crystallography

Jeffrey and Sundaralingam have reported details of the crystal structures of carbohydrates, nucleosides, and nucleotides published during 1974.[836] The following crystal structures have been reported during 1976:

[830] A. Kamei, H. Yoshizumi, S. Akashi, and K. Kagabe, *Chem. and Pharm. Bull. (Japan)*, 1976, 24, 1108.
[831] J. K. MacLeod, R. E. Summons, and D. S. Letham, *J. Org. Chem.*, 1976, **41**, 3959.
[832] J. G. Liehr, D. L. von Minden, S. E. Hattox, and J. A. McCloskey, *Biomed. Mass Spectrometry*, 1974, **1**, 281 (*Chem. Abs.*, 1976, **85**, 33 329x).
[832a] J. A. Lawson, J. I. Degraw, and M. Anbar, *J. Labelled Compounds*, 1975, **11**, 489 (*Chem. Abs.*, 1976, **84**, 17 664b).
[833] F. J. Winkler and A. V. Robertson, *Chem. Ber.*, 1976, **109**, 619, 633.
[834] P. J. L. Daniels, A. K. Mallams, J. Weinstein, J. J. Wright, and G. W. A. Milne, *J.C.S. Perkin I*, 1976, 1078.
[835] K. D. Haegele and D. M. Desiderio, jun., *Biomed. Mass Spectrometry*, 1974, **1**, 20 (*Chem. Abs.*, 1976, **85**, 21 761u).
[836] G. A. Jeffrey and M. Sundaralingam, *Adv. Carbohydrate Chem. Biochem.*, 1976, **32**, 353.

Free Sugars and Alditols.—α-D-Mannopyranose,[837] α-D-galactopyranose,[838, 839] β-D-galactopyranose,[839] β-D-fructopyranose.calcium bromide,[840] D-*threo*-hexo-2,5-diulose,[468] *meso*-L-*glycero*-L-*gulo*-heptitol.[841]

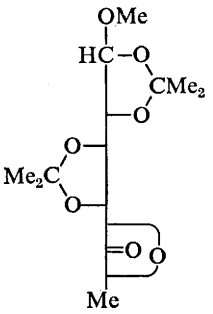
Acetylated and Benzoylated Derivatives of Sugars.—1,2,3,5-Tetra-*O*-acetyl-β-D-ribofuranose,[842] 3-*O*-acetyl-β-L-arabinofuranose 1,2,5-orthobenzoate,[843] (*Z*)-1-*O*-acetyl-2,3:4,5-di-*O*-isopropylidene-D-*erythro*- and -D-*threo*-pent-1-enitol,[439] 2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl chloride,[844] 2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl azide,[845] 1,3,4-tri-*O*-acetyl-2-deoxy-2-fluoro-α-D-xylopyranose,[846] 1,2,3,4-tetra-*O*-acetyl-α-D-lyxopyranose,[847] 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide,[848] 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-cyanoethylidene)-α-D-glucopyranose,[849] 3,4,6-tri-*O*-acetyl-β-D-mannopyranose 1,2-(methyl orthoacetate),[850] 2,3-di-*O*-acetyl-1,6-anhydro-β-D-galactopyranose,[851] 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl cyanide,[852] 1,2,3,4,6-penta-*O*-acetyl-α-D-idopyranose,[853] 1,4,5-tri-*O*-acetyl-2,3-*O*-isopropylidene-β-D-fructopyranose,[854] and 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl and 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl chlorides.[855]

Glycosides and Derivatives Thereof.—4-Nitrophenyl β-D-xylopyranoside,[856] methyl 3,4-*O*-isopropylidene-β-L-*erythro*-pentopyranosid-2-ulose,[857] dec-1-yl α-D-glucopyranoside (the hydrocarbon chains are fully extended and lie antiparallel, forming a hydrocarbon layer),[858] methyl 6-*O*-acetyl-β-D-glucopyranoside,[859] methyl α-D-glucopyranoside 4,6-phosphate (cyclohexylammonium salt),[235] methyl α-D-mannopyranoside 2,3:4,6-bis(ethylboronate),[267] methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-methylsulphinyl-α-D-glucopyranoside,[860] 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(*R*)-ethylene oxide,[861] methyl 6-*O*-acetyl-β-D-galactopyranoside,[862] methyl 3,4-*O*-(*exo*)-ethylidene-β-D-galactopyranoside,[863] ethyl 6-*O*-benzoyl-2,3,4-

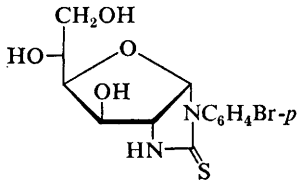
[837] F. Longchambon, D. Avenel, and A. Neuman, *Acta Cryst.*, 1976, **B32**, 1822.
[838] J. Ohanessian and H. Gillier-Pandraud, *Acta Cryst.*, 1976, **B32**, 2810.
[839] B. Sheldrick, *Acta Cryst.*, 1976, **B32**, 1016.
[840] W. J. Cook and C. E. Bugg, *Acta Cryst.*, 1976, **B32**, 656.
[841] K. Nimgirawath, V. J. James, and J. A. Mills, *J.C.S. Perkin II*, 1976, 349.
[842] B. J. Poppleton, *Acta Cryst.*, 1976, **B32**, 2702.
[843] L. G. Vorontsova, B. L. Tarnopol'skii, and Z. Sh. Safina, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 2258 (*Chem. Abs.*, 1976, **84**, 74 531e).
[844] P. Luger, G. Kothe, and H. Paulsen, *Chem. Ber.*, 1976, **109**, 1850.
[845] P. Luger and H. Paulsen, *Acta Cryst.*, 1976, **B32**, 2774.
[846] G. Kothe, P. Luger, and H. Paulsen, *Acta Cryst.*, 1976, **B32**, 2710.
[847] P. Herpin, R. Famery, J. Auge, and S. David, *Acta Cryst.*, 1976, **B32**, 215.
[848] M. Takai, H. Watanabe, J. Hayashi, and S. Watanabe, *Hokkaido Daigaku Kogakubu Kenkyu Hokoku*, 1976, **79**, 101 (*Chem. Abs.*, 1976, **85**, 85 882s).
[849] C. Foces-Foces, F. H. Cano, and S. García-Blanco, *Acta Cryst.*, 1976, **B32**, 3029.
[850] J. L. Flippen, *Cryst. Struct. Comm.*, 1976, **5**, 157.
[851] C. Foces-Foces, F. H. Cano, and S. García-Blanco, *Acta Cryst.*, 1976, **B32**, 427.
[852] C. Foces-Foces, F. H. Cano, and S. García-Blanco, *Acta Cryst.*, 1976, **B32**, 964.
[853] P. Luger and H. Paulsen, *Carbohydrate Res.*, 1976, **51**, 169.
[854] P. Köll and J. Kopf, *Chem. Ber.*, 1976, **109**, 3346.
[855] P. Herpin, R. Famery, J. Augé, S. David, and L. Guibé, *Acta Cryst.*, 1976, **B32**, 209.
[856] K. Harata, *Acta Cryst.*, 1976, **B32**, 1932.
[857] H. T. Palmer and R. A. Palmer, *Acta Cryst.*, 1976, **B32**, 377.
[858] P. C. Moews and J. R. Knox, *J. Amer. Chem. Soc.*, 1976, **98**, 6628.
[859] K. B. Lindberg, *Acta Cryst.*, 1976, **B32**, 642.
[860] K. B. Lindberg, *Acta Cryst.*, 1976, **B32**, 2017.
[861] A. D. Vasil'ev, V. I. Adrianov, V. I. Simonov, S. D. Shiyan, and M. L. Shul'man, *Bioorg. Khim.*, 1976, **2**, 601 (*Chem. Abs.*, 1976, **85**, 94 624m).
[862] K. B. Lindberg, *Acta Cryst.*, 1976, **B32**, 645.
[863] K. B. Lindberg, *Acta Cryst.*, 1976, **B32**, 639.

trideoxy-4-iodo-α-D-*threo*-hex-2-enopyranoside,[434] and 5,7-anhydro-8-deoxy-1(*S*),2:3,4-di-*O*-isopropylidene-1-*O*-methyl-D-*glycero*-D-*galacto*-octos-6-ulose (514).[864]

Amino-sugar Derivatives.—3-Amino-2,3,6-trideoxy-L-*lyxo*-hexose (daunosamine, in a structural investigation of carminomycin),[865] 4-amino-4-deoxy-D-glucose, 2-deoxystreptamine, and a 2,7-diamino-2,3,7-trideoxyoctadialdose (all components of apramycin),[535] 5-*O*-benzoyl-α-D-arabinofuranosylcarboxamide,[865a]



(514)



(515)

1-deoxy-2,3:4,6-di-*O*-isopropylidene-1-phthalimido-α-D-sorbofuranose,[866] 2-acetamido-2,3-dideoxy-D-*threo*-hex-2-enono-1,4-lactone,[867] and 1,4,6-tri-*O*-acetyl-2-(*N*-acetylacetamido)-2,3-dideoxy-α-D-*threo*-hex-2-enopyranose.[868]

The crystal structures of the following heterocyclic derivatives have also been reported: 1-(4-bromophenyl)-4,5-(1,2-*cis*-D-glucofurano)imidazolidine-2-thione (515),[869] 1-phenyl-4,5-(1,2-*cis*-D-glucofurano)imidazolidine-2-thione,[870] 1-(4-methoxyphenyl)-3-methyl-4-(D-*arabino*-tetrahydroxybutyl)imidazoline-2-thione,[871] and 4,5-(1,2-*cis*-D-ribofurano)-1,3-oxazolidone.[872]

Carboxylic Acid Derivatives.—Calcium D-glucarate,[873] D-glucaro-1,4-lactone,[874] calcium sodium α-D-galacturonate,[875] methyl (1,2,3,4-tetra-*O*-acetyl-β-D-galactopyranosyl)uronate,[876] calcium D-*arabino*-hexulopyranosonate,[877] the 2-*C*-benzyl derivative (326) from L-ascorbic acid (prepared by treating L-ascorbic acid with benzyl chloride and then with methanol),[505] and 2-(4-bromophenylhydrazono)-dehydro-L-ascorbic acid.[878]

[864] A. Ducruix, C. Pascard, S. David, and J.-C. Fischer, *J.C.S. Perkin II*, 1976, 1678.
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Di- and Tri-saccharides and their Derivatives.—β-Cellobiose octa-acetate,[879] phenyl α-maltoside and phenyl 6-deoxy-6-iodo-α-maltoside,[880] α-melibiose,[881–883] strontium 4-*O*-(4-deoxy-β-L-*threo*-hex-4-enopyranosyluronate)-α-D-galacturonate,[884] and melezitose.[885]

Nucleosides and their Derivatives and Related Compounds.—2′-*O*-Methyladenosine,[886] adenosine 5′-phosphate (an orthorhombic form)[887] and its barium salt,[736] 3-deaza-adenosine,[888] 2′,6-anhydro-1-β-D-arabinofuranosylcytosine,[889] 5,6-dihydroisocytidine,[890] 5,6-dihydro-2,4-dithiouridine,[891] 2,2′-anhydro-1-α-D-xylofuranosyluracil,[733] guanosine 5′-phosphate [the copper(II)[892] and cadmium salts[893]], xanthosine,[894] 6-methylthio-9-β-D-ribofuranosylpurine,[895] 6-benzylamino-9-β-D-ribofuranosylpurine,[896] 4-amino-5-β-D-ribofuranosyl-5-azaindole,[735] 2-(3,4-di-*O*-acetyl-2-deoxy-β-L-*erythro*-pentopyranosyl)-5,6-dimethylbenzotriazole,[897] and 4,5-bis(carboxymethyl)-3-(2,3-*O*-isopropylidene-β-D-erythrofuranosyl)-1-(4-nitrophenyl)pyrazole.[898]

Antibiotics.—Formycin B and 2-oxoformycin,[899] coformycin,[900] deaza-1-isotubercidin picrate,[901] apramycin,[535] and carminomycin.[865]

Ultrasonic Relaxation Measurements

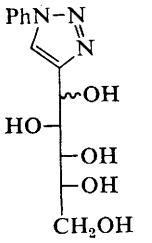
Ultrasonic absorption measurements have clearly indicated that adenosine 3′,5′-phosphate exists in aqueous or 7M-urea solutions (pH 8.0) as a rapidly equilibrating mixture of *syn* and *anti* conformers about the *N*-glycosidic bond.[902]

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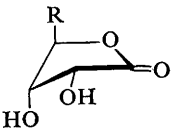
25
Polarimetry

Techniques have been developed for measuring the vacuum c.d. spectra (to 165 nm) of the individual anomers of monosaccharides (*e.g.* α- and β-D-glucopyranose, α- and β-D-galactopyranose, and α-D-xylopyranose) in deuterium oxide.[903] The results showed that it is not meaningful to compare the c.d. spectra of different sugars at equilibrium, even when the equilibrium mixtures contain roughly the same proportion of anomers, because one of the anomers may contribute more than the other to the intensity of the c.d. spectrum. Instead, the spectra of corresponding anomers should be compared (*i.e.* α-D-glucopyranose with α-D-galactopyranose) in relating the optical rotatory properties to the molecular structure. The vacuum c.d. spectra of aqueous solutions of twelve methyl aldopyranosides were also recorded, and, from difference spectra, it was possible to assign tentatively specific chromophores to the first three bands in the c.d. spectra of the methyl pyranosides at ~185 nm (due to the ring-oxygen atom), ~175 nm (due to the methoxy-group), and <165 nm (due, in part, to the methoxy-group).[904] The first band at ~180 nm in the c.d. spectra of pyranoses is apparently due to the ring-oxygen atom.

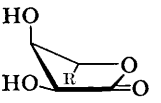
The optical rotatory behaviour of triazole derivatives having an unsubstituted sugar chain attached at C-4 [*e.g.* (516)] accords with the Generalized Heterocycle Rule – which relates the optical rotation of polyhydroxy derivatives of aromatic heterocycles with the stereochemistry of the CHOH group attached to the heterocycle – but the Rule does not hold for some of the derivatives having substituents on the sugar chain.[381] The c.d. spectrum of methyl 4,6-*O*-ethylidene-α-D-glucopyranoside in the presence of $Pr(dpm)_3$ exhibited a Cotton effect of opposite sign to that predicted (see Vol. 9, p. 190), showing that this method is not always reliable for assigning the chirality of glycols.[905]



(516)



(517) R = CH_2OH or HO– 



(518) R = 

[903] R. G. Nelson and W. C. Johnson, *J. Amer. Chem. Soc.*, 1976, **98**, 4290.
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The chiroptical properties of aldohexono- and aldopentono-1,4-lactones and some of their 2- and 3-deoxy derivatives have been discussed in terms of the conformation of the five-membered lactone ring.[906] It is possible to decide whether these γ-lactones exist in solution in an ^3E or E_3 conformation from c.d. measurements – for example, D-ribono- and D-allono-1,4-lactones prefer an E_3 conformation (517), while D-talono-1,4-lactone prefers an ^3E conformation (518).

The c.d. spectra of oligosaccharides composed of 2-acetamido-2-deoxy-D-glucopyranosyl and D-mannopyranosyl residues have been examined in an effort to correlate the spectral variations with the structures of the oligosaccharides.[907] The type of linkage between these monosaccharides was shown to affect the magnitude of the c.d. bands. Thus c.d. curves appear to be useful for determining the sequence and the location of *O*-glycosyl linkages of the various components of oligosaccharides, provided that one of the components is a 2-acetamido-2-deoxyhexose or *N*-acetylneuraminic acid.

An empirical rule that predicts the sign of the Cotton effect of glycosylated uracil, cytosine, thymine, adenine, and guanine has been developed.[908] The rule is not restricted to pentofuranosyl residues linked to N-1 of pyrimidines and to N-9 of purines, but can be applied to cyclic and acyclic sugars linked to any position on these bases. The c.d. spectra of the 'double-headed' nucleosides 5′-deoxy-5′-(indol-1-yl)- and 5′-deoxy-5′-(6-methylthiopurin-9-yl)-adenosine and -uridine have been reported,[909] and 3-deazapurine nucleosides have been shown to prefer a *syn* conformation about the *N*-glycosidic bond by comparison of their c.d. spectra with those of common nucleosides.[910]

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26
Separatory and Analytical Methods

Chromatographic Methods

The chromatography of carbohydrates and related compounds has been reviewed.[911]

Gas–Liquid Chromatography.—Phthalic esters – which are extensively used as plasticizers in the formulation of plastics used to make containers for solvents, *etc.* – have been found as contaminants during the analysis of polysaccharides by gas chromatography of the alditol acetate derivatives; for example, dibutyl phthalate behaves as a tetritol tetra-acetate on g.l.c. and di(2-ethylhexyl) phthalate has a retention time close to that for xylitol penta-acetate.[912] The contamination by phthalic esters can be minimized by distilling all solvents used in the analytical steps or by using smaller volumes of potentially contaminated solvents.

Mono-, di-, and tri-saccharides can be separated satisfactorily by g.l.c. of their trimethylsilylated oxime derivatives,[913] and an investigation has been conducted into the most selective stationary phases for the separation of mixtures of pentoses and hexoses as their TMS derivatives.[914] Traces of D-glucose and other related impurities in samples of D-glucitol can be detected by g.l.c. of the *O*-acetyl derivatives.[915]

The components of glycoproteins have been identified and determined by hydrolysis of the glycoprotein with trifluoroacetic acid, separation (by ion-exchange chromatography) and then reduction ($NaBH_4$) of the neutral- and amino-sugar fractions, and g.l.c. of the *O*-trifluoroacetylalditols.[916] Alternatively, the amino-sugars in such hydrolysates can be deaminated and determined by g.l.c. of the 2,5-anhydrohexononitrile acetates, while the neutral sugars are also determined as the aldonitrile acetates.[917]

Partially methylated derivatives of 2-deoxy-2-methylamino-D-glucose have been separated and identified as the corresponding alditol acetates by combined

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g.l.c.–m.s.[918] The TMS derivatives of methyl mono-, di-, and tri-*O*-benzyl-α-D-glucopyranosides have also been separated by g.l.c.[156] Although neither methyl 2,3- and 2,4-di-*O*-benzyl-α-D-glucopyranosides nor the corresponding 2,3,6- and 2,4,6-tri-*O*-benzyl ethers could be separated by g.l.c. of their TMS derivatives, the compositions of mixtures containing them can be determined (± 5%) from the intensities of 'characteristic' fragments observed in the mass spectra of the TMS derivatives. Iridoid and secoiridoid D-glucosides[919] and the free acids, sodium salts, and lactones of several hexuronic acids[920] have been examined as their TMS derivatives by g.l.c. The oximes[920] and aldonitrile acetates[920a] of hexuronic acids have been examined as alternative derivatives for g.l.c. D-Glucuronic acid conjugates of 1- and 2-naphthols and of 2-, 3-, and 4-hydroxybiphenyls have been separated by g.l.c. of the permethylated derivatives.[921]

Optimum reaction conditions for the silylation of nucleosides with bis(trimethylsilyl)trifluoroacetamide and the behaviour of the silylated nucleosides on g.l.c. have been investigated.[922]

Column and Ion-exchange Chromatography.—The separation of carbohydrates using column chromatography has been reviewed.[923]

The retention data for anion-exchange chromatography in tetraborate solution of alditols and deoxyalditols have been determined; the order of elution of 1-deoxyalditols can be related to chromatographic data for the corresponding aldonic acids.[924] Anion-exchange chromatography of borate complexes has also been used to separate amino-sugars and neutral sugars from complex mixtures of oligosaccharides,[925] and mono-, di-, and tri-*O*-methyl ethers of D-xylose, D-glucose, and D-mannose.[926] Separations of D-glucose from D-gluconic, D-glucaric, and D-*xylo*-hex-5-ulosonic acids,[927] of unsaturated oligohexuronides,[928] of the methyl ethers of methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides (obtained by partial methylation),[929] of the products obtained on hydrolysis of nucleic acids,[930] and of ribo- and deoxyribo-nucleoside 5′-mono-, 5′-di-, and 5′-triphosphates[931] have been accomplished by anion-exchange chromatography.

Paper Chromatography.—Steroidal D-glucuronides have been separated by reversed-phase partition chromatography on paper impregnated with organic

[918] T. Tai, K. Yamashita, and A. Kobata, *J. Biochem.* (*Japan*), 1975, **78**, 679.
[919] H. Inouye, K. Uobe, M. Hirai, Y. Masada, and K. Hashimoto, *J. Chromatog.*, 1976, **118**, 201.
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[920a] T. T. Gorovits and N. K. Abubakirov, *Khim. prirod. Soedinenii*, 1975, **11**, 523 (*Chem. Abs.*, 1976, **84**, 12 094f).
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[922] C. W. Gehrke and A. B. Patel, *J. Chromatog.*, 1976, **123**, 335.
[923] K. Capek and J. Stanek, jun., *J. Chromatog. Library*, 1975, **3**, 465 (*Chem. Abs.*, 1976, **84**, 12 066y).
[924] K. Larsson and O. Samuelson, *Carbohydrate Res.*, 1976, **50**, 1.
[925] V. A. Derevitskaya, N. P. Arbatskii, and N. K. Kochetkov, *Doklady Akad. Nauk S.S.S.R.*, 1975, **223**, 1137 (*Chem. Abs.*, 1976, **84**, 31 320t).
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[928] B. A. Davé, R. H. Vaughn, and I. B. Patel, *J. Chromatog.*, 1976, **116**, 395.
[929] A. K. Allen, R. C. Davies, A. Neuberger, and D. M. L. Morgan, *Carbohydrate Res.*, 1976, **51**, 149.
[930] T. Uematsu and R. J. Suhadolnik, *J. Chromatog.*, 1976, **123**, 347.
[931] J. X. Khym, *J. Chromatog.*, 1976, **124**, 415.

amines or quaternary ammonium salts and irrigated with a solution of potassium chloride.[932]

Thin-layer Chromatography.—A useful discussion of the factors (*e.g.* solvents and sorbents) affecting the t.l.c. of carbohydrates has appeared.[933]

T.l.c. on cellulose has been used to separate mixtures of monosaccharides.[934] Other separations accomplished by t.l.c. include those of the steroidal glycosides (saponins) from *Agave americana* L.,[935] [^{3}H]inositol and [^{3}H]inositol monophosphates (on sheets of silica gel–glass fibre),[936] purine nucleoside 3′,5′-phosphates from their respective nucleoside 5′-phosphates and nucleosides [on plastic-backed sheets of (polyethyleneimine)cellulose],[937] 2′-deoxythymidine and the corresponding 5′-mono-, 5′-di-, and 5′-tri-phosphates [on plastic-backed sheets of (polyethyleneimine)cellulose],[938] 2′-deoxy-5-halogenouridines,[939] and erythromycin stearate and allied antibiotics.[940]

High-pressure Liquid Chromatography.—The following separations have been achieved using high-pressure (-performance) liquid chromatography: common sugars (on microparticulate silica [941] and on silica having a permanently bonded polar phase attached),[942, 943] mono- and di-saccharides (on anion-exchange resins) [944, 945] and their benzoylated derivatives,[946] D-gluco-oligosaccharides,[947] the products of acetolysis of D-mannans,[948] bleomycin,[949] kanamycins A and B,[950] digitalis glycosides,[951] and, using reversed-phase partitioning, steroidal D-glucuronides,[952] and ATP, ADP, adenosine, and other nucleosides.[953, 954]

Other Analytical Methods

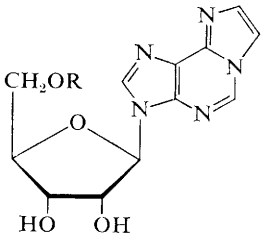
Aldoses reacted with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole in alkaline solution to give, after oxidation with hydrogen peroxide, stable 3-monosubstituted mercaptotetrazine derivatives that are violet, and which absorb at

[932] R. Mattox, R. D. Litwiller, J. E. Goodrich, and W. C. Tan, *J. Chromatog.*, 1976, **120**, 435.
[933] M. Ghebregzabher, S. Rufini, B. Monaldi, and M. Lato, *J. Chromatog.*, 1976, **127**, 133.
[934] M. Hoton-Dorge, *J. Chromatog.*, 1976, **116**, 417.
[935] B. Wilkomirski, W. Bobeyko, and P. Kintia, *J. Chromatog.*, 1976, **116**, 482.
[936] M. Hokin-Neaverson and K. Sadeghian, *J. Chromatog.*, 1976, **120**, 502.
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[938] P. S. Fitt, P. I. Peterkin, and V. L. Grey, *J. Chromatog.*, 1976, **124**, 137.
[939] P. G. Olafsson, A. M. Bryan, and K. Lau, *J. Chromatog.*, 1976, **124**, 388.
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[941] J. L. Rocca and A. Rouchouse, *J. Chromatog.*, 1976, **117**, 216.
[942] R. Schwarzenbach, *J. Chromatog.*, 1976, **117**, 206.
[943] F. M. Rabel, A. G. Caputo, and E. T. Butts, *J. Chromatog.*, 1976, **126**, 731.
[944] W. Voelter and H. Bauer, *J. Chromatog.*, 1976, **126**, 693.
[945] J. G. Lawrence, *Scan*, 1974, **5**, 19 (*Chem. Abs.*, 1976, **85**, 71 766v).
[946] J. Lehrfeld, *J. Chromatog.*, 1976, **120**, 141.
[947] J. A. Boundy, K. L. Smily, C. L. Swanson, and B. T. Hofrieter, *Carbohydrate Res.*, 1976, **48**, 239.
[948] F. R. Seymour, M. E. Slodki, R. D. Plattner, and R. M. Stodola, *Carbohydrate Res.*, 1976, **48**, 225.
[949] W. J. Rzeszotarski, W. C. Eckelman, and R. C. Reba, *J. Chromatog.*, 1976, **124**, 88.
[950] D. L. Mays, R. J. van Apeldoorn, and R. G. Lauback, *J. Chromatog.*, 1976, **120**, 93.
[951] F. Nachtmann, H. Spitzy, and R. W. Frei, *J. Chromatog.*, 1976, **122**, 293.
[952] M. Lafosse, G. Kéravis, and M. H. Durand, *J. Chromatog.*, 1976, **118**, 283.
[953] F. S. Anderson and R. C. Murphy, *J. Chromatog.*, 1976, **121**, 251.
[954] R. A. Hartwick and P. R. Brown, *J. Chromatog.*, 1976, **126**, 679.

520—555 nm.[955] The molar absorptivity decreases linearly, without significant change in λ_{max}, as the chain-length of the aldose increases, so that it is possible to determine the number of carbon atoms in simple aldoses and also to distinguish between aldoses and ketoses, which yield 3-disubstituted mercaptotetrazines having appreciably higher molar absorptivities than the 3-monosubstituted mercaptotetrazines obtained from the isomeric aldoses. 4-Hydroxybenzoylhydrazine has been used as the colour reagent in determinations of the available carbohydrates in foodstuffs [956] and partially methylated D-glucoses and other water-soluble ethers of D-glucose.[957]

2-Aminobiphenyl in acetic and phosphoric acids has been recommended as a spray reagent for detecting mono- and oligo-saccharides following t.l.c. on silica gel.[958] Aldopentoses in solution can be determined spectrophotometrically (λ_{max} 540 nm) after heating with 4-aminobenzoic acid, thiourea, and acetic acid, without serious interference from hexoses, ketoses, and alditols.[959] D-Arabinose and L-fucose have been determined in the presence of other pentoses and hexoses and alditols by an enzymic procedure using D-arabinose (L-fucose) dehydrogenase,[960] and a spectrophotometric method for the quantitative measurement of L-rhamnose is based on its reaction with 4-hydroxybiphenyl in the presence of Cu^{2+} ions, although L-fucose and lactic acid interfere with the determination.[961]

A potentially useful method for determining the position of the glycosidic linkage in plant glycosides (*e.g.* 3-β-D-xylopyranosylcimigineol) involves oxidation of the sugar residue with periodate in the presence of cyclohexylamine and acetic acid.[962] The resulting formates (*e.g.* cimigineol 3-formate) contain C-1 of the original sugar residue. D-Gluconic and lactobionic acids in pharmaceutical preparations have been measured by a fluorometric method that is based on oxidation of the acids with lead tetra-acetate and reaction of the oxidized products with dichlorofluorescein.[963]



(519) R = H, PO_3H_2, *etc.*

[955] E. Humeres, F. Nome, and R. Aguirre, *Carbohydrate Res.*, 1976, **46**, 284.
[956] G. J. Hudson, P. M. V. John, B. S. Bailey, and D. A. T. Southgate, *J. Sci. Food Agric.*, 1976, **27**, 681.
[957] D. W. Leedy, *Carbohydrate Res.*, 1976, **47**, 337.
[958] A. Lombard and M. L. Tourn, *Atti Accad. Sci. Torino, Rend. Classe Sci. fis. mat. nat.*, 1974, 319 (*Chem. Abs.*, 1976, **85**, 13 484r).
[959] A. Nanba and Y. Matsuo, *Hiroshima Daigaku Kogakubu Kenkyu Hokoku*, 1976, **24**, 11 (*Chem. Abs.*, 1976, **85**, 71 808k).
[960] K. Yamanaka, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2227.
[961] O. Hadzija and M. Tonkovic, *Mikrochim. Acta*, 1976, **2**, 49 (*Chem. Abs.*, 1976, **85**, 108 904s).
[962] M. Nagai, N. Sakurai, T. Inoue, and K. Kawai, *Yakugaku Zasshi*, 1975, **95**, 1350 (*Chem. Abs.*, 1976, **84**, 74 572n).
[963] G. Gübitz, R. W. Frei, and H. Bethke, *J. Chromatog.*, 1976, **117**, 337.

The reactivity of 5-acetamido-3,5-dideoxy-L-*arabino*-2-heptulopyranosonic acid (a seven-carbon analogue of *N*-acetylneuraminic acid) in the resorcinol and thiobarbituric acid procedures – which are commonly used for the determination of sialic acids – has been evaluated.[348] The chemistry involved in the formation of the characteristic chromophores in these assays was also discussed.

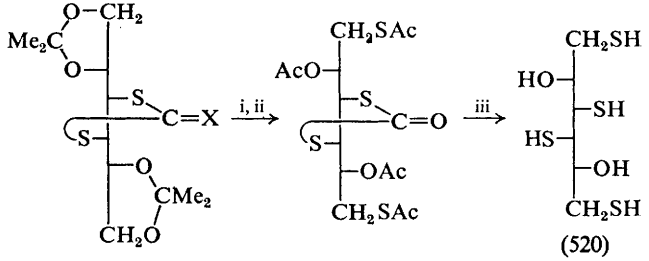
A fluorometric determination of adenosine, AMP, ATP, and adenosine 3′,5′-phosphate, following their separation by high-pressure liquid chromatography, relies on their conversion into fluorescent 1,N^6-dietheno derivatives (519) on heating with monochloroacetaldehyde.[964]

[964] M. Yoshioka and Z. Tamura, *J. Chromatog.*, 1976, **123**, 220.

27
Alditols

Fractionation of extracts of *Convolvulus glomeratus* afforded a new branched-chain alditol, 2-*C*-methyl-D-erythritol, whose constitution and absolute configuration were deduced by ^{1}H and ^{13}C n.m.r. spectroscopy, c.d. measurements, and synthesis.[965, 966]

1,3,4,6-Tetrathio-D-iditol (520) has been synthesized from 1,2:5,6-di-*O*-isopropylidene-3,4-dithio-D-iditol 3,4-dithiocarbonate or the corresponding 3,4-trithiocarbonate by the sequence of reactions shown in Scheme 99.[967]



(520)

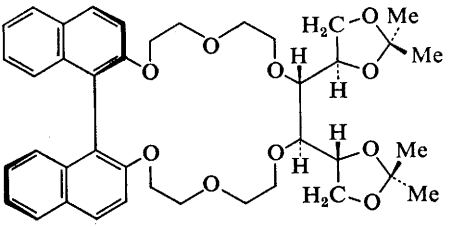
X = O or S

Reagents: i, HBr–AcOH; ii, MeCOSK–Me_2CO; iii, $LiAlH_4$

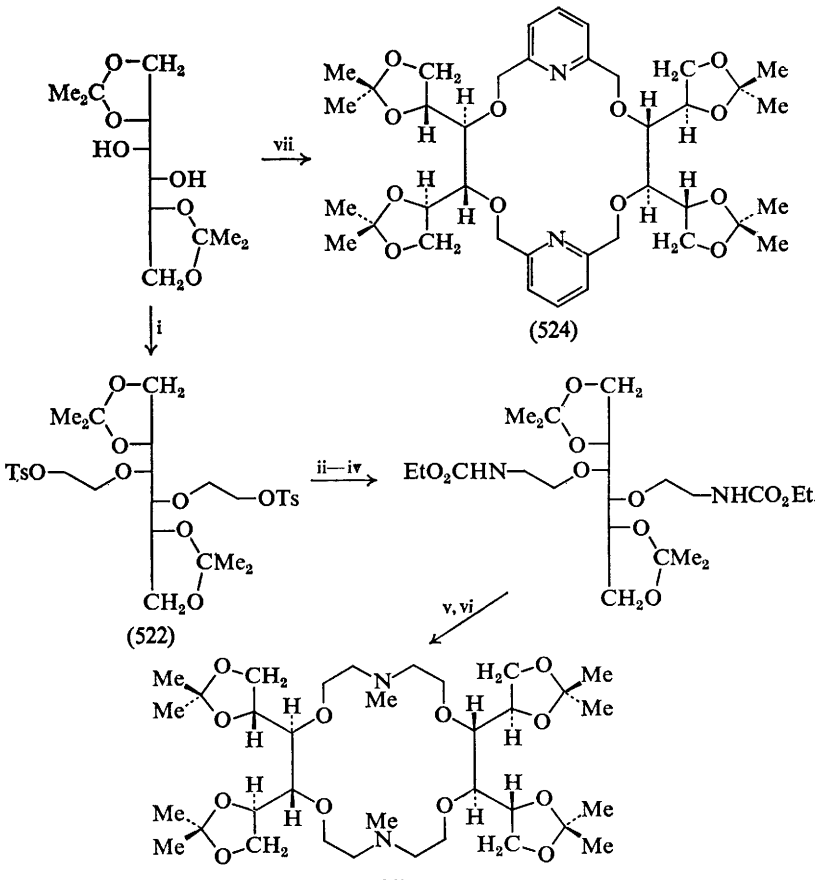
Scheme 99

The chiral macrocyclic polyether (*R*)-D-(521) and its (*S*)-D-diastereoisomer have been derived from D-mannitol and (*R*)- or (*S*)-binaphthol, respectively.[968] Significant changes were observed in the ^{1}H n.m.r. spectra of (*R*)-D-(521) and its diastereoisomer in the presence of primary alkylammonium salts [*e.g.* (+)-(*R*)-, (−)-(*S*)-, and (±)-(*RS*)-α-phenylethylammonium hexafluorophosphate], indicating that both diastereoisomers act as hosts to suitable guest molecules. The chiral hosts DD-(523) and DD-(524) have been obtained from 1,2:5,6-di-*O*-isopropylidene-D-mannitol by the routes outlined in Scheme 100.[969] The temperature dependence of the ^{1}H n.m.r. spectrum of the 1:1 complex of DD-(524) and benzylammonium thiocyanate in [$^{2}H_2$]dichloromethane was interpreted

[965] T. Anthonsen, S. Hagen, M. A. Kazi, S. W. Shah, and S. Tagar, *Acta Chem. Scand.* (*B*), 1976, **30**, 91.
[966] S. W. Shah, S. Brandänge, D. Behr, J. Dahmén, S. Hagen, and T. Anthonsen, *Acta Chem. Scand.* (*B*), 1976, **30**, 903.
[967] G. E. McCasland, A. B. Zanlungo, and L. J. Durham, *J. Org. Chem.*, 1976, **41**, 1125.
[968] W. D. Curtis, R. M. King, J. F. Stoddart, and G. H. Jones, *J.C.S. Chem. Comm.*, 1976, 284
[969] D. A. Laidler and J. F. Stoddart, *J.C.S. Chem. Comm.*, 1976, 979.



(521)



(522) (523) (524)

Reagents: i, see Vol. 9, p. 198; ii, NaN_3–DMF; iii, H_2–Pt–C; iv, $EtCO_2Cl$–NaOH; v, (522)–DMSO; vi, $LiAlH_4$; vii, 2,6-bis(bromomethyl)pyridine–DMSO–NaH

Scheme 100

in terms of slow dissociation of the complex, which renders the isopropylidene groups on one side of the host diastereotopic in relation to those on the other side in a face-to-face complex.

The volume distribution coefficients of alditols and deoxyalditols on anion-exchange chromatography in tetraborate solution have been measured and shown to increase with increasing stability and charge of the borate complex.[924] Vicinal hydroxy-groups in a *gauche* arrangement formed strong complexes with borate, provided that the steric conditions are otherwise favourable. Xylitol has been shown to react with boric acid under reduced pressure to give mixtures of boric esters and polymeric xylitol–borate complexes.[271]

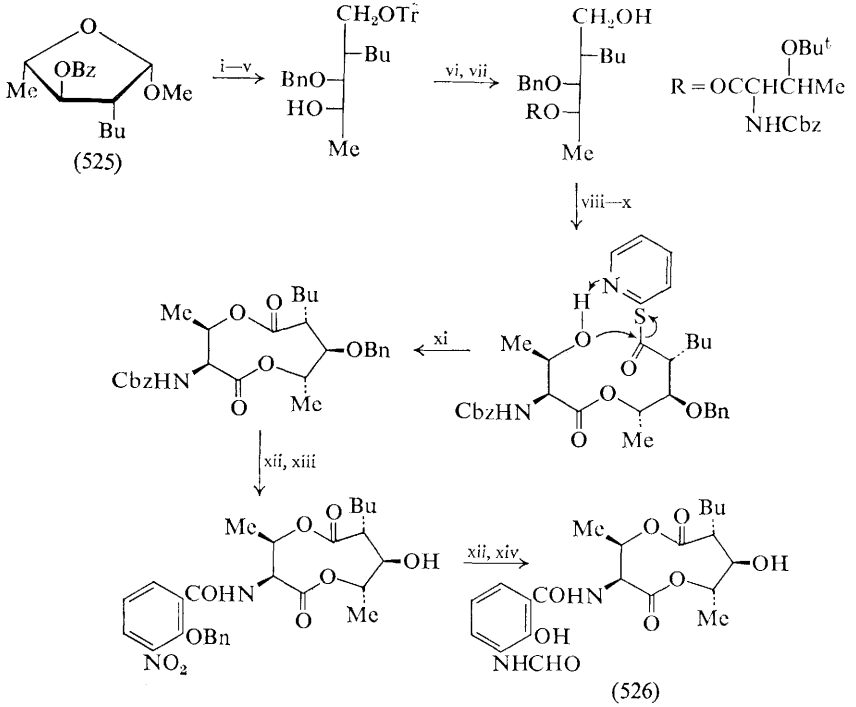
The complete degradation of alditols into formic acid with alkaline hydrogen peroxide and a ferrous salt is referred to in Chapter 22,[741] and the determination of pentitols by a procedure involving periodate oxidation is also mentioned in that chapter.[754]

28

The Synthesis of Optically Active Non-carbohydrate Compounds

The use of 2,5-dicarbonyl sugars in the synthesis of heterocyclic compounds has been reviewed.[970]

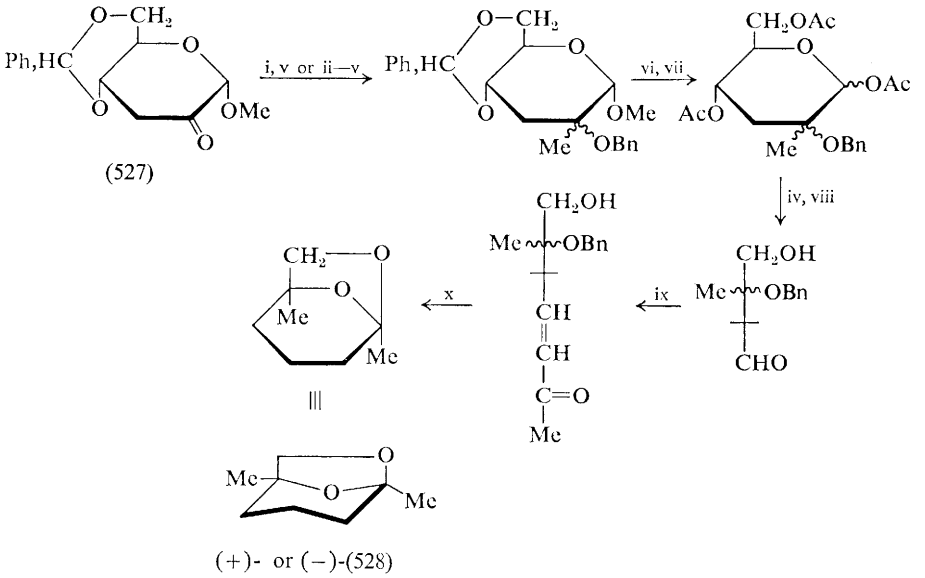
The branched-chain sugar methyl 3-*O*-benzoyl-2-butyl-2,5-dideoxy-β-L-arabinofuranoside (525) (see Vol. 9, p. 203) has been transformed into deisovalerylblastmycin (526) (Scheme 101).[971]



Reagents: i, MeONa; ii, BnBr–NaH–THF; iii, H_3O^+; iv, $NaBH_4$; v, TrCl–py; vi, ROH–DCC; vii, 90% AcOH; viii, CrO_3; ix, CF_3CO_2H; x, 2,2′-dithiodipyridine–Ph_3P–PhH; xi, $AgClO_4$–PhH; xii, H_2–Pd–C; xiii, $OCCH_2CH_2CONO_2CC_6H_3(OBn)NO_2$; xiv, p-$NO_2C_6H_4OCHO$

Scheme 101

[970] K. Imada, *Kagaku To Yakugaku No Kyoshitsu*, 1975, **46**, 12 (*Chem. Abs.*, 1976, **84**, 105 918t).
[971] S. Aburaki and M. Kinoshita, *Chem. Letters*, 1976, 701.

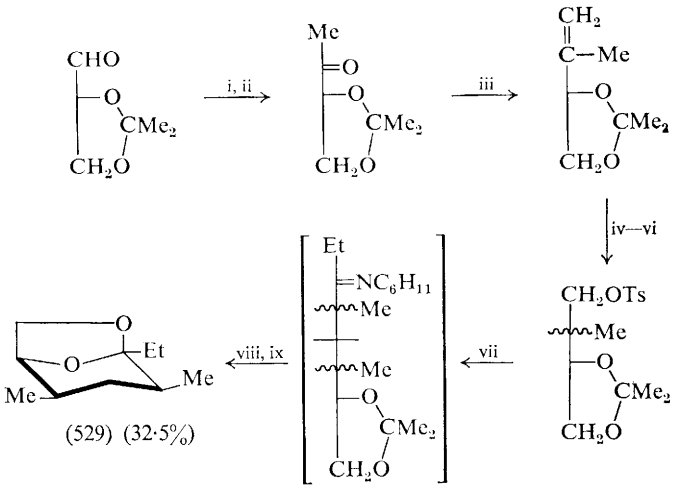


(527)

(+)- or (−)-(528)

Reagents: i, MeMgI; ii, $Ph_3P{=}CH_2$; iii, $Hg(OAc)_2$; iv, $NaBH_4$; v, BnCl–NaH–DMF; vi, H^+; vii, Ac_2O–BF_3,Et_2O; viii, $NaIO_4$; ix, $Ph_3P{=}CHCOMe$; x, H_2–Pd–C

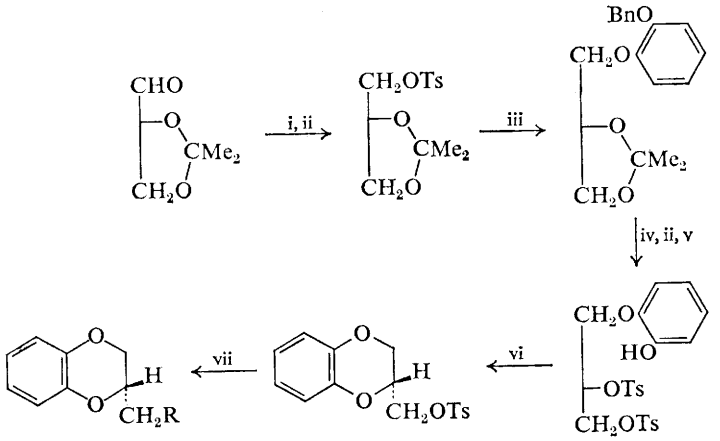
Scheme 102



(529) (32·5%)

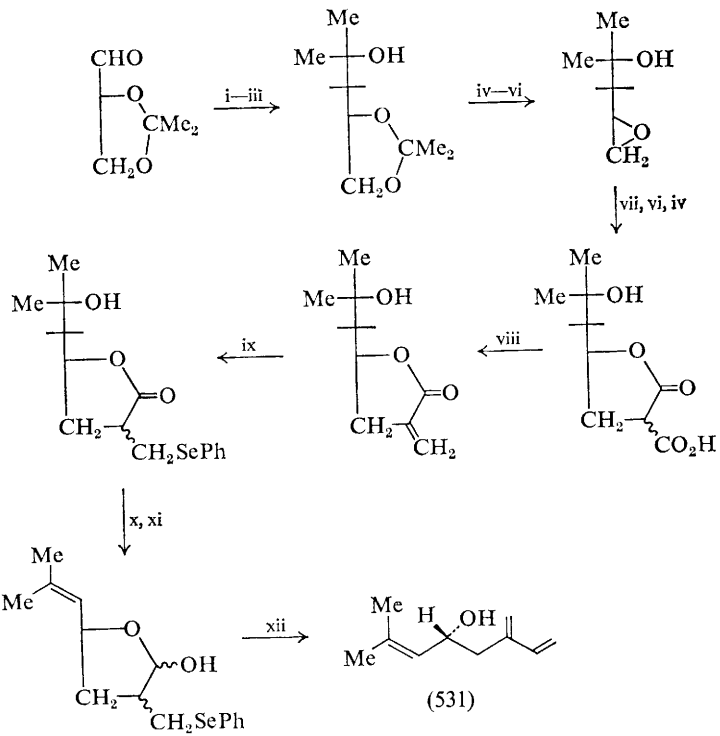
Reagents: i, MeMgI–Et_2O; ii, CrO_3–Me_2CO; iii, $Ph_3P{=}CH_2$; iv, B_2H_6–THF; v, H_2O_2–NaOH; vi, TsCl–py; vii, $[C_6H_{11}N{=}CEtCHMe]^-$; viii, dil. HCl; ix, g.l.c.

Scheme 103



Reagents: i, $NaBH_4$; ii, TsCl–py; iii, *o*-$BnOC_6H_4OH$–MeONa; iv, H_3O^+; v, H_2–Pd–C; vi, MeONa; vii, Et_2NH or piperidine

Scheme 104



Reagents: i, $Ph_3P{=}CMe_2$; ii, $Hg(OAc)_2$; iii, $NaBH_4$; iv, H_3O^+; v, TsCl (1 mol)–py; vi, aq. KOH; vii, $CH_2(CO_2Et)_2$–EtONa; viii, HCHO–Et_2NH; ix, PhSeH; x, $POCl_3$–py; xi, $Bu^i{}_2AlH$–THF; xii, $Ph_3\overset{+}{P}Me\ Br^-$–NaH–DMSO (+retro-Michael reaction)

Scheme 105

Methyl 4,6-*O*-benzylidene-3-deoxy-α-D-*erythro*-hexopyranosidulose (527) has been converted into (*S*)-(−)-frontalin (528) – a pheromone of several species of beetles belonging to the genus *Dendroctonus* – and its (*R*)-(+)-enantiomer by the reactions outlined in Scheme 102.[972] (−)-α-Multistriatin (529), the pheromone of the smaller European elm-bark beetle (*Scolytus multistriatus* Marsham), has been synthesized from 2,3-*O*-isopropylidene-D-glyceraldehyde (derived from D-mannitol) by the route shown in Scheme 103; the absolute stereochemistry of the pheromone was thereby established as 1(*S*):2(*R*):4(*S*):5(*R*).[973] 2,3-*O*-Isopropylidene-D-glyceraldehyde has also been used as the starting material in syntheses of such *NN*-dialkylaminomethylbenzodioxans as prosymal (530; R = NEt_2) and piperoxan (530; R = piperidinyl) (Scheme 104), which are competitive antagonists of α-adrenergic receptors,[974] and (*R*)-(−)-ipsdienol (531) (Scheme 105), which is enantiomeric with one of the pheromones of the bark beetle *Ips paraconfusus* Lanier.[975]

[972] D. R. Hicks and B. Fraser-Reid, *J.C.S. Chem. Comm.*, 1976, 869.
[973] K. Mori, *Tetrahedron*, 1976, **32**, 1979.
[974] W. L. Nelson and J. E. Wennerstrom, *J.C.S. Chem. Comm.*, 1976, 921.
[975] K. Mori, *Tetrahedron Letters*, 1976, 1609.

Part II

MACROMOLECULES

1
Introduction

The objectives and format of Part II remain the same as those of previous Reports.

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2
General Methods

BY R. J. STURGEON

Analysis

A data-handling system that is able to perform all the functions required in processing the data from amino-acid and carbohydrate analyses by an on-line technique has been reported.[1] This technique removes the need to store data in an intermediate form, and good agreement was observed between automatically and manually computed values derived from the same chromatogram.

Submicrogram amounts of an acid polysaccharide have been detected using techniques including rocket immunoelectrophoresis and rocket affinoelectrophoresis.[2] Glycoproteins containing non-reducing, terminal residues of either D-glucose or D-mannose have been separated by electrophoresis on polyacrylamide gel, prior to their detection using a concanavalin A-peroxidase labelling technique.[3] Concanavalin A binds to the glycoprotein in the gel and the free binding sites on the lectin can then bind to horseradish peroxidase, which is visualized by reaction with diaminobenzidine. Brain D-galactosylceramides have been separated by high-performance liquid chromatography (h.p.l.c.) of their fully benzoylated derivatives.[4] Neutral glycolipids derived from human erythrocytes have been separated by h.p.l.c. on columns of totally porous silica beads.[5] Chemical analyses demonstrated that ceramide di- and tri-hexosides were each separated into two fractions, due to differences in the compositions of the fatty acids and the long-chain bases. Ascending dry-column chromatography has been used in the preparation of glycolipids.[6] Brain gangliosides, G_{M_1}, $G_{D_{1a}}$, $G_{D_{1b}}$, and G_{T_1}, have been isolated in good yield after high-resolution chromatography on columns of either DEAE-Sephadex or porous silica beads.[7]

Two new ion-exchange polysaccharides have been developed for use in the fractionation of polysaccharides and other biopolymers.[8] Pectins from the bark of *Salix alba* were separated on columns of *O*-(3-diethylamino-2-hydroxypropyl)starch (DEAHP-starch) into six components differing in uronic acid content and in molecular size. The corresponding cellulose derivative (DEAHP-cellulose) was used in the fractionation of glucomannans from the same source. The methodology and the instrumentation used in ion-exchange chromatography

[1] J. E. Fox and J. M. Wilkinson, *Analyt. Biochem.*, 1976, **76**, 387.
[2] P. Owen and M. R. J. Salton, *Analyt. Biochem.*, 1976, **73**, 20.
[3] J. G. Wood and F. O. Sarinana, *Analyt. Biochem.*, 1975, **69**, 320.
[4] R. H. McCluer and J. E. Evans, *J. Lipid Res.*, 1976, **17**, 412.
[5] S. Ando, M. Isobe, and Y. Nagai, *Biochim. Biophys. Acta*, 1976, **424**, 98.
[6] C. V. Viswanathan and A. Hayashi, *J. Chromatog.*, 1976, **123**, 243.
[7] T. Momoi, S. Ando, and Y. Magai, *Biochim. Biophys. Acta*, 1976, **441**, 488.
[8] M. Antal and R. Toman, *J. Chromatog.*, 1976, **123**, 434.

of carbohydrates have been reviewed.[9] A review of the t.l.c. of carbohydrates includes details of the separations of many mono- and oligo-saccharides, as well as derivatives of oligosaccharides, on a wide range of solid supports.[10] Carbohydrates have been separated on silica gel coated with a bonded polar phase; acetonitrile–water was used as the mobile phase, but the selectivity of the separations of mono-, di-, and tri-saccharides can be affected by the addition of acids or salts.[11] The effects of operational parameters—for example, solute concentration, column length, flow rate, and temperature—upon the gel-chromatographic separation of oligosaccharides of D-glucose have been examined.[12] A linear relationship is observed between the peak-height and the concentration of saccharides up to heptasaccharides. Mono- and di-saccharides in complex mixtures of carbohydrates have been separated as their fully benzoylated derivatives by h.p.l.c.[13]

Separations of mono- and di-saccharides on ion-exchange resins have been reviewed.[14] Parameters for the elution of D-glucose from ion-exchange columns by alcohol–water mixtures[15] and automated procedures for the separation of monosaccharides on ion-exchange resins[16] are included in a collection of historic publications on the ion-exchange chromatography of carbohydrates. Retention values for numerous alditols and deoxyalditols have been determined in tetraborate solution.[17] The order of elution of 1-deoxyalditols was correlated with the chromatographic behaviour of the corresponding aldonic acid. The distribution coefficient for all types of polyhydroxy-compound examined increased with increasing stability and charge of the borate complex. Vicinal hydroxy-groups in a *gauche* conformation form strong complexes, provided that the steric conditions are otherwise favourable. Mono- and oligo-saccharides have been separated by t.l.c. on cellulose.[18] The sugars were detected by a sensitive reagent consisting of aminohippuric and thiobarbituric acids. Two columns with different exclusion limits have been used in the separation of oligosaccharides, containing up to fifteen residues of D-glucose, by low-pressure chromatography.[19]

Membrane-bound sugars and amino-sugars have been determined as their alditol acetates by a procedure involving the use of t.l.c., g.l.c., and radio-gas chromatography.[20] Losses occurring during the release of sugars from the membrane and in the preparation of their volatile derivatives were assessed by comparison with those of added, radio-labelled internal standards. The carbohydrate contents of glycoproteins have been measured using a spectrophoto-

[9] J. F. Kennedy, *Biochem. Soc. Trans.*, 1974, **2**, 54.
[10] M. Ghebregzabher, S. Rufini, B. Monaldi, and M. Lato, *J. Chromatog.*, 1976, **127**, 133.
[11] F. M. Rabel, A. G. Caputo, and E. T. Butts, *J. Chromatog.*, 1976, **126**, 731.
[12] N. K. Sabbagh and I. S. Fagerson, *J. Chromatog.*, 1976, **120**, 55.
[13] J. Lehrfeld, *J. Chromatog.*, 1976, **120**, 141.
[14] J. G. Lawrence, *Chimia*, 1975, **29**, 367.
[15] H. Rückert and O. Samuelson, in 'Ion Exchange Chromatography', ed. H. F. Walton, Dowden, Hutchinson, and Ross, Pennsylvania, 1976, p. 370.
[16] K. Larsson and O. Samuelson, in ref. 15, p. 382.
[17] K. Larsson and O. Samuelson, *Carbohydrate Res.*, 1976, **50**, 1.
[18] M. Hoton-Dorge, *J. Chromatog.*, 1976, **116**, 417.
[19] A. W. Wight, *Stärke*, 1976, **28**, 311.
[20] J. Stadler, *Analyt. Biochem.*, 1976, **74**, 62.

metric method based on the alkaline ferricyanide reaction; the method is accurate in the 1—25 nmole range.[21] The use of a simple, two-step, ion-exchange procedure enabled the total sugar content, and also the proportion of neutral sugars and amino-sugars, to be measured. Amino-sugar components of glycoproteins have been estimated using an amino-acid analyser; since mild acid conditions were used to hydrolyse the glycoproteins, liberated peptides and tryptophan or its degradation products were removed by ion-exchange chromatography before the amino-sugars were determined.[22] Ion-exchange chromatography has been used in the separation and identification of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose.[23] Conditions for differential and total analyses of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and hexosaminitols, using an amino-acid analyser, have been summarized.[24] An improved method has been reported for the quantitative determination of amino-sugars by the Elson–Morgan reaction.[25] In order to obtain conclusive identification of the various peaks appearing during the g.l.c. of monosaccharides, TMS derivatives of the methyl glycosides have been investigated by both g.l.c.–m.s. and ^{1}H n.m.r. spectroscopy.[26] The resulting information permits unambiguous interpretation of the gas chromatograms obtained in the application of this method to the analysis of glycoproteins and glycopeptides. The use of capillary columns in the g.l.c. analysis of monosaccharides has been described.[27] TMS derivatives of isomeric sugars were well resolved.

Phthalic esters – which are used extensively as plasticizers and which are sometimes found in laboratory solvents and equipment – have been shown to have retention times on g.l.c. similar to those of some alditol acetates.[28] Least contamination by phthalic esters was obtained after distillation of the solvents used in any analytical steps. Aldononitrile acetates and alditol acetates derived from free aldoses and alditols, respectively, in cataractous human-lens tissue have been separated on open-tubular capillary columns coated with a non-polar phase containing dispersed particles of silanized silicic acid.[29] A system has been described for the separation of complex mixtures of carbohydrates by h.p.l.c.; its efficiency depends on the use of anion-exchange columns and a specially designed gradient chamber.[30] An analytical procedure has been developed for the separation of D-glucose and D-mannose on cation-exchange resins.[31] The use of an acid-resistant reagent pump has enabled hexoses and pentoses to be determined, with a sensitivity of 10^{-10} mol, by anion-exchange chromatography.[32, 33] An aminoalkyl-substituted silica gel has been used in the separation

[21] G. Krystal and A. F. Graham, *Analyt. Biochem.*, 1976, **70**, 336.
[22] T. H. Plummer, *Analyt. Biochem.*, 1976, **73**, 532.
[23] K. Murayama, N. Shindo, and H. Koide, *Analyt. Biochem.*, 1976, **70**, 537.
[24] F. Downs and W. Pigman, *Methods in Carbohydrate Chem.*, 1976, **7**, 244.
[25] H. M. van de Loo, *Analyt. Biochem.*, 1976, **76**, 556.
[26] J. P. Kamerling, G. J. Gerwig, J. F. G. Vliegenthart, and J. R. Clamp, *Biochem. J.*, 1975, **151**, 491.
[27] N. Swaminathan, B. Apon, and F. Aladjem, *Analyt. Biochem.*, 1976, **75**, 646.
[28] W. F. Dudman and C. P. Whittle, *Carbohydrate Res.*, 1976, **46**, 267.
[29] C. D. Pfaffenberger, J. Szafranek, and E. C. Horning, *J. Chromatog.*, 1976, **126**, 535.
[30] W. Voelter and H. Bauer, *J. Chromatog.*, 1976, **126**, 693.
[31] J. Thomas and L. H. Lobel, *Analyt. Biochem.*, 1976, **73**, 222.
[32] W. H. Morrison, M. F. Lou, and P. B. Hamilton, *Analyt. Biochem.*, 1976, **71**, 415.
[33] W. H. Morrison, M. F. Lou, and P. B. Hamilton, *Analyt. Biochem.*, 1976, **76**, 657.

of mono- and oligo-saccharides by liquid chromatography.[34] A comparison has been made of the separations of methyl ethers of D-glucose, D-xylose, D-galactose' and D-mannose by paper chromatography, t.l.c., and liquid chromatography.[35] One of the advantages of separating methyl ethers by liquid chromatography is that the coefficient of variation of the retention time for individual sugars does not vary very much over several months. Mixtures of D-glucose, D-galactose, and D-mannose have been separated by t.l.c. on activated silica gel, and the individual hexoses were estimated by densitometry, following treatment with ceric sulphate.[36] A general, automated ^{13}C n.m.r. procedure has been developed both for rapid qualitative and quantitative analyses of simple sugars, and the results were compared with those obtained using such techniques as g.l.c.[37]

An automated procedure reported for the determination of monosaccharides uses 4-hydroxybenzoic acid hydrazide as the reagent.[38] This reagent is less hazardous than the traditional one of orcinol–sulphuric acid. In the reaction of 3-aminophenol with sugars in the thiourea–acetic acid system, the addition of borate ions suppressed the formation of chromogens with pentoses and hexoses, but enhanced that with disaccharides.[39] The relatively greater intensity of the chromogen formed in the reaction of 3-aminophenol with maltose was used in an assay of serum amylase, and the results compared favourably with those using dyed starches. A method has been described for the radiochromatographic analysis of [^3H]alditols obtained by reduction of aldoses with sodium borotritide.[40]

A modified procedure, using D-glucose oxidase-guaiacum, has been recommended for the measurement of D-glucose in plasma.[41] Interference in the estimation of D-glucose by the D-glucose oxidase–peroxidase system has been noted with 4-hydroxyacetanilide, 4-aminophenol, and oxyphenylbutazone, but not with structurally related compounds (for example, acetanilide, aniline, and phenylbutazone) that lack a phenolic hydroxy-group.[42] None of these compounds interfered with the measurement of D-glucose using hexokinase-D–glucose 6-phosphate dehydrogenase. The particular procedure used for deproteinization of blood, prior to the estimation of D-glucose with D-glucose oxidase, has been shown to be important.[43] When deproteinization was carried out using perchloric or trichloroacetic acids, substances that oxidized the chromogen in the presence of peroxidase were solubilized, resulting in abnormally high values. This type of interference is not encountered when protein is removed by means of solutions of barium hydroxide and zinc sulphate. Some enzymic methods used for the determination of D-glucose, such as the oxidase–peroxidase method and the

[34] R. Schwarzenbach, *J. Chromatog.*, 1976, **117**, 206.
[35] M. Sinner, *J. Chromatog.*, 1976, **121**, 122.
[36] B. B. Pruden and G. Pineault, *J. Chromatog.*, 1975, **115**, 477.
[37] J. W. Blunt and M. H. G. Munro, *Austral. J. Chem.*, 1976, **29**, 975.
[38] C. M. Mundie, M. V. Cheshire, H. A. Anderson, and R. H. E. Inkson, *Analyt. Biochem.*, 1976, **71**, 604.
[39] J. F. Goodwin, *Clinical Chem.*, 1976, **22**, 169.
[40] H. E. Conrad, *Methods in Carbohydrate Chem.*, 1976, **7**, 71.
[41] W. A. Brunton and I. W. Percy-Robb, *Clinica Chim. Acta*, 1976, **69**, 131.
[42] I. Kaufmann-Raab, H. G. Jonen, E. Jähnchen, G. F. Kahl, and U. Groth, *Clinical Chem.*, 1976, **22**, 1729.
[43] L. Van Hung, *Compt. rend.*, 1976, **282**, *D*, 751.

hexokinase–D-glucose 6-phosphate dehydrogenase method, give low values when D-glucose has been lyophilized in the presence of protein.[44] Low values can also be obtained from the use of commercial kits in which D-glucose has been 'weighed in' to a quantity of protein base.

A novel fluorometric technique for the rapid and economic determination of D-glucose uses an immobilized form of D-glucose oxidase–peroxidase.[45] A cylindrical magnetic stirrer was specially designed to hold the immobilized enzymes firmly and to enable the reaction mixture to pass through the enzyme layer, thereby catalysing a rapid enzymic transformation. Blood plasma could be assayed by this procedure without pretreatment, and the immobilized enzyme was stable for several months – several hundred assays were performed during this period. D-Glucose oxidase immobilized on the internal wall of tubing has been used in an autoanalyser for the continuous determination of D-glucose in sera.[46] A continuous flow method for the determination of D-glucose in haemolysates and in other materials, using hexokinase–D-glucose 6-phosphate dehydrogenase, has been described.[47] D-Glucose dehydrogenase (β-D-glucose-NAD-oxidoreductase, E.C. 1.1.1.47) from *Bacillus megaterium* has been used in assays of the levels of D-glucose in body fluids; D-xylose and D-mannose were the only common monosaccharides to interfere in this reaction.[48] The sensitivity of the kinetic determination of D-glucose with D-glucose dehydrogenase has been improved by the addition of potassium thiocyanate as a competitive inhibitor,[49, 50] and results with accuracy comparable to the D-glucose oxidase and hexokinase procedures have been obtained.[51]

The uronic acid contents of polysaccharides have been measured by decarboxylation of the polysaccharide with hydriodic acid in a flask connected directly to the gas circuit of a gas–liquid chromatograph.[52] Following the hydrolysis of glycosaminoglycans with formic acid, the resulting hexuronic acids have been determined by means of automated ion-exchange chromatography.[53] A new analysis of carboxylic acids, which is more sensitive than existing methods, has been reported.[54] It is based on liquid-chromatographic separation of the acids and specific detection of the hydroxamic acids formed after reaction of the carboxylic acids with hydroxylamine in the presence of DCC. The equilibrium compositions of the various forms of hexuronic acids in DMSO have been investigated by g.l.c. of the silylated forms.[55] A g.l.c. method for analysis of the hexuronic acids, using either TMS or oxime derivatives, was also developed. Uronides supplemented with 6-[^{14}C]labelled uronic acid have been decarboxylated under acidic conditions, and the specific activity of the carbon dioxide evolved was

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measured following gas chromatography.[56] Several hexuronic acids and oligosaccharides containing hexuronic acid residues have been separated by high-voltage paper electrophoresis.[57] Stoicheiometric reduction of the carboxy-groups of polyuronides has now been achieved; reaction with a water-soluble carbodi-imide was used to activate the carboxy-groups so that they could be reduced with sodium borohydride.[58] D-Gluconic and lactobionic acids present in pharmaceutical preparations have been measured using an *in situ* fluorometric method that is based on the cleavage of vicinal glycol groups with lead tetra-acetate, followed by treatment with dichlorofluorescein.[59] An enzyme electrode, consisting of invertase, D-glucose oxidase, and mutarotase immobilized on a collagen membrane, has been used in assays of sucrase.[60] The method monitors the decrease in dissolved oxygen, resulting from the enzymic reactions shown in Scheme 1, by continuous amperometric measurements.

$$\text{sucrose} + H_2O \xrightarrow{\text{sucrase}} \alpha\text{-D-glucose} + \text{D-fructose}$$

$$\alpha\text{-D-glucose} \xrightarrow{\text{mutarotase}} \beta\text{-D-glucose}$$

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow[\text{oxidase}]{\text{glucose}} \text{D-gluconic acid} + H_2O_2$$

Scheme 1

An enzymic method for the determination of [1-^{14}C]lactose is based on the removal of the radio-label as $^{14}CO_2$.[61] Treatment of lactose with β-galactosidase released D-galactose and D-glucose, the latter hexose releasing $^{14}CO_2$ on enzymic decarboxylation. The preparation of TMS ethers of isomeric kestoses in aqueous solution has been reported.[62]

The addition of DMSO to solutions of lead tetra-acetate in acetic acid gave improved results in the histochemical detection of polysaccharides.[63] A number of lipopolysaccharides containing 2-acetamido-2-deoxyhexoses have been successfully *N*-deacetylated by treatment with sodium hydroxide in aqueous DMSO at *ca.* 100 °C.[64] Sodium thiophenolate, which was added as an oxygen scavenger, seems also to have a catalytic effect. In the presence of 2,2′-bipyridine, sodium azide, and manganese(II) ions, periodate ion forms a yellow chromogen which can be extracted with methyl isobutyl ketone and measured; this is a sensitive test for periodate ion.[65] Glycosidases that release simple reducing sugars can be located, following electrophoresis on polyacrylamide gels, by incubation with appropriate substrates (for example, yeast mannan for α-mannosidase and sucrose for invertase); the liberated reducing sugars give a precipitate of Prussian Blue with potassium hexacyanoferrate.[66]

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Structural Methods

The methyl ethers of methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside have been isolated on a preparative scale by chromatography on a strongly basic ion-exchange resin, and the procedure efficiently separates the methyl ethers obtained by partial methylation of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside.[67] The separations are thought to depend on an ion-exchange process in which all the free hydroxy-groups are involved, and it was concluded that the following acidity sequence holds: 4-OH > 3-OH > 6-OH. Unexpected products have been identified following the methylation analysis of aminosugars.[68] Permethylation (by the Hakomori procedure) of 2-acetamido-2-deoxy-D-glucitol, followed by hydrolysis with acid and acetylation, furnished 2-*N*-acetylacetamido-2-deoxy-1,3,4,5,6-penta-*O*-methyl-D-glucitol, and similar treatment of 6-*O*-substituted 2-acetamido-2-deoxy-D-glucitols yielded 6-*O*-acetyl-2-*N*-acetylacetamido-2-deoxy-1,3,4,5-tetra-*O*-methyl-D-glucitol. However, the product isolated from the corresponding 4-*O*-substituted aminohexitol (for example, di-*N*-acetylchitobi-itol) gave the corresponding *N*-methylacetamido derivative, as well as the *N*-acetylacetamido derivative, suggesting that the hydroxy-group at C-4 partly inhibits the hydrolysis of the *N*-methyl group with acid.

Chemical-ionization m.s. has been used to complement electron-impact m.s. in the structural determination of oligosaccharides.[69] When ammonia and isobutane were used as the reagent gases, intense ions corresponding to $(M + NH_4)^+$ were obtained with the peracetates of some di-, tri-, and tetrasaccharides. The thermal fragmentations of these ions permit the masses of subunits within the parent saccharide to be established unequivocally, while glycosyl-fragment ions in the spectra provide clear information about the nature of the non-reducing end of the oligosaccharide chain. Chemical-ionization mass spectra, using isobutane as the reagent gas, have also been recorded for a number of fully methylated glycosyl- and diglycosyl-alditols.[70] The ratio of the intensities for the alditol (A^+) ions to the alditol hydrate (AH_2O^+) ions, which are produced by cleavage of the glycosidic linkage on the alditol or glycosyl side of the oxygen atom, respectively, depends strongly on the position of the glycosidic linkage. G.l.c.–m.s. analysis has been used in the linkage analysis of some sialic acid-containing meningococcal polysaccharides.[71] Methanolysis of the *O*,*N*-methylated polysaccharides having the sialic acid residue in (2 → 9)-, (2 → 8)-, and (1 → 4)-linkage furnished the respective 4,7,8-, 4,7,9-, and 7,8,9-tri-*O*-methyl derivatives of methyl *N*-acetyl-*N*-methyl-β-D-neuraminate methyl glycoside, which could be identified on the basis of their typical fragmentation patterns. Improved separations of methylated alditol acetates have been achieved by g.l.c.[72]

Complexes of lanthanide shift reagents with permethylated aldohexopyranosides and their 6-deoxy analogues having the *gluco*, *galacto*, and *manno* con-

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[71] A. K. Bhattacharjee and H. J. Jennings, *Carbohydrate Res.*, 1976, **51**, 253.
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figurations have been examined by ^{1}H n.m.r. spectroscopy.[73] On the basis of the shift data obtained upon addition of Eu(fod)$_3$ and Pr(fod)$_3$, and the broadening of signals produced by the addition of Gd(fod)$_3$, it was concluded that the lanthanide ion binds preferentially to neighbouring oxygen atoms of methoxy-groups having an axial–equatorial disposition. Methods for determining the anomeric configuration of sugars by ^{1}H n.m.r. spectroscopy have been published.[74]

O-Alkylation of methyl pentofuranosides displaced the signal of the appended ^{13}C nucleus downfield, whereas the adjacent ^{13}C nuclei were usually shifted upfield to a small extent.[75] The effect of *O*-methylation was appreciably larger than that of either *O*-isopropylation or *O*-glycosylation, and both the *O*-methyl and the *O*-isopropyl derivatives can be used as models for interpreting the ^{13}C n.m.r. spectra of oligo- and poly-saccharides containing furanoid sugars. The ^{13}C n.m.r. spectra of two cyclodextrins and several linear glucans have been completely assigned by comparison with those of D-glucose, *O*-methylated derivatives of D-glucose, and various oligosaccharides of D-glucose containing different glycosidic linkages.[76] This technique enabled the composition, structure, and principal sequences of the glucans to be determined. A selective, double-irradiation technique has been used in assigning most of the ^{13}C-signals in the spectra of six fully acetylated D-glucobioses containing (1 → 3)-, (1 → 4)-, or (1 → 6)-linkages.[77] Comparison of the spectra of cellobiose and maltose octa-acetates with those of cellulose and amylose triacetates underlined both the value of, and the limitations imposed by, the technique in interpreting the ^{13}C n.m.r. spectra of polysaccharides. The ^{13}C n.m.r. spectra of several β-linked disaccharides containing residues of D-glucose, D-galactose, L-rhamnose, 2-acetamido-2-deoxy-D-glucose, or 2-acetamido-2-deoxy-D-galactose have been completely assigned.[78] These disaccharides are model compounds for interpreting the ^{13}C n.m.r. spectra of immunological polysaccharides.

Reasons have been advanced to account for the present difficulties in using the far-i.r. region of the spectra of low-molecular-weight oligosaccharides for determining the position of the glycosidic linkage.[79] Inspection of the far-i.r. spectra of disaccharides – differing in monosaccharide composition and in the position and configuration of the glycosidic linkage – indicated that the paucity of information obtained could be attributed to the interaction of vibrations and to the high sensitivity of bending skeletal and twisting vibrations to small changes in the molecule, in addition to the fact that, on passing from one oligo-saccharide to another, at least two structural factors are changed, making the search for any correlations, and the assignment of vibrations, impossible.

The influence of intrinsic and extrinsic Cotton effects on the o.r.d. and c.d. spectra of acetate and xanthate derivatives of polysaccharides has been studied.[80]

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Differences in the c.d. spectra of oligosaccharides containing 2-acetamido-2-deoxy-D-glucopyranosyl and 2-acetamido-2-deoxy-D-mannopyranosyl residues have been related to the presence of β-(1 → 4)- and β-(1 → 2)-linkages.[81] This technique should prove helpful in the identification of glycosidic bonds in carbohydrates that exhibit a c.d. spectrum. The c.d. spectra of several sialic acid-containing polysaccharides have been shown to be influenced by the state of ionization of the carboxy-group contained in the sialic acid, by the location within the sialic acid residues of the inter-saccharide linkages, and by changes in the configuration of hydroxy-groups remote from the carboxy-group of the sialic acid.[82]

A Fourier-transform method has been used to measure the spin-lattice relaxation times (T_1 values) of the anomeric protons of a small selection of oligo- and polysaccharide derivatives.[83] For disaccharides, at least, the T_1 values appear to have the same general stereospecificity and diagnostic potential as for monosaccharides, with the important additional feature that inter-ring relaxation contributions appear to be detectable and thereby offer a powerful new method for studying the conformations of oligosaccharides. The situation for higher oligosaccharides is less clear.

The anomeric configuration of D-xylose liberated from β-D-xylopyranosides by β-D-xylosidase from *Bacillus pumilus* has been determined by an enzymic procedure, based on the stereospecificity of D-xylose isomerases.[84] The initial hydrolysis product is α-D-xylose, indicating that this β-D-xylosidase, unlike other glycosidases, acts by inversion of configuration.

Details of a number of established chemical methods used in determining various aspects of the fine structures of polysaccharides – for example, the specific degradation of polysaccharides containing uronic acid residues by base-catalysed elimination,[85] the location of acyl substituents,[86] and the distribution of single-unit side-chains of D-galactose in galactomannans by alkaline hydrolysis of 6-deoxy-6-(4-tolylsulphonyl)hexopyranosides[87] – are reported in the latest volume in the series 'Methods in Carbohydrate Chemistry'.

The behaviour of a number of linear and branched β-glucans during gel chromatography on cross-linked dextrans and agarose has been shown to be unpredictable; thus, the results of molecular-weight determinations using the technique must be treated with caution.[88] Dextrans of mol. wt. $\leqslant 6 \times 10^6$ have been fractionated by gel-permeation chromatography on porous glass, in order to obtain precise information on the molecular-weight distribution.[89] Several methods were used to correct for peak-broadening; for example, peak-broadening was described by Gaussian functions, which enabled straightforward corrections for peak-broadening to be applied.

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A microchemical method for determining the DP of neutral oligo- and polysaccharides has been reported.[90] After reduction of the polysaccharide with sodium borohydride and hydrolysis with acid, the alditol (from the reducing end) is separated from other aldoses (from non-reducing residues) by ion-exchange chromatography and their relative proportion is measured by g.l.c. of the corresponding acetates. Linkage sequencing of D-gluco-oligosaccharides has been investigated by measuring the rates of degradation of the oligosaccharides in calcium hydroxide solution (β-alkoxycarbonyl elimination).[91] The component aldehydes in dialdehyde fragments formed by periodate oxidation of oligosaccharides have been converted quantitatively into the corresponding 2,4-dinitrophenylhydrazones, which were then separated by chromatography and analysed spectrophotometrically.[92] Using this procedure, it was possible to determine the positions of glycosidic substituents in various types of D-glucobioses, oligosaccharides of senega, and some synthetic β-(1 → 6)-linked D-gluco-oligosaccharides. Several murine antigalactan immunoglobulins have been shown to interact with polysaccharides containing β-(1 → 6)-linked D-galactopyranosyl residues and with polysaccharides whose structures are compatible with the presence of such a linkage.[93]

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3
Plant and Algal Polysaccharides

BY R. J. STURGEON

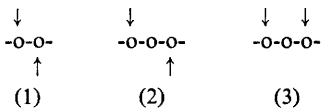
Starch

A new type of maize starch, called 'amylo-waxy' starch, has been isolated and characterized as a product of the cross-fertilization of waxy- and amylo-maizes.[1] Maize starch has been fractionated by gel chromatography with aqueous perchloric acid solution.[2] The inorganic ions did not interfere with the iodine-staining reaction or detection of the polysaccharide with the phenol–sulphuric acid reagent. The viscosity characteristics and enzymic (α- and β-amylase) hydrolysis of the starch from the fruit of *Annona reticulata* are similar to those of maize starch.[3] The number-average molecular weights of starches have been determined by a procedure that involved oxidation of the polysaccharide with potassium hexacyanoferrate solution and potentiometric titration of ferrous ions with ceric sulphate.[4] The molecular-weight distribution of amylose has been measured after gel-permeation chromatography on deactivated silica gel; the values obtained are in good agreement with those obtained by other methods.[5] The structures of amylose-B and -V have been investigated by i.r. and Raman spectroscopy, and the reactivities of both forms towards acetylation, sulphation, and xanthation were compared with those of cellulose.[6] The higher reactivity of amylose-V was attributed, in part, to the extent of intermolecular hydrogen-bonding. γ-Irradiation of tapioca starch was accompanied by a decrease in viscosity, as well as by a decrease in the temperature of gelatinization.[7] The polysaccharide was also degraded, with the production of acids and reducing sugars. γ-Irradiation of maize starch was also accompanied by decreases in viscosity and in average chain-length, and by the formation of acidic and reducing units.[8] The disappearance of malonaldehyde after γ-irradiation of maize starch is considered to take place by reaction with amino-acids, rather than by reaction with hydrogen peroxide.[9] The amount of glycolaldehyde formed during γ-irradiation of maize starch has been found to depend on the moisture content of the grain and on temperature.[10]

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Amylose prepared from starch dispersed in 10 M-urea was unaffected by the actions of β-amylase and phosphorylase, whereas it was degraded by α-amylase.[11] The physical and chemical properties of the modified amylose were generally comparable to those of natural amylose. Removal of the urea, either by rapid dialysis or by extraction with aqueous ethanol, may aggregate the amylose molecules, possibly forming a structure that is stabilized by hydrogen-bonding; molecules associated in this way could acquire resistance to β-amylase and phosphorylase, since the non-reducing end-groups would be inaccessible to the active sites of the enzymes. Monoglycerides have been used to influence the gelatinization and enzymic breakdown of wheat and cassava starches.[12] The amylose–monoglyceride complex was stable to α-amylase.

A singly-branched heptaose was produced as a limit dextrin when the β-limit dextrin obtained from rice starch was digested with α-amylase from *Bacillus amylolique faciens*.[13] Enzymic analysis of this apparently homogeneous oligosaccharide revealed it to be a mixture of singly-branched heptaoses having different branch-points. Digestion of the branched heptaoses with pullulanase before, and after, reduction with sodium borohydride showed that the main chains consist of malto-triose, -tetraose, and -pentaose bearing side-chains of malto-tetraose, -triose, and maltose, respectively. Two other extensively branched dextrins, containing nine and ten D-glucosyl residues, respectively, were also isolated following hydrolysis of the β-limit dextrin with α-amylase.[14] Structural analysis revealed that each is a mixture of four triply-branched dextrins having structures containing 6^3-α-D-glucosylmaltotriose and/or 6^2-α-D-glucosylmaltose as a linking unit. However, the branching configuration and the minimum number of α-(1 → 4)-D-glucosidic linkages between the two branches can be represented by one of the three structures (1)—(3).



(1) (2) (3)

-o- = α-(1 → 4)-D-glucopyranosyl residue; ↓ = α-(1 → 6)-inter-chain linkage

Two glucoamylases from *Aspergillus niger* and one from *Rhizopus niveus* have been used to hydrolyse the granules of native wheat and maize starches.[15] One of the *A. niger* enzymes and the *R. niveus* enzyme attacked the surface of the grains relatively uniformly, leaving large disc-like depressions. Both enzymes released the same amount of D-glucose from the starch granules, and this amount was roughly twice that released by the other *A. niger* glucoamylase. Maltose and glyceric acid 3-phosphate were the principal radioactive products obtained when intact spinach chloroplasts, labelled with ^{14}C mainly in the starch, were incubated in the dark under aerobic conditions.[16] Maltose appears to be a

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[16] C. Levi and M. Gibbs, *Plant Physiol.*, 1976, **57**, 933.

degradation product, since labelled D-glucose was not detected, although it is possible that the disaccharide might be formed from unlabelled D-glucose within the chloroplast and D-[^{14}C]glucose 1-phosphate derived from starch labelled during photosynthesis. It was suggested that maltose phosphorylase catalyses the synthetic reaction, while an amylolytic reaction operates in the reverse direction. Star-shaped amylopectins, which contain elongated branches introduced by potato phosphorylase, have been characterized by measurement of their viscosities, iodine-binding properties, and rates of retrogradation.[17] All the non-reducing end-groups appear to be used in the synthesis (*cf.* only 15—20% of the corresponding groups of glycogen), yielding materials with higher rates of retrogradation and different iodine-absorption spectra.

The mechanism of starch–sugar interconversion in potato tubers stored at different temperatures has been investigated by monitoring the changes in the sugar phosphates involved in the biosynthetic pathways between sugar and starch.[18] It appears that starch–sugar interconversion is regulated at the amyloplast membrane and, possibly, involves electron-transfer. In potatoes sweetened through senescence, electron micrographs showed that the amyloplast membranes had disintegrated. The degree of polymerization (DP_n) of linear, amylose-type polysaccharides has been determined by an enzymic method that measures the ratio of the concentration of total D-glucose to that of maltotetraose released on hydrolysis of the polysaccharide with phosphorylase.[19] The method may also be used to determine the average chain-length of branched, amylopectin-type polysaccharides, provided they are completely debranched before degradation with phosphorylase. Methods based on ion-exchange chromatography, gel-permeation chromatography, and enzymic degradation have been used to assess the purity and amylopectin content of samples of amylose, and the suitability of dyed amylose derivatives in assays of α-amylase.[20] A new method for estimating the number of α-(1 → 6)-linkages in starch is based on extensive hydrolysis of the polysaccharide with β-amylase. An attempt has been made to measure the extent of complex formation between amylose and various milk proteins by means of iodine-staining.[21] Ambiguous results have been obtained, since iodine is taken up both by the polysaccharide and by the protein. Residues containing carbohydrate and protein were isolated after acetylation of potato, maize, and rice starches; acid hydrolysis liberated D-glucose, maltose, and amino-acids, including arginine, leucine, and histidine.[22] Starch-derived oligosaccharides containing both α-(1 → 4)- and α-(1 → 6)-linkages have been identified following ion-exchange chromatography of wort and beer; identification was aided by the fact that oligosaccharides containing the same type of glycosidic linkage exhibited a straight-line relationship between the logarithm of the distribution coefficient and the number of monosaccharide residues present.[23]

[17] B. Pfannemüller, W. Burchard, and I. Franken, *Stärke*, 1976, **28**, 1.
[18] F. A. Isherwood, *Phytochemistry*, 1976, **15**, 33.
[19] K. M. Khan, *Carbohydrate Res.*, 1976, **51**, 145.
[20] J. F. Kennedy, *Stärke*, 1976, **28**, 196.
[21] M. G. Jones and K. Wilson, *Stärke*, 1976, **28**, 338.
[22] M. Lüdtke, *Stärke*, 1976, **28**, 83.
[23] J. Havlicek and O. Samuelson, *J. Inst. Brewing*, 1975, **81**, 466.

The developing grains of three wheat cultivars have been analysed for sucrose synthase, sucrase, and amylase, and for the rate of deposition of starch.[24] The relationship between sucrose synthase and amylase and the role of these and other enzymes in the synthesis of starch were discussed. Soluble primed phosphorylase and ADP-D-glucose: α-1,4-D-glucan-4-glucosyltransferase (starch synthase) have been detected during the accumulation of starch in developing rice grains.[25, 26] The activity of starch synthase bound to the starch granules increased as the starch content of the grains increased. Two isoenzymes of soluble starch synthase have been isolated from rice grains by means of ion-exchange and gel-permeation chromatography.[27] One of them (mol. wt. 1.1×10^5) is a protein that requires the presence of a primer to manifest its activity, whereas the other (mol. wt. 6.9×10^4) is a glycoprotein that does not require a primer. Q-Enzyme [α-1,4-D-glucan:α-1,4-D-glucan-6-α-(1,4-α-glucano)-transferase, E.C. 2.4.1.18] – which synthesizes the α-(1 → 6)-D-glucosidic branch-linkages of amylopectin – has been isolated from potatoes and purified.[28] Past reports have suggested that Q-enzyme consists of two components, a hydrolase (mol. wt. 7×10^4) and a transferase (mol. wt. 2×10^4), whereas the purified preparation is a monomer (mol. wt. 8.5×10^4) exhibiting only transferase activity. The average unit chain-length, the extent of β-amylolysis, and the iodine-staining properties of the products resulting from the enzyme's action on native and synthetic amyloses resembled those of amylopectin. Additional branch-linkages were introduced by the enzyme into potato amylopectin, although the product did not resemble phytoglycogen. The conversion of amylose into an amylopectin-like molecule by potato Q-enzyme occurs by a random, *endo*-type transglycosylation of the substrate chains.[29] Inter-chain transfer takes place during the formation of the amylopectin branch-linkage. The minimum length of amylose chain that can act as an acceptor in the trans-glycosylation reaction is 40 D-glucose units. That the enzyme requires substrate chains of this length indicates that stabilized secondary and tertiary structures must be established in the substrate before Q-enzyme can act on it, and that the forces responsible for maintaining this conformation are sufficiently strong only when the chains exceed a minimum length. Inter-chain transfer is suggested to take place by one of two mechanisms; by reaction with either a single chain or a double chain, each having a stabilized and, possibly, helical conformation. The occurrence of intra-chain transfer cannot be discounted.

Cellulose

Changes in definition of the i.r. spectra of cellulose and related oligosaccharides with temperature have been attributed to the effect on the superstructure of changes in hydrogen-bonding.[30] The marked effects that changes in temperature

[24] P. Meredith and L. D. Jenkins, *Stärke*, 1976, **28**, 189.
[25] C. M. Perez, A. A. Perdon, A. P. Recurreccion, R. M. Villareal, and B. O. Juliano, *Plant Physiol.*, 1975, **56**, 579.
[26] C. M. Perez, A. A. Perdon, A. P. Recurreccion, R. M. Villareal, and B. O. Juliano, *Plant Physiol.*, 1976, **58**, 602.
[27] R. A. Pisigan and E. J. Del Rosario, *Phytochemistry*, 1976, **15**, 71.
[28] D. Borovsky, E. E. Smith, and W. J. Whelan, *European J. Biochem.*, 1975, **59**, 615.
[29] D. Borovsky, E. E. Smith, and W. J. Whelan, *European J. Biochem.*, 1976, **62**, 307.
[30] H. Hatakeyama, C. Nagasaki, and T. Yurugi, *Carbohydrate Res.*, 1976, **48**, 149.

have on the frequency and intensity of bands in the 3400 cm^{-1} region are ascribed to changes in intra- and inter-molecular hydrogen-bonding. Decreases in the ratio of the absorbances at 1372 cm^{-1} and 2900 cm^{-1} at temperatures above ambient may reflect conformational changes resulting from greater freedom of movement of the hydroxy-groups when a particular type of hydrogen-bond is broken. Conflicting values for the DP were obtained from viscosity measurements on cellulose in aqueous solutions of cupriethylene diamine.[31] Different viscosity coefficients were obtained with identical solutions of cellulose when the measurements were made with a capillary viscometer and with an oscillating viscometer. Cellulose from the woods of *Picea excelsa*, *Fagus sylvatica*, and *Pinus sylvestris* has an apparent chain-length which is longer than that from the barks.[32] Cellulose has been prepared in the form of regular beads by solidification of an oil suspension of a cellulose solution.[33] The bead form of cellulose, which resembles macroporous materials, is suitable as a packing for gel chromatography and can also be transformed into ion-exchange materials.

Native cellulose from the green alga *Valonia ventricosa* has been shown to consist of a crystalline array of parallel chains by *X*-ray analysis.[34] A two-fold screw axis has been proposed for the rigid components of the D-glucose ring of this polysaccharide, although there is some freedom for rotation about the primary hydroxy-group.[35] Under conditions of sonic irradiation, the fibrillation of cellulose microfibrils takes place when the polarity of the chains is antiparallel, but not when it is parallel; for example, cellulose microfibrils from the cells of *Valonia macrophysa* and bacterial cellulose remained intact.[36] Electron-diffraction techniques have confirmed that *V. ventricosa* cellulose is arrayed in parallel chains.[37] Microfibrils of *V. ventricosa* cellulose have been used to nucleate the crystallization of supersaturated solutions of cellulose of low DP.[38] Epitaxial crystallization of cellulose II occurred on the *V. ventricosa* cellulose microfibrils, with the lamellar crystals oriented at right angles to the microfibrils. After hydrolysis with acid, regenerated cellulose fibres exhibited small-angle *X*-ray interferences, corresponding to long periods of 150—200 Å.[39, 40] A review has dealt with changes in the *X*-ray crystallinity index of cellulose resulting from treatment with alkali and sulphite during woodpulping.[41]

The DP of γ-irradiated cellulose, as measured by viscometry in cuprammonium solution, has been shown to be lower than that of the untreated cellulose.[42] Irradiation not only induced direct scission of the polysaccharide chains but also the formation of alkali-labile groups, which subsequently underwent scission in the solvent used for viscometry.

[31] V. L. Frampton, W. J. Evans, and W. B. Carney, *Cellulose Chem. Technol.*, 1976, **10**, 519.
[32] K. Garves, *Cellulose Chem. Technol.*, 1976, **10**, 249.
[33] J. Peška, J. Štamberg, J. Hradil, and M. Ilavský, *J. Chromatog.*, 1976, **125**, 455.
[34] K. H. Gardener and J. Blackwell, *Biopolymers*, 1974, **13**, 1975.
[35] A. Sarko and R. Muggli, *Macromolecules*, 1974, **7** 486.
[36] J. R. Colvin, *J. Polymer Sci., Part A-1, Polymer Chem.*, 1976, **14**, 2377.
[37] W. Claffey and J. Blackwell, *Biopolymers*, 1976, **15**, 1903.
[38] A. Buleon, H. Chanzy, and E. Roche, *J. Polymer Sci., Part A-2, Polymer Phys.*, 1976, **14**, 1913.
[39] J Schurz and K. John, *Cellulose Chem. Technol.*, 1975, **9**, 493.
[40] J. Haase, R. Hosemann, and B. Renwanz, *Cellulose Chem. Technol.*, 1975, **9**, 513.
[41] G. Jayme, *Cellulose Chem. Technol.*, 1975, **9**, 477.
[42] I. Sakurada, K. Kaji, T. Okada, and A. Tsuchiya, *Cellulose Chem. Technol.*, 1975, **9**, 503.

Oligosaccharides containing 2—7 D-glucopyranosyl residues have been isolated following the bleaching of cellulose with sodium hypochlorite.[43] Smaller proportions of the oligosaccharides (4)—(6), containing end-groups of

β-D-Glc*p*-(1 → 3)-D-Ara
(4)

β-D-Glc*p*-(1 → 4)-β-D-Glc*p*-(1 → 3)-D-Ara
(5)

β-D-Glc*p*-(1 → 4)-β-D-Glc*p*-(1 → 4)-β-D-Glc*p*-(1 → 3)-D-Ara
(6)

D-arabinose, and erythrose were also formed. Aldobionic acids through to aldo-octaonic acids composed of D-glucose and end-groups of erythronic, D-arabinonic, or D-gluconic acids comprised an appreciable proportion of the acidic material.

Treatment of hydrocellulose with hot alkali in the absence of oxygen gave a number of mono- and di-carboxylic acids, which were separated by ion-exchange chromatography and identified by g.l.c.–m.s. of the TMS derivatives.[44] Among the compounds characterized were 3,4-dideoxy-, 2,3,4-trideoxy-, and 2,3-dideoxy-hexaric acids. Cotton cellulose has been partially hydrolysed with hydrogen chloride in benzene containing various proportions of water.[45] Both the rate and site of hydrolysis were strongly dependent on the amount of water present. The determining factors were recognized as the partition of the hydrogen chloride between the water in the benzene and that adsorbed by cellulose, and the relative accessibility of glycosidic linkages near, and far removed from, chain-ends. Hydrolysis tended to be confined to the ends of the cellulose chains when little water was present.

Two cellulases isolated from culture filtrates of *Trichoderma viride* caused only a slight decrease in the DP of native cellulose, but a rapid decrease in that of reprecipitated cellulose.[46] Both enzymes have the ability to release free fibres from filter paper.

Genetic manipulation of the white rot fungus *Sporotrichum pulverulentum* has produced mutants that do not contain enzymes capable of hydrolysing cellulose and hemicelluloses.[47] These mutants were used to remove the lignin from birch wood, leaving the cellulose and hemicelluloses essentially unaltered in the 'biological pulp'. One of the mutants produced no cellulase, but it degraded lignin (31%) and hemicelluloses (32%).[48] The products obtained when *S. pulverulentum* grows on spruce fibres and cellulose have been investigated.[49] Cellobionic acid was the principal acidic product obtained from the degradation

[43] S. I. Andersson and O. Samuelson, *Cellulose Chem. Technol.*, 1976, **10**, 209.
[44] L. Löwendahl, G. Petersson, and O. Samuelson, *Cellulose Chem. Technol.*, 1976, **10**, 471.
[45] T. P. Nevell and W. R. Upton, *Carbohydrate Res.*, 1976, **49**, 163.
[46] L. E. R. Berghem, L. G. Petersson, and U. Baxio-Fredriksson, *European J. Biochem.*, 1976, **61**, 621.
[47] P. Ander and K. Eriksson, *Svensk Papperstidn.*, 1975, **78**, 641.
[48] P. Ander and K. Eriksson, *Svensk Papperstidn.*, 1976, **78**, 643.
[49] U. Westermark and K. Eriksson, *Svensk Papperstidn.*, 1975, **78**, 653.

of spruce fibres, while cellobiose was the principal neutral product. Less cellobionic acid was produced when the organism grows on cellulose. It was established that cellobionic acid is derived by oxidation of cellobiose to cellobiono-1,4-lactone by the enzyme cellobiose:quinone oxidoreductase in the presence of a hydrogen-acceptor (presumably oxidized lignin or degradation products of lignin). Cellobionic acid is metabolized intracellularly by the fungus, rather than being hydrolysed by extracellular enzymes. The rate of degradation of lignin by *Pleurotus ostreatus* is enhanced in the presence of cellulose.[50] Cellobiose:quinone oxidoreductase is induced only when the fungus grows in media containing cellulose, and the enzyme may facilitate the degradation of lignin by preventing polymerization reactions catalysed by laccase (monophenol oxidase). Lignosulphonates are degraded during the growth of *P. ostreatus*, and they undergo both polymerization and depolymerization.[51] However, depolymerization is more prevalent in a lignosulphonate–cellulose medium than in one containing no cellulose. The polymerization of lignin is inhibited by the presence of the oxidoreductase, which reduces the lignin-derived quinones.

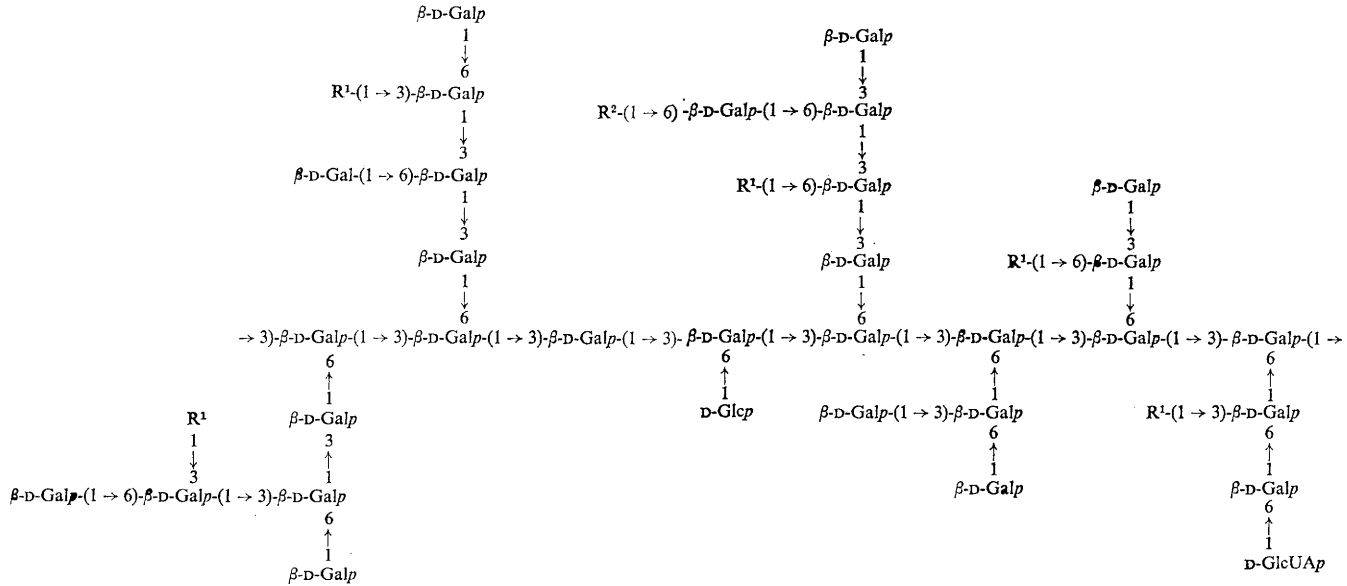
The roles of cytoplasmic organelles and the plasma membrane in the biosynthesis of cellulose have been reviewed.[52]

Gums and Mucilages

Structural studies on gums have been reviewed.[53] Further progress in the structural chemistry of gums is considered to depend on the development of new precise methods of structural analysis and on elucidation of the biological functions of these polysaccharides. Studies of the molecular-weight distribution of the raw gum exudate from *Acacia baileyana* F. Muell., of partially hydrolysed material, and of the products of successive Smith degradations of the gum have suggested the occurrence of sub-units having a molecular weight of $\sim 4 \times 10^3$.[54] These subunits are composed of β-(1 → 3)-linked D-galactopyranosyl residues, to most of which are attached L-arabinofuranosyl or D-galactopyranosyl residues; most of the pendant groups were removed by a single Smith degradation to give a monodisperse fragment (mol. wt. 2.5×10^3). Further degradation by successive oxidation, reduction, and hydrolysis with acid yielded a linear galactan containing about ten hexose residues. *A. cyanophylla* has been shown to be an anomalous member of the Phyllodineae in view of the unusual chemical composition and structure of the exuded gum.[55] Methylation and hydrolysis of the gums from *A. difformis*, *A. mabellae*, *A. retinodes*, and *A. rubida* showed that their structures are similar to those of gums from *A. podalyriifolia* and *A. pycnantha*. A homogeneous, degraded polysaccharide isolated from purified bael (*Aegle marmelos*) gum consists of residues of L-arabinose, D-galactose, L-rhamnose, and D-galacturonic acid (1 : 19 : 1 : 2).[56] From the results of periodate oxidation,

[50] T. Hiroi and K. Eriksson, *Svensk Papperstidn.*, 1976, **79**, 157.
[51] T. Hiroi, K. Eriksson, and B. Stenlund, *Svensk Papperstidn.*, 1976, **79**, 162.
[52] M. J. Chrispeels, *Ann. Rev. Plant Physiol.*, 1976, **27**, 19.
[53] Y. S. Ovodov, *Pure Appl. Chem.*, 1975, **42**, 351.
[54] S. C. Churms and A. M. Stephen, *Carbohydrate Res.*, 1975, **45**, 291.
[55] D. M. W. Anderson and P. C. Bell, *Phytochemistry*, 1976, **15**, 301.
[56] A. Roy, S. B. Bhattacharya, A. K. Mukherjee, and C. V. N. Rao, *Carbohydrate Res.*, 1976, **50**, 87.

D-Galp
1
↓
4
→ 3)-D-Galp-(1 → 3)-D-Galp-(1 → 3)-D-Galp-(1 → 3)-D-Galp-(1 → 3)-D-Galp- (1 → 3)-D-Galp-(1 → 3)-D-Galp-(1 → 3)-D-Galp-(1 →
6 6 6 6 6 6
↑ ↑ ↑ ↑ ↑ ↑
1 1 1 1 1 1
D-Galp L-Rhap D-Galp D-Galp L-Araf D-Galp D-Galp
3 2 3(5) 3 3
↑ ↑ ↑ ↑ ↑
1 1 1 1 1
D-Galp D-Galp D-Galp D-Galp D-Galp
3 3 3
↑ ↑ ↑
1 1 1
D-GalUAp D-GalUAp
(7)



R^1 represents D-mannose, D-xylose, L-rhamnose, L-arabinose, or (1→2)-linked chains of L-arabinose
R^2 represents D-glucose or D-glucuronic acid

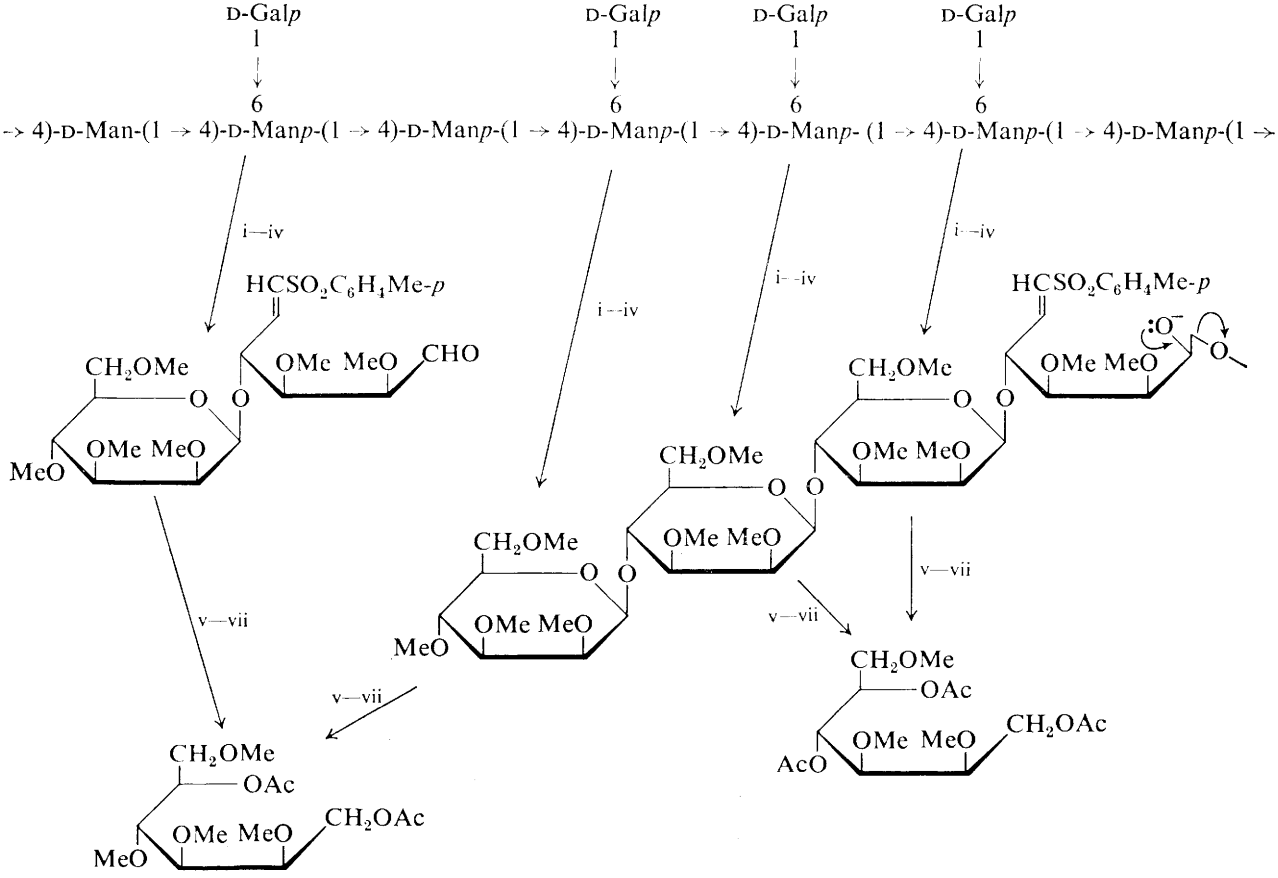
(8)

methylation analysis, and Smith and Barry degradations, a tentative structure (7) was proposed for the average repeating-unit of the degraded gum.

The gum exudate of *Anacardium occidentale* (the cashew-nut tree) contains residues of D-galactose, L-arabinose, L-rhamnose, D-glucose, and D-glucuronic acid, in addition to trace amounts of D-mannose, D-xylose, and 4-*O*-methyl-D-glucuronic acid.[57] The main aldobiouronic acid unit has been characterized as 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose, and a smaller proportion of the 4-*O*-methyl analogue is also present. Successive Smith degradations and methylation of the gum and of its degradation products indicated a highly-branched galactan framework composed of chains of β-(1 → 3)-linked D-galactopyranosyl residues that are branched and interspersed with β-(1 → 6)-linkages. L-Arabinose is present either as end-groups or in short (1 → 2)-linked chains containing up to five residues, while D-glucose, L-rhamnose, D-mannose, D-xylose, and D-glucuronic acid are all present as end-groups. A possible structural fragment (8) of *A. occidentale* gum was proposed. A methylated and degraded form of leiocarpan A, the principal polysaccharide in the gum exudate of *Anogeissus leiocarpus*, has been subjected to base-catalysed degradation with DBU and acetic anhydride in benzene.[58] Mild hydrolysis with acid and de-*O*-acetylation then gave 3,4,6-tri-*O*-methyl-D-mannose and 3,4-di-*O*-methyl-6-*O*-(2,3,4-tri-*O*-methyl-β-D-xylopyranosyl)-D-mannose. The characterization of the methylated disaccharide provided evidence for the site of attachment and anomeric configuration of the D-xylopyranosyl end-groups in the parent polysaccharide.

Methylation analysis, Smith degradation, and controlled hydrolysis with acid revealed that significant structural differences are to be found in the gum polysaccharides from *Combretum hartmannianum* and *C. leonense*.[59] Preliminary investigation of *C. hartmannianum* gum showed that all the uronic acid residues are end-groups; there are substantial proportions of terminal L-arabinofuranosyl residues and of side-chains consisting of 3-*O*-β-L-arabinopyranosyl-L-arabinose; intra-chain D-mannosyl residues are linked at position 3; and all the L-rhamnosyl residues are located at non-reducing, terminal positions. The presence of 6-*O*-β-D-galactopyranosyl-D-galactose in all partial acid hydrolysates indicates a structure based on (1 → 6)-linked D-galactose chains. By contrast, *C. leonense* gum contains very few uronic acid end-groups and no D-mannosyl residues, while most of the L-rhamnosyl residues are intra-chain. The distribution of the α-D-galactopyranosyl side-chains in guaran and locust-bean gum has been determined by measurement of the *O*-acetyl-*O*-methyl-D-mannitol derivatives obtained from the corresponding primary *C*-(4-tolylsulphonyl)ated polysaccharide.[60] These derivatives result from base-catalysed β-elimination and methylation [with sodium (methylsulphinyl)methide and methyl iodide] of the primary *C*-(4-tolylsulphonyl)ated galactomannans, followed by sequential acid hydrolysis, reduction, and acetylation of the partially degraded 4-tolylsulphones (Scheme 1). The resulting *O*-acetyl-*O*-methyl-D-mannitol derivatives are

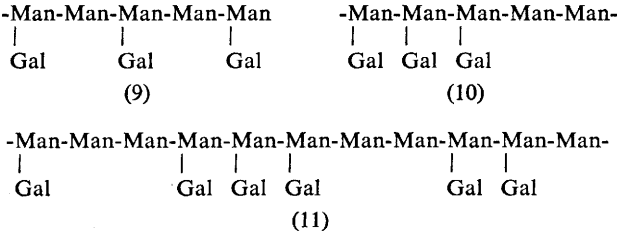
[57] D. M. W. Anderson and P. C. Bell, *Analyt. Chim. Acta*, 1975, **79**, 185.
[58] G. O. Aspinall and A. S. Chaudhari, *Canad. J. Chem.*, 1975, **53**, 2189.
[59] D. M. W. Anderson and P. C. Bell, *Carbohydrate Res.*, 1976, **49**, 341.
[60] C. W. Baker and R. L. Whistler, *Carbohydrate Res.*, 1975, **45**, 237.



Reagents: i, *N*-iodosuccinimide–Ph_3P–HMPT; ii, *p*-$MeC_6H_4SO_2Na$–HMPT; iii, DMSO–NaH; iv, MeI; v, H_3O^+; vi, $NaBH_4$; vii, Ac_2O–py

Scheme 1

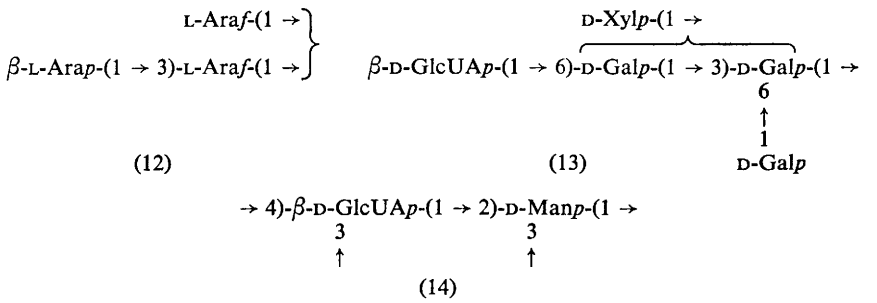
characteristic of the distribution pattern of the α-D-galactopyranosyl side-chains in the parent galactomannan; thus, D-mannopyranosyl residues substituted at O-6 with α-D-galactopyranosyl groups and at O-4 by a D-mannopyranosyl group without substitution at O-6 will produce 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol, whereas, if the D-mannopyranosyl residue attached at O-4 bears a D-galactopyranosyl group at O-6, this residue will give rise to 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol. The results indicate that guaran has a primary structure consisting of regular repetitions of the trisaccharide 4-*O*-(6-*O*-α-D-galactopyranosyl-β-D-mannopyranosyl)-β-D-mannose, whereas locust-bean gum (mol. wt. 2.1×10^5) is composed of α-D-galactopyranosyl side-chains distributed on D-mannopyranosyl residues in blocks. The relative ratio of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-mannitol produced from the first non-reducing β-D-mannopyranosyl group in the block of consecutively substituted β-D-mannopyranosyl units indicates that each substituted block in locust-bean gum contains 25 β-D-mannopyranosyl residues. If the substituted blocks are assumed to be separated by non-substituted β-D-mannopyranosyl units of equal length, the average length of each unsubstituted block is 85 linearly linked β-D-mannopyranosyl residues. The results of periodate oxidation of these polysaccharides and of methylation of the periodate-oxidized, borohydride-reduced polysaccharide indicated that the simple alternating structure (9) and the simple block structure (10) are excluded for both polysaccharides.[61] The results are consistent with near-random arrangement (11), which is the simplest interpretation of the available data.



The constituent sugars of methylated cherry gum have been identified and quantitatively determined by g.l.c.–m.s. of the methylated alditol acetates formed from the methylated gum, the carboxy-reduced, methylated gum, and the carboxy-reduced, methylated gum after remethylation.[62] The results of this and previous studies can be assessed in terms of three regions in the structure of cherry gum: the outer residues of L-arabinose (12), branched-chains of D-galactopyranosyl residues mutually joined by (1 → 3)- and (1 → 6)-linkages and bearing D-glucuronic acid residues (13), and residues of the aldobiouronic acid, 2-*O*-(β-D-glucopyranosyluronic acid)-D-mannopyranose (14).

An increase in the secretion of polysaccharides has been observed in the walls of cotyledon cells of *Nicotiana tabacum* after *X*-irradiation.[63] An acid-stable core polysaccharide, consisting of residues of D-glucose and D-mannose (10 : 2),

[61] J. Hoffman, B. Lindberg, and T. Painter, *Acta Chem. Scand.* (*B*), 1976, **30**, 365.
[62] G. A. Ospinall, A. S. Chaudhari, and C. C. Whitehead, *Carbohydrate Res.*, 1976, **47**, 119.
[63] P. Posso and H. Poussel, *Compt. rend. Soc. Biol.*, 1975, **169**, 1346.

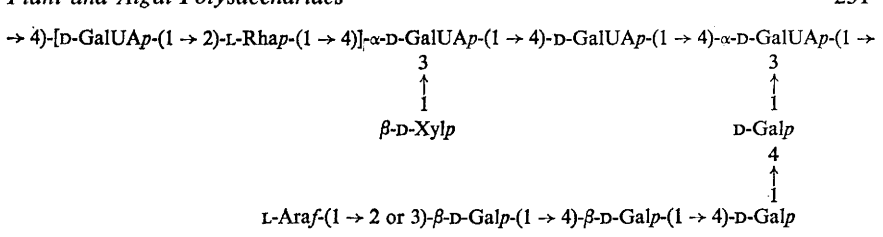


has been isolated from the seed mucilage of *Ocimum basilicum*.[64] Two fractions were isolated from Hakomori methylation of the polysaccharide; they were shown by g.l.c.–m.s. to be derived from a linear, cellulose-like polysaccharide containing (1 → 4)-linked β-D-glucopyranosyl residues and a linear polysaccharide containing (1 → 4)-linked β-D-glucopyranosyl and (1 → 4)-linked β-D-mannopyranosyl residues. Data from kinetic and organelle-fractionation experiments with *Zea mays*, and the autoradiographic localization *in situ* of labelled L-[^{3}H]fucose, have supported the suggestion that secretion of the root-cap slime occurs *via* the dictyosomes and the dictyosome vesicles.[65]

Pectins

Fractionation of the pectin isolated from rapeseed (*Brassica campestris*) cotyledon meal gave two, closely related, pectic polysaccharides, which were shown to be homogeneous by ultracentrifugation.[66] The principal fraction is composed of residues of L-arabinose, D-xylose, D-galactose, and L-rhamnose (5.3 : 3.2 : 1.0 : 0.57), and of D-galacturonic acid (33%) having a high degree (36%) of esterification. Both polysaccharides have structural features in common with the pectinic acids from soybean and mustard cotyledon meal, and form a group that is characterized by the presence of an unusually high proportion of neutral sugars. The principal pectinic acid fraction from rapeseed meal contains a (1 → 4)-linked D-galacturonan chain, and 75% of the D-galacturonic acid residues carry side-chains of L-arabinosyl residues. Pectins with a sequence of, at least, four D-galacturonic acid units have been shown to be hydrolysed by the poly-(galacturonic acid) transeliminase of *Bacillus pumilus*.[67] The unsaturated trimer formed is not attacked by the enzyme, thus it accumulates as the principal end-product. A partially hydrolysed polysaccharide from soy sauce has been further degraded with an *endo*-polygalacturonase, and the resulting sugars were identified as D-galacturonic acid and its α-(1 → 4)-linked dimer, 3-*O*-β-D-xylopyranosyl-D-galacturonic acid, and β-D-xylopyranosyl-(1 → 3)-α-D-galactopyranosyluronic acid-(1 → 4)-D-galactopyranosyluronic acid.[68] The structure (15) proposed for the polysaccharide in soy sauce is based on the results of degradative studies on the polysaccharide methyl ester with pectin lyase and on other studies.

[64] R. N. Tharanathan and Y. V. Anjaneyalu, *Austral. J. Chem.*, 1975, **28**, 1345.
[65] R. E. Paull and R. L. Jones, *Plant Physiol.*, 1976, **57**, 249.
[66] I. R. Siddiqui and P. J. Wood, *Carbohydrate Res.*, 1976, **50**, 97.
[67] B. A. Dave, R. H. Vaughn, and I. B. Patel, *J. Chromatog.*, 1976, **116**, 395.
[68] T. Kikuchi and H. Sugimoto, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 87.



(15)

The pectin in soybean cell walls was partially solubilized during fermentation of the sauce by *Aspergillus sojae*, leaving a non-dialysable component that could be separated by gel chromatography into two fractions of similar structure.[69] The presence of four acid polysaccharides originating from pectin has been reported among the insoluble residues obtained from fermentation of soy sauce.[70] Three of the polysaccharides contained about 90% of the D-galacturonic acid residues, and could be solubilized by an *endo*-polygalacturonase. Some of the difficulties encountered in the press-filtration of residues during the manufacture of soy sauce are thought to be caused by the presence of these water-insoluble acid polysaccharides. Treatment of soybeans before fermentation with a crude enzyme preparation of *A. sojae endo*-polygalacturonase allowed easier filtration of the residues.

The composition of the neutral sugars comprising the pectins of *Phaseolus vulgaris* has been shown to fluctuate during the culture cycle of suspensions of cotyledon cells, whereas only small changes were observed in the monosaccharide composition of the neutral polysaccharides.[71] The pectin from the bark of the white willow *Salix alba* has been partially depolymerized with acid.[72] Neutral disaccharides [(16) and (17)] and acidic oligosaccharides [(18)—(20)] isolated from the hydrolysates were characterized by g.l.c.–m.s. of their methylated derivatives.

D-Gal*p*-(1 → 3)-D-Gal*p*

(16)

L-Ara*f*-(1 → 6)-D-Gal*p*

(17)

α-D-GalUA*p*-(1 → 2)-β-L-Rha*p*-(1 → 2)-L-Rha*p*

(18)

α-D-GalUA*p*-(1 → 2)-β-L-Rha*p*-(1 → 4)-D-GalUA*p*

(19)

α-D-GalUA*p*-(1 → 4)-α-D-GalUA*p*-(1 → 4)-α-D-GalUA*p*-(1 → 4)-α-D-GalUA*p*-(1 → 2)-L-Rha*p*

(20)

[69] T. Kikuchi, *J. Agric. Chem. Soc. Japan*, 1976, **50**, 273.
[70] T. Kikuchi, H. Sugimoto, and T. Yokotsuka, *J. Agric. Chem. Soc. Japan*, 1976, **50**, 279.
[71] S. Mante and W. G. Boll, *Canad. J. Botany*, 1976, **54**, 198.
[72] R. Toman, Š. Karácsonyi, and M. Kubačková, *Cellulose Chem. Technol.*, 1976, **10**, 561.

Both water-soluble pectins and water-insoluble glycoproteins have been isolated from sunflower heads.[73] The biosynthesis of pectin has been followed during wall-regeneration of plasmolysed cells of tobacco leaves.[74] Although the incorporation of L-arabinose either was increased or was unaffected by plasmolysis, the incorporation of other sugars decreased. Separate mechanisms for the biosynthesis of arabinan and poly(galacturonorhamnan) are therefore likely to exist, and they must differ in their responses to physicochemical, structural, and organizational changes that accompany plasmolysis. D-[^{14}C]Glucose has been incorporated *in vivo* into the roots of *Zea mays*; sufficient time was allowed for the membrane compartments to become saturated, so that labelled substances isolated from pellets rich in either dictyosomes or the endoplasmic reticulum represent material in various stages of biosynthesis and transport across the membrane system.[75] Water-soluble polysaccharides and glycoproteins of molecular weight $>4 \times 10^4$ were isolated from fractions rich in dictyosomes and endoplasmic reticulum, and, possibly, represent exportable material in the final stages of synthesis. The relatively high proportions of uronic acid, D-galactose, and L-arabinose in the water-soluble polysaccharide indicated the presence of pectin. Water-insoluble carbohydrate material from both fractions could be solubilized using proteolytic enzymes; the material isolated from the dictyosome's pellet has a molecular weight $>4 \times 10^3$, whereas that obtained from the endoplasmic reticulum has a molecular weight $<4 \times 10^3$ and does not contain L-fucose. Since maize root-cap cells produce slime polysaccharides containing L-fucose, the absence of L-fucose from the small polymers within the endoplasmic reticulum indicates that the synthesis of some slime polysaccharides is initiated in the endoplasmic reticulum.

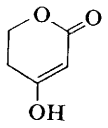
Hemicelluloses

The isolation of polysaccharide fractions from depectinated primary cell walls of lupin hypocotyls by successive extractions at two different temperatures has been examined.[76] The extent that such denaturants as guanidinium thiocyanate and urea extract the same polysaccharides was also investigated. That sequential disruption of the cell wall took place indicated that the constituent polymers are involved in a greater variety of linkages and in a different way from that suggested by a recent model of the primary cell wall.[77] Tobacco cell walls grown in tissue culture in the dark are friable and contain large proportions of pectins and hemicelluloses.[78] Friability appears to be associated, in part, with a significant increase in the proportion of L-arabinose, and with a large decrease in the amount of D-xylose, in the cell-wall hemicelluloses. Ion-exchange chromatography has been used to isolate two lignin–arabinoxylan complexes from alkaline extracts of spruce hemicellulose.[79] Examination of one of the complexes by electron

[73] A. F. Abdel-Fattah, S. S. Mabrouk, M. Edress, and M. S Shaulkamy, *Carbohydrate Res.*, 1976, **50**, 109.
[74] S. A. Boffey and D. H. Northcote, *Biochem. J.*, 1975, **150**, 433.
[75] D. J. Bowles and D. H. Northcote, *Planta*, 1976, **128**, 101.
[76] J. A. Monro, R. W. Bailey, and D. Penny, *Phytochemistry*, 1976, **15**, 175.
[77] K. Keegstra, K. W. Talmadge, W. D. Bauer, and P. Albersheim, *Plant Physiol.*, 1973, **51**, 188.
[78] P. Halmer and T. A. Thorpe, *Phytochemistry*, 1976, **15**, 1585.
[79] D. Fengel and M. Pryklenk, *Svensk Papperstidn.*, 1975, **78**, 617.

microscopy indicated the presence of thick fibrils in various states of aggregation, while very fine fibrils were observed in a galactoglucomannan fraction.[80] Polysaccharide fractions containing high levels of lignin appeared to be extensively wound and coiled. The hemicelluloses of larch wood (*Larix decidua*) are composed of equal amounts of pentosans and hexosans.[81]

A xylan extracted from the holocellulose present in the stalks of *Nicotiana tabacum* has been purified by ion-exchange chromatography.[82] The results of methylation analysis, partial hydrolysis with acid, and enzymic hydrolysis indicated that the xylan is composed of a straight chain of approximately 100 β-(1 → 4)-linked D-xylopyranosyl residues. Curie point–g.l.c. of the xylan yielded 2-furaldehyde, which is probably formed *via* 3-deoxy-D-*glycero*-pent-2-ulose.[83] Another major product was identified as 3-hydroxy-2-penteno-1,5-lactone (21) by high-resolution m.s.; this lactone has also been prepared by pyrolysis of a hexuronic acid-containing xylan from beech. Since the tobacco-stalk xylan contains no acidic components, the lactone (21) must arise by

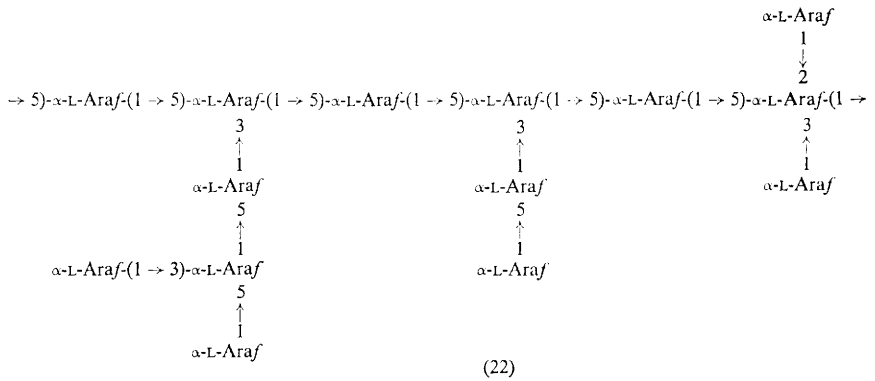


(21)

degradation of D-xylopyranosyl residues in the xylan molecule. The water-soluble polysaccharides extracted from bamboo shoots with DMSO consist of xylan, arabinogalactan, and a non-starchy α-D-glucan of comparatively low mol. wt. (8×10^3).[84] Methylation analysis indicated that the xylan consists of a (1 → 4)-linked D-xylopyranosyl chain, to which are attached L-arabinofuranosyl residues.[85] An unusual finding is that the arabinogalactan contains (1 → 3)-linked β-D-galactopyranosyl residues; methylation analysis showed that the L-arabinofuranosyl residues are present as single-unit side-chains attached to O-6 of D-galactopyranosyl units in a linear chain. A structure has been proposed for a water-soluble arabinan from rapeseed (*Brassica napus*) on the basis of the results of methylation, partial hydrolysis with acid, and Smith degradation; the arabinan has a highly branched structure, possibly (22), containing (1 → 5)-linked L-arabinofuranosyl residues, about one-third of which are further substituted at O-3.[86] However, the lengths of the side-chains were not determined, and it seems unlikely that the polysaccharide has an ordered structure.

An arabinoxylan (mol. wt. 5×10^4) has been isolated by gel filtration and ion-exchange chromatography of aqueous extracts of wheat flour.[87] This polysaccharide was able to agglutinate a number of normal cell types (for example,

[80] D. Fengel, *Svensk Papperstidn.*, 1976, **79**, 24.
[81] B. S. Il and C. I. Simionescu, *Cellulose Chem. Technol.*, 1976, **10**, 441.
[82] S. Eda, A. Ohnishi, and K. Katō, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 359.
[83] A. Ohnishi, E. Takagi, and K. Katō, *Carbohydrate Res.*, 1976, **50**, 275.
[84] E. Maekawa, *Agric. and Biol. Chem. (Japan,)* 1975, **39**, 2281.
[85] E. Maekawa, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2291.
[86] O. Larm, O. Theander, and P. Aman, *Acta Chem. Scand. (B)*, 1975, **29**, 1011.
[87] M. Minetti, P. Aducci, and A. Teichner, *Biochim. Biophys. Acta*, 1976, **437**, 505.



(22)

human red cells) regardless of their blood grouping, but it did not agglutinate tumour cells. The agglutinating activity can be destroyed by oxidation of the polysaccharide with sodium periodate, and also by treatment with crude 'cellulase' preparations, which presumably contained pentosanase activity. Normal haptenic inhibitors of lectins, such as monosaccharides and their derivatives, did not affect the agglutination reaction, which is inhibited by D- and L-tryptophan.

Non-cellulosic β-D-glucans containing both (1 → 3)- and (1 → 4)-linked D-glucopyranosyl residues have been isolated from the leaves and stems of the bamboos *Arundinaria japonica* and *A. anceps*.[88] A β-D-glucan (DP ~26) of similar structure was isolated from the endosperm of sorghum grain.[89] A hemicellulosic D-glucan has been isolated from the delignified cell walls of roses cultured on an agar medium.[90] The polysaccharide consists of roughly 120 β-(1 → 3)-linked D-glucopyranosyl residues and is strongly bound to microfibrillar cellulose and lignin in the cell walls. Another hemicellulosic polysaccharide that binds to microfibrillar cellulose was also isolated; this xyloglucan consists of a main chain of β-(1 → 6)-linked D-glucopyranosyl residues, some of which have D-xylopyranosyl residues at O-4.[91]

The quantities of non-cellulosic D-glucan and cellulose present in cell walls of the cortex tissue of apples vary little during ripening.[92] Cell-plate formation in *Zea mays* seedlings and in tuber slices of Jerusalem artichokes involves a (1 → 3)-linked β-D-glucan.[93] Histochemical staining showed the absence of this polysaccharide from older walls, although it appears to be present in the early stages of cell-wall formation during cytokinesis. The activity, extraction, and stability of a number of enzymes involved in the biosynthesis of *Citrus* polysaccharides have been studied.[94] The key enzyme regulating the metabolic pathway appears to be β-(1 → 3)-D-glucan synthase. Enzymes that incorporate D-glucose from

[88] K. C. B. Wilkie and S. L. Woo, *Carbohydrate Res.*, 1976, **49**, 399.
[89] G. R. Woolard, E. B. Rathbone, and L. Novellie, *Carbohydrate Res.*, 1976, **51**, 249.
[90] A. Mollard and F. Barnoud, *Physiol. Végétale*, 1976, **14**, 233.
[91] A. Mollard and F. Barnoud, *Physiol Végétale*, 1976, **14**, 241.
[92] I. M. Bartley, *Phytochemistry*, 1976, **15**, 625.
[93] R. G. Fulcher, M. E. McCully, G. Setterfield, and J. Sutherland, *Canad. J. Botany*, 1976, **54**, 539.
[94] J. Carbonell, J. P. Beltrán, and V. Conejero, *Phytochemistry*, 1976, **15**, 1873.

UDP-D-glucose into β-(1 → 3)- and β-(1 → 4)-linked D-glucans in pea roots have been shown not to be associated specifically with the Golgi apparatus.[95] Glucan synthase activity is not localized in the Golgi cisternae, the mitochondria, or fractions of the rough endoplasmic reticulum, but is more likely to be associated with smooth-membrane vesicles found in all of the fractions, and these might be derived from any of the smooth organelles of the cell. The membrane fractions also incorporated D-glucose from UDP-D-glucose into a lipid-monosaccharide, a lipid-oligosaccharide, and protein-linked oligosaccharides. The distribution of the enzymic activities and the presence of (1 → 3)- and (1 → 4)-linkages in both the lipid- and protein-linked oligosaccharides suggest that they are involved in the synthesis of β-glucan. Several of the kinetic properties of UDP-D-glucose:β-(1 → 4)-glucan glucosyltransferase [β-(1 → 4)-glucan synthase] isolated from oats have been described.[96]

Eight oligosaccharides containing D-fructose have been isolated from the roots of asparagus (*Asparagus officinalis*).[97] Identification of the constituent monosaccharides, g.l.c. of their methylated derivatives, partial hydrolysis with acid, and enzymic studies showed that the oligosaccharides are members of a series of 1^F(1-β-D-fructofuranosyl)$_n$-sucroses of the 1-kestose and neokestose type.

A glucomannan isolated from white willow (*Salix alba*) is composed of 20 β-D-glucopyranosyl and β-D-mannopyranosyl residues (ratio 1 : 1.4) mutually joined by (1 → 4)-linkages.[98] (1 → 4)-Linkages are also present in a glucomannan (DP ~45) isolated from the fibres of sunn hemp (*Crotalaria juncea*); the molecule appears to have an average of one branch-point at O-6 of both D-glucosyl and D-mannosyl residues of the main chain.[99] Both D-glucopyranosyl (87%) and D-mannopyranosyl (13%) residues are present as non-reducing end-groups, and D-glucose and D-mannose occur in the chain both as alternate and contiguous units. A purified glucomannan from the bulbs of *Lilium maximowiczii* is composed of β-(1 → 4)-linked D-glucopyranosyl and β-(1 → 4)-linked D-mannopyranosyl residues in the ratio of 1 : 2; branching occurs at O-3 of a few of the D-mannopyranosyl residues.[100] A glucomannan was the only product obtained when appropriate proportions of GDP-D-mannose and GDP-D-glucose were incubated with the GDP-D-glucose:glucosyltransferase from *Pisum sativum*.[101] The properties of the enzyme are very similar to those of the glucomannan synthase previously isolated from *Phaseolus aureus*.[102] The particulate preparation of *P. sativum* glucomannan synthase also contained an enzyme that catalyses the incorporation of D-[^{14}C]mannose from GDP-D-[^{14}C]mannose into a [^{14}C]-mannolipid.

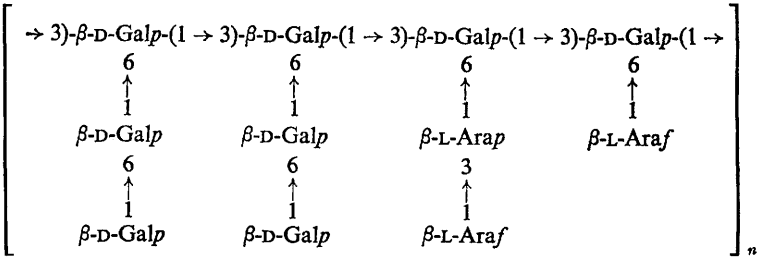
The Sepharose-immobilized form of a lectin from the seeds of *Bandeiraea simplicifolia* has been used in a single-step purification of a galactomannan from

[95] C. T. Brett and D. H. Northcote, *Biochem. J.*, 1975, **148**, 107.
[96] C. M. Tsai, *J. Carbohydrates, Nucleosides, Nucleotides*, 1975, **2**, 419.
[97] N. Shiomi, J. Yamada, and M. Izawa, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 567.
[98] S. Karácsonyi and M. Pašteka, *Coll. Czech. Chem. Comm.*, 1975, **40**, 1240.
[99] P. C. Das Gupta, S. K. Sen, and A. Day, *Carbohydrate Res.*, 1976, **48**, 73.
[100] K. Kato, Y. Yamaguchi, K. Mutoh, and Y. Ueno, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1393.
[101] M. B. Hinman and C. L. Villemez, *Plant Physiol.*, 1975, **56**, 608.
[102] C. L. Villemez, *Arch. Biochem. Biophys.*, 1974, **165**, 407.

Cassia alata seeds.[103] Several murine antigalactan immunoglobulins have been found to precipitate with willow-bark galactan, presumably by interaction with the (1 → 6)-linked, non-reducing, D-galactopyranosyl end-groups of the polysaccharide.[104] The chemistry of seed galactomannans has been reviewed, with sections devoted to their occurrence and function, structural aspects, the interactions with other polysaccharides, and commercial uses.[105]

Additional evidence has been obtained that the galactomannan from *C. occidentalis* seeds is composed of a main chain of (1 → 4)-linked β-D-mannopyranosyl residues, to which α-D-galactopyranosyl side-chains are attached.[106] The principal products afforded by partial acid hydrolysis of the polysaccharide are di- and tri-saccharides containing β-(1 → 4)-linked D-mannopyranosyl residues. The presence of single-unit, non-reducing D-galactopyranosyl residues attached to the main chain is supported by the recovery from the hydrolysate of 6-*O*-α-D-galactopyranosyl-D-mannose and α-D-galactopyranosyl-(1 → 6)-β-D-mannopyranosyl-(1 → 4)-D-mannopyranose. D-Galactose and D-mannose released on enzymic hydrolysis of the galactomannan present in the endosperm of various germinating seeds have been shown to be absorbed by the cotyledons, where they are further metabolized.[107]

The structure (23) has been reported for an arabinogalactan from larch (*Larix decidua*).[108] The polysaccharide contains residues of D-galactose and L-arabinose in the ratio of 3 : 1 and has a main chain of (1 → 3)-linked β-D-galactopyranosyl residues, to which side-chains of β-L-arabinofuranosyl residues and of β-D-galactopyranosyl residues are attached. Most of the pentose occurs as 3-*O*-β-L-arabinofuranosyl-L-arabinopyranosyl side-chains.



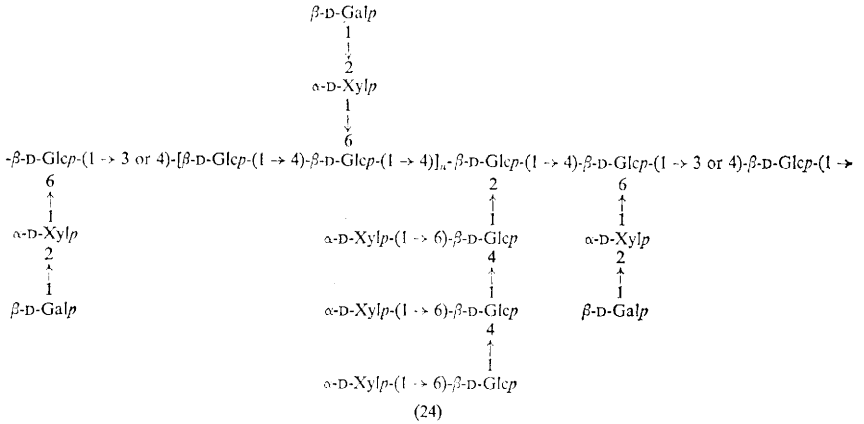
(23)

A galactomannan isolated from the leaves of *Symplocos spicata* contains residues of L-arabinose and D-galactose (5 : 3).[109] Methylation and periodate-oxidation studies suggested that this polysaccharide has an unusual structure: the main chain is composed of (1 → 4)-linked β-L-arabinopyranosyl and (1 → 3)-linked β-D-galactopyranosyl residues, with single-unit side-chains of L-arabinopyranose attached at O-6 of some of the D-galactosyl residues.

[103] T. T. Ross, C. E. Hayes, and I. J. Goldstein, *Carbohydrate Res.*, 1976, **47**, 91.
[104] B. N. Manjula and C. P. J. Glaudemans, *Immunochemistry*, 1976, **13**, 469.
[105] I. C. M. Dea and A. Morrison, *Adv. Carbohydrate Chem. Biochem.*, 1975, **31**, 241.
[106] D. S. Gupta and S. Mukherjee, *Indian J. Chem.*, 1975, **13**, 1152.
[107] B. V. McCleary and N. K. Matheson, *Phytochemistry*, 1976, **15**, 43.
[108] C. Simionescu, B. S. Il, and A. Cernătescu-Asandei, *Cellulose Chem. Technol.*, 1976, **10**, 535.
[109] R. D. Tiwari and A. L. Tripathi, *Planta Medica*, 1976, **29**, 376.

Gel chromatography has been used to separate the oligosaccharides released from soybean arabinogalactan by a purified (1 → 4)-β-D-galactanase; oligosaccharides eluted in the void volume of the column contained L-arabinose (87%) and D-galactose (6%).[110] Closer examination of this polysaccharide indicated that some of the L-arabinosyl units are substituted at positions 5, 2,5, 3,5, and 2,3,5, whereas others occur at non-reducing termini. This evidence cannot be reconciled with the published structure for the soybean arabinogalactan, *viz.* a (1 → 4)-linked D-galactan backbone bearing single L-arabinose or L-arabinosyl-L-arabinose side-chains.[110a]

Seeds from *Balsamina*, *Tropaeolum*, and *Tamarindus* contain amyloids (D-galacto-D-xylo-D-glucans) that are depolymerized by fungal and plant glycosidases.[111] Dialysable and non-dialysable fragments obtained by depolymerization of *Balsamina* amyloid with a purified cellulase from *Penicillium notatum* have been characterized, and, based on the results of these and chemical studies, the structure (24) was proposed for the amyloid.



(24)

Structures have been assigned to various oligosaccharides isolated following partial acid hydrolysis of the polysaccharide from defatted seeds of *Cassia tora*.[112] The results suggest that the polysaccharide has a linear chain of β-(1 → 4)-linked D-glucopyranosyl and D-mannopyranosyl residues; D-galactopyranosyl residues form single-unit branches attached to D-mannopyranosyl residues only by α-(1 → 6)-linkages; D-xylopyranosyl residues are present as single-unit branches attached to β-(1 → 4)-linked D-glucopyranosyl residues by α-(1 → 6)-linkages; and β-(1 → 6)-linked D-glucopyranosyl residues are present only in the branches.

The glucuronoarabinoxylan from sorghum-grain husks is composed of L-arabinose, D-xylose, D-glucuronic acid, and 4-*O*-methyl-D-glucuronic acid (15 : 18 : 2 : 1).[113] Structural studies indicated that the polysaccharide has a main chain of (1 → 4)-linked β-D-xylopyranosyl residues, with the uronic acid

[110] J. M. Labavitch, L. E. Freeman, and P. Albersheim, *J. Biol. Chem.*, 1976, **251**, 5904.
[110a] M. Morita, *Agric. and Biol. Chem. (Japan)*, 1965, **29**, 626.
[111] J. E. Courtois, P. Le Dizet, and D. Robic, *Carbohydrate Res.*, 1976, **49**, 439.
[112] S. C. Varshney, S. A. I. Rizvi, and P. C. Gupta, *J.C.S. Perkin I*, 1976, 1621.
[113] G. R. Woolard, E. B. Rathbone, and L. Novellie, *Carbohydrate Res.*, 1976, **51**, 239.

residues attached to O-2 of some of the D-xylopyranosyl residues; most of the L-arabinofuranosyl residues occur as non-reducing end-groups attached to O-3, and in some cases both to O-2 and O-3, of some of the D-xylopyranosyl residues.

An acid polysaccharide isolated from the rhizomes and young fronds of bracken (*Pteridium aquilinum*) contains residues of D-galactose, D-xylose, L-fucose, L-arabinose, D-mannose, and D-glucuronic acid.[114] Partial hydrolysis with acid released an aldobiouronic acid, which was tentatively identified as 2-*O*-(α-D-glucopyranosyluronic acid)-D-mannose. A method that corrects for the loss of D-mannose during hydrolysis of the aldobiouronic acid was also reported. Decrease in the DP of a glucuronoxylan from hornbeam (*Carpinus beiulus*) during thermolysis is brought about by the random cleavage of β-glycosidic linkages and by the decomposition of some of the sugar residues.[115] Dehydration occurred at 150 °C, with the formation of γ-lactones and sugars containing a carbonyl group at either C-2 or C-3.

Monosaccharides and β-(1 → 4)-linked disaccharides composed of D-glucose and D-mannose have been degraded by alkali in the presence, and in the absence, of oxygen.[116] Oxygen enhanced the rate of alkaline degradation of monosaccharides, but had little effect on that of disaccharides. A 4-*O*-methyl-D-glucuronoxylan was solubilized when birch (*Betula verrucosa*) meal was heated under pressure with an aqueous solution of sodium hydrogen carbonate.[117] Xylan also dissolved under similar conditions, but only in the presence of oxygen. Twenty-five monocarboxylic acids and eleven dicarboxylic acids produced on treatment of pine (*Pinus sylvestris*) wood with alkali have been separated by ion-exchange chromatography and identified.[118] The effects produced by manganese and iron salts on the degradation of *B. verrucosa* meal by oxygenated alkali have been investigated.[119] Addition of manganese salts increased the rate of delignification, whereas the rate of depolymerization of polysaccharides was retarded; iron salts arrested these effects.[120]

Algal Polysaccharides

Agar.—A galactan sulphate from the red alga *Ceramium rubrum* has an alternating structure of the agar-type, with D-galactose or 6-*O*-methyl-D-galactose as one alternating unit and L-galactose, 3,6-anhydro-L-galactose, and their respective 2-*O*-methyl ethers as the other unit.[121] Both D- and L-galactosyl residues have sulphate hemi-ester groups at O-6, with smaller amounts at O-2 and O-4 of, probably, D-galactosyl residues. The polysaccharide differs from others previously examined insofar as most of the L-galactosyl residues are non-sulphated. The size, shape, and, probably, the ultrastructure of beaded agarose have been preserved during dehydration and embedding in a resin.[122] The

[114] M. C. Jarvis and H. J. Duncan, *Phytochemistry*, 1976, **15**, 171.
[115] A. Ebringerova, *Cellulose Chem. Technol.*, 1976, **10**, 121.
[116] R. Malinen and E. Sjöström, *Cellulose Chem. Technol.*, 1975, **9**, 651.
[117] O Samuelson and L. A. Sjöberg, *Svensk Papperstidn.*, 1976, **79**, 90.
[118] L. Löwendahl, G. Petersson, and O. Samuelson, *Tappi*, 1976, **59**, 118.
[119] K. Abrahamsson and O. Samuelson, *Svensk Papperstidn.*, 1976, **79**, 281.
[120] D. Pal and O. Samuelson, *Svensk Papperstidn.*, 1976, **79**, 311.
[121] J. R. Turvey and E. L Williams, *Carbohydrate Res.*, 1976, **49**, 419.
[122] A. Amsterdam, Z. Er-El, and S. Shaltiel, *Arch. Biochem. Biophys.*, 1975, **171**, 673.

structure, as revealed by electron microscopy, accounts for the known physico-chemical properties of beaded agar – for example, its low matrix-volume and high water-content – and correlates with a number of the structural features of agar proposed by Rees on the basis of optical and *X*-ray data.[123]

Alginic Acid.—New developments in the chemistry of alginates, and in the uses of alginates in food, have been reviewed.[124] Periodate-induced degradation of alginic acid has been shown to proceed by two mechanisms: one fast – but not initiated by hydroxy-radicals – and the other slow.[125] This dual mechanism of degradation implies that the alginate molecule contains infrequent and atypical monomers, which can be cleaved by periodate ions with rupture of the chain. No evidence was forthcoming to indicate whether or not the abnormalities are introduced during biosynthesis of the polysaccharide or by subsequent *in vivo* or *in vitro* processes. The random coil-to-helix transition of the sodium alginate from a brown seaweed has been investigated by potentiometric titration.[126] Information on the helical structure was derived from studies on the polysaccharide–iodine complex. Acid polysaccharides (for example, alginic acid, carrageenan, and furcellaran) are used as precipitants of protein in brewing, and methods have been developed for the quantitative measurement of low concentrations of these polysaccharides in beer.[127]

Carrageenans.—Carrageenans in gametangial (both male and female) and tetrasporangial plants of the red alga *Iridaea cordata* have been isolated, fractionated, and characterized on the basis of their i.r. spectra and anhydro-galactose and sulphate contents.[128] As in the case of *Chondrus crispus*, λ-carrageenan is produced only by tetrasporangial plants, whereas κ-carrageenan is produced by gametangial plants. Antibodies prepared against specific carrageenans from *C. crispus* have been used in quantitative precipitin tests with carrageenans extracted from certain red algae at various stages of their life cycle.[129] Specific anti-(κ-carrageenan) serum was precipitated by carrageenans extracted from gametangial, but not tetrasporangial, plants from three genera of Gigartinaceae. Carrageenans and related polysaccharides from species outside this family did now show this difference. 3,6-Anhydrogalactose appears to be present in the determinant site of the antigen. The disorder-to-order transition occurring at the gel-point of κ-carrageenan solutions has been monitored by the change in optical rotation and by light-scattering methods.[130] It appears that the sol-to-gel transition is accompanied by a conformational change that involves the formation of double helices. Measurements of the heat of gelation showed that the enthalpy of the process increases with ionic strength; this is ascribed to a secondary process in which the double helices assemble into larger aggregates.

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[125] J. E. Scott, M. J. Tigwell, C. F. Phelps, and I. A. Nieduszynski, *Carbohydrate Res.*, 1976, **47**, 105.
[126] C. I. Simionescu, V. I. Popa, and V. Rusan, *Cellulose Chem. Technol.*, 1975, **9**, 641.
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[128] E. L. McCandless, J. S. Craigie, and J. E. Hansen, *Canad. J. Botany*, 1975, **53**, 2315.
[129] S. P. C. Hosford and E. L. Candless, *Canad. J. Botany*, 1975, **53**, 2835.
[130] T. H. M. Snoeren and T. A. J. Payens, *Biochim. Biophys. Acta*, 1976, **437**, 264.

Laminarin.—In a study of the conformations of polysaccharide chains in solution, values for the characteristic ratio (C_∞) and the steric factor (σ) have been computed for laminarin [a β-(1 → 3)-linked D-glucan] and an α-(1 → 3)-linked D-glucan.[131] Laminarin exhibits lower values for C_∞ and σ than does the α-(1 → 3)-linked D-glucan; this differs from the situation encountered with α- and β-(1 → 4)-linked D-glucans, suggesting that the chain dimensions of polysaccharides depend, among other factors, on the type and orientation of the inter-unit glycosidic bonds.

A new method for fractionating laminarin into its components on a preparative scale has been described.[132] This method, which uses DEAE–Sephadex–molybdate, separates laminarins with mannitol-terminated chains from those with D-glucose-terminated chains. Two glucans formed in the green alga *Caulerpa simpliciuscula* during photosynthesis have been separated by gel chromatography.[133] The principal glucan, which exhibits properties similar to those of soluble laminarin, has a DP of 37, whereas the minor glucan has a DP of 270.

Miscellaneous Algal Polysaccharides.—The cell walls of the siphonous green algae *Bryopsis plumosa* and *Derbesia tenuissima* probably contain a β-(1 → 4)-linked D-mannan as the principal polysaccharide.[134] A close taxonomic relationship was demonstrated between the genera; both have stepanokontic zoospores and contain a xylan and cellulose as the main cell-wall polysaccharides of the gametophytic phase, whereas a mannan is the main cell-wall polysaccharide of the sporophytic phase. The branched α-D-glucan (glycogen) from the blue-green alga *Anacystis nidulans* was degraded by a bacterial isoamylase by hydrolysis of all the α-(1 → 6)-linkages.[135] Examination of the resulting mixture of linear chains by gel chromatography showed that *A. nidulans* glycogen is similar to sweet-corn glycogen and only superficially similar to other bacterial glycogens. The rate of deposition of new walls has been measured in a detailed study of the formation of cell walls in *Fucus* zygotes.[136] In the initial stages (*i.e.* four hours after fertilization) a single fucan is detected, whereas another fucan is present only in a localized region of the wall at a later stage. The ratio of D-mannuronic acid to L-guluronic acid in the alginic acid did not vary during assembly of the wall, although the intracellular level of laminarin decreased, probably by the action of a membrane-bound *exo*-β-(1 → 3)-glucanase. The complete hydrolysis of laminarin by this enzyme accounts for at least part of the D-glucose available for cell-wall biosynthesis and for the increase in respiration triggered by fertilization. Highly-branched, complex polysaccharides have been isolated from the heterocyst and spore envelopes of *Anabaena cylindrica*.[137] Identification of the oligosaccharides liberated by partial acid hydrolysis indicated that the principal polysaccharides of both envelopes contain repeating units of 3-*O*-β-laminaritriosyl-D-mannose, and that D-glucose, D-mannose, D-galactose,

[131] N. Yathindra and V. S. R. Rao, *Current Sci.*, 1973, **42**, 809.
[132] J. R. Stark, *Carbohydrate Res.*, 1976, **47**, 176.
[133] R. J. Howard, S. W. Wright, and B. R. Grant, *Plant Physiol.*, 1976, **58**, 459.
[134] H. J. Huizing and H. Rietema, *Brit. Phycol. J.*, 1975, **10**, 13.
[135] M. Weber and G. Wöber, *Carbohydrate Res.*, 1975, **39**, 295.
[136] R. S. Quatrano and P. T. Stevens, *Plant Physiol.*, 1976, **58**, 224.
[137] L. Cardemil and C. P. Wolk, *J. Biol. Chem.*, 1976, **251**, 2967.

and D-xylose occupy terminal positions on the branches. Sulphated polysaccharides in *Laminaria saccharina* have been located by *X*-ray microanalysis.[138]

Polysaccharide-containing vesicles of the unicellular red alga *Porphyridium aerugineum* have been shown to accumulate near the cell membrane during the logarithmic phase of growth.[139] During the stationary phase, a sulphated polysaccharide is secreted by exocytosis at the cell surface, where it forms a capsule. Acid hydrolysis of an extracellular polysaccharide from *P. cruentum* has yielded a hexuronic acid, identified as 2-*O*-methyl-D-glucopyranosyluronic acid.[140] Two sulphated polysaccharides isolated from the thallus of *Undaria pinnatifida* are composed of residues of L-arabinose, D-xylose, D-galactose, and D-glucuronic acid.[141] The water-soluble, sulphated polysaccharide from *Ulva lactuca* formed a soft gel when dialysed against sea water.[142] Experiments with 'artificial' sea water or components therein indicated that both boric acid and calcium ions are essential for gelation, which appears to involve the formation of a polysaccharide–borate complex, leading to intermolecular linkages that are somehow stabilized by calcium ions. The polysaccharide contains residues of L-rhamnose, D-xylose, D-glucose, and D-glucuronic acid in a highly branched molecule that has no well-defined structure. If the sulphated polysaccharide exists in *U. lactuca* as a gel stabilized by complexation with borate, this is the first example of a polysaccharide–borate complex in Nature.

[138] M. E. Callow and L. V. Evans, *Planta*, 1976, **131**, 155.
[139] J. Ramus and D. M. Robins, *J. Phycol.*, 1975, **11**, 70.
[140] J. H. Kieras, F. J. Kieras, and D. V. Bowen, *Biochem. J.*, 1976, **155**, 181.
[141] T. Fujikawa, T. Anno, and M. Wada, *J. Agric. Chem. Soc. Japan*, 1975, **49**, 667.
[142] A. Haug, *Acta Chem. Scand.* (*B*), 1976, **30**, 562.

4
Microbial Polysaccharides

BY R. J. STURGEON

Teichoic Acids

A review has appeared outlining the origin of the glycerol phosphate residues that occur in the linkage between ribitol teichoic acid and peptidoglycan in cell walls.[1] The relations between the synthesis of cell-wall and membrane teichoic acids, and, possibly, between the synthesis of phospholipids and teichoic acids were also discussed. The chemistry and biochemistry of lipoteichoic acids have been reviewed.[2]

The lipoteichoic acid from *Bacillus licheniformis* contains lipid, 1,3-poly-(glycerol phosphate), and 2-amino-2-deoxy-D-glucose.[3] Treatment with hydrofluoric acid released the lipid moiety, which was identified as a diacyl derivative of β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 3)-glycerol, a structure identical with that of the membrane glycolipid. The lipoteichoic acid isolated from the Gram-negative anaerobe *Butyrivibrio fibrisolvens* consists of conventional 1,3-linked chains of glycerol phosphate attached to a glycosyl 1-glyceride; a non-micellar, deacylated lipoteichoic acid of lower mol. wt. was also isolated.[4]

A fully acylated lipoteichoic acid isolated from *Streptococcus faecalis* inhibited the autolysis (by the muramidase activity) of cell walls of the organism at concentrations comparable to those found in intact cells.[5, 6] The muramidase of *S. faecalis* was also inhibited by commercially available lipids and by several of the lipids and phospholipids commonly found in bacteria. Autolysis was inhibited to a greater extent by the phospholipids than by the lipoteichoic acid, suggesting possible differences in the accessibility of the enzyme to the inhibitors.[7] The presence of a poly(glycerol phosphate) backbone and fatty acids in the lipoteichoic acid from *Streptococcus pyogenes* is essential for maximum immunosuppression of the primary immunoglobulin IgM response in mice to sheep cells.[8] Membrane lipoteichoic acid continues to be synthesized, but in lower yield, by an osmotically fragile, stabilized L form of *S. pyogenes*.[9] The lipid

[1] J. Baddiley, *Biochem. Soc. Trans.*, 1975, 3, 840.
[2] N. Shaw, *Adv. Microbial Physiol.*, 1975, **12**, 141.
[3] D. Button and N. L. Hemmings, *Biochemistry*, 1976, **15**, 989.
[4] M. J. Hewett, A. J. Wicken, K. W. Knox, and M. E. Sharpe, *J. Gen. Microbiol.*, 1976, **94**, 126.
[5] R. F. Cleveland, A. J. Wicken, L. Daneo-Moore, and G. D. Shockman, *J. Bacteriol.*, 1976, **126**, 192.
[6] R. F. Cleveland, A. J. Wicken, L. Daneo-Moore, and G. D. Shockman, *J. Bacteriol.*, 1976, **126**, 1355.
[7] R. F. Cleveland, L. Daneo-Moore, A. J. Wicken, and G. D. Shockman, *J. Bacteriol.*, 1976, **127**, 1582.
[8] G. A. Miller, J. Urban, and R. W. Jackson, *Infection and Immunity*, 1976, **13**, 1408.
[9] B. M Slabyj and C. Panos, *J. Bacteriol.*, 1976, **127**, 355.

component of the L form and of the parent Streptococcus is glycerophosphoryl-diglucosyl diglyceride and not phosphatidylkojibiosyl diglyceride. The level of teichoic acid is unaltered when *S. pyogenes* grows in the presence of bacitracin, although the level of lipoteichoic acid is suppressed.

The teichoic acid synthesized when *Bacillus subtilis* grows in a Mg^{2+}-limited medium is composed of glycerol, phosphate, and D-glucose in the ratio 1 : 1 : 0.88.[10] Treatment of the teichoic acid with alkali released a 1-*O*-β-D-glucosylglycerol phosphate; examination of this fragment and its dephosphorylated form by ^{13}C n.m.r. spectroscopy confirmed that the monomeric unit is 1-*O*-β-D-glucopyranosylglycerol 3-phosphate. Assignment of the signals in the ^{13}C n.m.r. spectrum showed that the undegraded teichoic acid must be a poly-(glycerol 2- or 3-phosphate) substituted at O-1 of the glycerol with β-D-glucopyranosyl residues.

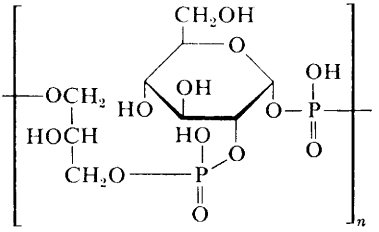
A glycerol-requiring mutant of *B. subtilis* altered its morphology when grown in the presence of limited supplies of glycerol or phosphate or Mg^{2+} ions.[11] Teichoic acid was identified as the principal anionic polymer present in the walls of glycerol- and Mg^{2+}-limited cells, whereas only teichuronic acid was found in the cell wall during phosphate-limited growth. There are no apparent differences between the teichoic acids synthesized by the mutant organism and the parent strain when grown under phosphate- and Mg^{2+}-limited conditions, and the observed morphological differences could not be correlated with a change in the content of anionic polymers in the cell walls. The addition of an excess of phosphate to cultures of *B. subtilis* W23 grown under phosphate-limited conditions resulted in the synthesis, and incorporation into the cell wall, of material containing teichoic acid. The conditions necessary for incorporation of this material can readily be identified, since its presence in the cell wall restored the ability to bind the bacteriophage SP50.[12] Location of the adsorbed bacteriophage showed that recently incorporated receptor material first appeared at the cell surface along the length of the cylindrical portion of the wall. The results are consistent with models for assembly of the cell wall in which newly-synthesized material is intercalated at a large number of sites distributed along the length of the cell. The material is incorporated first at the inner (cytoplasmic) surface of the cell walls and becomes exposed at the surface only during subsequent growth. The ability to bind bacteriophage did not develop until between a half and one generation time after the incorporation of newly-synthesized material into the cell wall.[13]

The ribitol teichoic acid from the cell walls of *Staphylococcus aureus* in solution binds Mg^{2+} ions univalently to phosphate groups and to a counter-ion, in contrast to the cell wall where Mg^{2+} ions form bridges across phosphate groups of adjacent chains of teichoic acid.[14] Differences in the affinities between cell walls with or without alanyl ester residues were much greater at low concentrations than they were at high concentrations of Mg^{2+} ions. Thus, at very low concentrations of Mg^{2+} ions, effective binding to the cell wall is significantly improved

[10] W. R. De Boer, F. J. Kruyssen, and J. T. M. Wouters, *European J. Biochem.*, 1976, **62**, 1.
[11] J. T. M. Wouters and M. P. M. Leegwater, *Arch. Mikrobiol.*, 1976, **110**, 295.
[12] A. R. Archibald and H. E. Coapes, *J. Bacteriol.*, 1976, **125**, 1195.
[13] A R. Archibald, *J. Bacteriol.*, 1976, **127**, 956.
[14] P. A. Lambert, I. C. Hancock, and J. Baddiley, *Biochem. J.*, 1975, **151**, 671.

by the loss of alanyl ester residues. The extracellular slime material synthesized by *Staph. aureus* grown in a medium containing high concentrations of carbohydrate and salts contained ribitol teichoic acid and, in two of three strains examined, a basic protein that reacted with antisera raised against whole cells or cell walls of *Staph. aureus*.[15] A Gram-negative, rod-shaped soil bacterium having properties very similar to those of *B. circulans* was able to release phosphate, but not 2-acetamido-2-deoxy-D-glucose, from the 2-acetamido-2-deoxy-D-glucosylribitol teichoic acid of *Staph. aureus*.[16]

A polysaccharide fraction of canine coagulate-positive staphylococci contains glycerol, 2-amino-2-deoxy-D-glucose, D-glucose, muramic acid, phosphate, and the usual amino-acids found in peptidoglycans, but it did not cross-react serologically with standard teichoic acids.[17] The results of chemical and immunochemical investigations suggested that (1) is the most likely structure for the teichoic acid.[18]



(1)

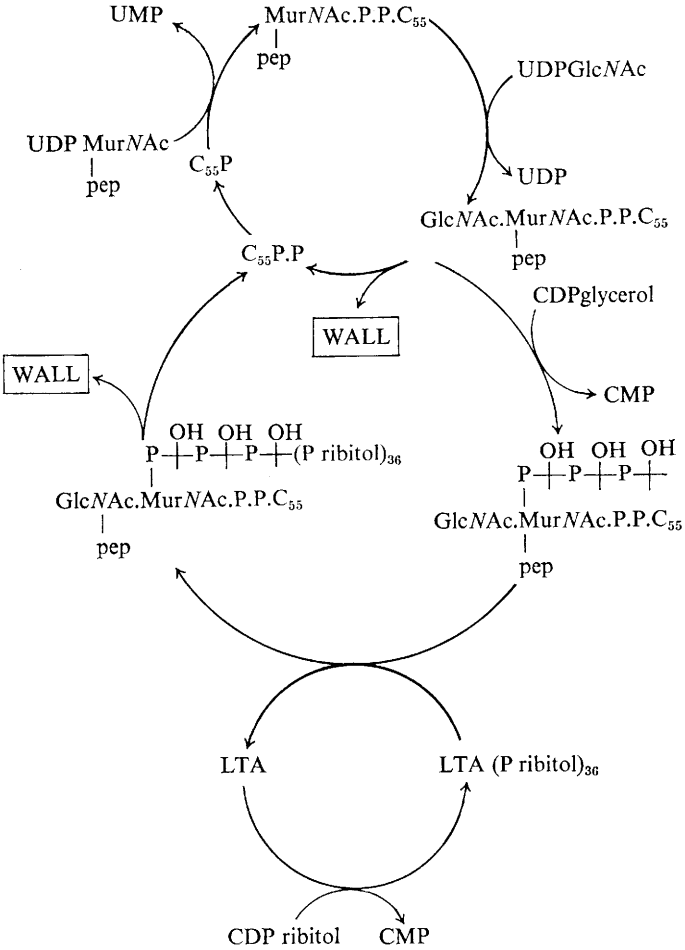
The cell wall of *Sporolactobacillus inulinus* contains a peptidoglycan of the diaminopimelic acid type and a teichoic acid of the ribitol type containing D-galactose and D-glucose, but no alanine.[19] Based on the cell-wall composition, the phylogenic position of *Sporolactobacillus* is considered to lie between the ordinary Lactobacilli and the Bacillaceae.

The location of the D-glucosylated teichoic acid in whole cells and isolated cell walls of *S. faecalis* has been investigated by electron microscopy after staining.[20] The presence of teichoic acid in certain regions of the cell wall could be demonstrated by binding concanavalin A, but not in regions that were densely stained.

A crude cell-wall preparation from *Staph. aureus* H catalysed the synthesis of poly(ribitol phosphate) – which is linked to the cell-wall peptidoglycan – from CDP-ribitol, CDP-glycerol, UDP-2-acetamido-2-deoxy-D-glucose, and ATP.[21] Glycerol phosphate could be incorporated into the cell wall in the absence of CDP-ribitol in a reaction that was greatly stimulated by UDP-2-acetamido-2-deoxy-D-glucose. Poly(ribitol phosphate) appears to be linked to the cell-wall peptidoglycan by an oligomer containing 2-acetamido-2-deoxy-D-glucose and glycerol phosphate. Other work has supported these conclusions.[22] Vancomycin

[15] J. H. Brock and B. Reiter, *Infection and Immunity*, 1976, **13**, 653.
[16] S. Iwata, K. Tochikubo, K. Kato, T. Hirata, S. Kotani, and K. Yagi, *Jap. J. Microbiol.*, 1976, **20**, 123.
[17] C. Endresen and A. Grov, *Acta Pathol. Microbiol. Scand.*, 1976, **84B**, 300.
[18] C. Endresen and A. Grov, *Acta Pathol. Microbiol. Scand.*, 1976, **84B**, 305.
[19] S. Okada, T. Toyoda, M. Kozaki, and K. Kitahara, *J. Agric. Chem. Soc. Japan*, 1976, **50**, 259.
[20] J. M. Garland, A. R. Archibald, and J. Baddiley, *J. Gen. Microbiol.*, 1975, **89**, 73.
[21] R. Bracha and L. Glaser, *J. Bacteriol.*, 1976, **125**, 872.
[22] I. Hancock and J. Baddiley, *J. Bacteriol.*, 1976, **125**, 880.

decreased the incorporation of glycerol from CDP-glycerol into the peptidoglycan by membrane preparations. The synthesis of poly(glycerol phosphate) depends at an early stage on the concomitant synthesis of cell-wall teichoic acid and at a later stage on the synthesis of peptidoglycan. A possible mechanism for the synthesis of the linkage unit and the incorporation of teichoic acid into cell-wall material is illustrated in Scheme 1, which indicates that two distinct lipid carriers are involved in the synthesis of the teichoic acid-linkage unit. The



pep = peptide; C_{55} = polyisoprenol lipid carrier
P = phosphate; LTA = lipoteichoic acid carrier
PP = pyrophosphate; $P\!\!\begin{array}{l}\text{OH}\\|\\-\end{array}$ = glycerol phosphate

Scheme 1

linkage unit is composed of three glycerol phosphate residues that are interposed between the phosphate-terminated end of the ribitol teichoic acid and a muramic acid residue of the peptidoglycan.[23] One of the two acceptors required for the synthesis and attachment of teichoic acid to the cell-wall peptidoglycan is either identical or similar to the membrane teichoic acid and accepts ribitol phosphate residues from CDP-ribitol in building up the main chain of the cell-wall teichoic acid.[24] The synthesis of the poly(ribitol phosphate)-lipoteichoic acid carrier is not inhibited by the antibiotic tunicamycin. The other acceptor receives three residues of glycerol phosphate from CDP-glycerol to form the linkage unit. The main chain of the teichoic acid is transferred from poly(ribitol phosphate)-lipoteichoic acid to the lipid containing the linkage unit, and this reaction is strongly inhibited by tunicamycin. The antibiotic also inhibited the formation of lipid intermediates involved in the synthesis of peptidoglycan by cell-free extracts of *Micrococcus lysodeikticus*.[25]

Peptidoglycans

The biological activities of bacterial peptidoglycans have been reviewed,[26] and the immunoadjuvant activities of a number of synthetic *N*-acetylmuramyl-peptides[27] and of derivatives and analogues of *N*-acetylmuramyldipeptide (*N*-acetylmuramyl-L-alanyl-D-isoglutamine)[28] have been reported.

The effects of endogenous and exogenous factors on the primary structures of peptidoglycans have been reviewed.[29] The structures of peptidoglycans in vegetative cell walls were compared with those in the spore cortex. Changes in the structures of the cell-wall peptidoglycans either during the morphological life-cycle or as a result of genetic variation were also recorded.

A radioimmunoassay based on binding a ^{125}I-labelled hapten (L-Ala-γ-D-Glu-L-Lys-D-Ala-D-Ala) has been developed for measuring antibodies to peptidoglycans in human sera.[30]

The i.r. spectra of a number of peptidoglycans of bacterial cell walls have been reported; the carbohydrate portion of the peptidoglycans is arranged similarly to chitin, and the peptide moiety does not occur in the β-structure.[31]

The synthesis of peptidoglycans by *Agmenellum quadruplicatum* is stimulated during the formation of the cross-walls.[32] There is no correlation between septum formation or cell separation and the rate of turnover of the peptide portion of the peptidoglycan. The peptidoglycans of a new strain of *Arthrobacter*

[23] J. Coley, A. R. Archibald, and J. Baddiley, *F.E.B.S. Letters*, 1976, **61**, 240.
[24] I. C. Hancock, G. Wiseman, and J. Baddiley, *F.E.B.S. Letters*, 1976, **69**, 75.
[25] G. Tamura, T. Sasaki, M. Matsuhashi, A. Takatsuki, and M. Yamasaki, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 447.
[26] B. Heymer, *Klin. Wochenschr.*, 1975, **53**, 81.
[27] S. Kotani, Y. Watanabe, F. Kinoshita, T. Shimono, I. Morisaki, T. Shiba, S. Kusimoto, Y. Tarumi, and K. Ikenaka, *Biken J.*, 1975, **18**, 105.
[28] A. Adam, M. Devys, V. Souvannavong, P. Lefrancier, J. Choay, and E. Lederer, *Biochem. Biophys. Res. Comm.*, 1976, **72**, 339.
[29] K. H. Schleifer, W. P. Hammes, and O. Kandler, *Adv. Microbial Physiol.*, 1976, **13**, 246.
[30] B. Heymer, K. H. Schleifer, S. Read, J. B. Zabriskie, and R. M. Krause, *J. Immunology*, 1976, **117**, 23.
[31] H. Formaneck, K. H. Schleifer, H. P. Seidl, R. Lindemann, and G. Zundel, *F.E.B.S. Letters*, 1976, **70**, 150.
[32] B. F. Dickens and L. O. Ingram, *J. Bacteriol.*, 1976, **127**, 334.

crystallopoieties showed a gradual loss of lysine and a gain of diaminopimelic acid during morphogenesis of the sphere rods.[33]

Critical amounts of cortex (a peptidoglycan layer of bacterial spores) are required for different properties of *Bacillus sphaericus* spores.[34] Characteristic properties of the spores, such as the refractility and the resistance to xylene, octanol, and heat, were measured. Reduction with sodium borotritide and acid hydrolysis, *etc.*, has been used to detect muramic lactam, which is located specifically in the cortical peptidoglycan of bacterial spores.[35] The cortex content of several sporulation-deficient mutants of *B. subtilis* was examined using this procedure.

Two periods of synthesis of the enzymes involved in the synthesis of peptidoglycan precursors occur during sporulation of *B. sphaericus*.[36] The first period involves the synthesis of those enzymes, including L-lysyl ligase, required for the synthesis of the precursor of vegetative-type peptidoglycan, whereas the synthesis of diaminopimelyl ligase and other enzymes needed for the synthesis of the precursor of cortical peptidoglycan occurs during the second period, which precedes the appearance of cortex. The distribution of peptidoglycan synthase activities between sporangia and forespores in sporulating cells of *B. sphaericus* has been measured.[37] A factor that is essential for the synthesis of peptidoglycans has been extracted from toluene-treated cells of *B. megaterium* with lithium chloride; it appears to be a glycoprotein requiring Mg^{2+} ions and, at least, one thiol group for stimulatory activity.[38]

In the presence of D-serine, D-valine, D-threonine, or D-methionine, a number of bacteria synthesized modified peptidoglycan precursors having D-alanyl residues replaced by one of the other D-amino-acids.[39] Many of the modified mucopeptides were not cross-linked, since they are poor substrates in the transpeptidation reaction.

Several organisms with a coryneform morphology contain LL-diaminopimelic acid and arabinose as the principal cell-wall components, whereas it is generally accepted that most pathogenic and commensal strains of Corynebacteriae of animal origin contain *meso*-diaminopimelic acid and arabinogalactan in the cell walls.[40]

Peptidoglycans of the Gram-negative bacteria *Escherichia coli*, *Moraxella glucidolytica*, *Neisseria perflava*, and *Proteus vulgaris* gave identical precipitin reactions, and inhibition studies showed that the antibodies are essentially directed against the peptide portion (2) of the peptidoglycans.[41] The peptide

L-Ala-D-Glu-(L)-*meso*-A_2pm-(L)-D-Ala

(2) A_2pm = 2,2′-diaminopimelic acid

[33] E. P. Previc and N. Lowell, *Biochim. Biophys. Acta*, 1975, **411**, 377.
[34] Y. Imae and J. L. Strominger, *J. Bacteriol.*, 1976, **126**, 907.
[35] Y. Imae and J. L. Strominger, *J. Bacteriol.*, 1976, **126**, 914.
[36] P. E. Linnett and D. J. Tipper, *J. Bacteriol.*, 1976, **125**, 565.
[37] D. J. Tipper and P. E. Linnett, *J. Bacteriol.* 1976, **126**, 213.
[38] A. Taku and D. P. Fan, *J. Biol. Chem.*, 1976, **251**, 1889.
[39] B. Trippen, W. P. Hammes, K. H. Schleifer, and O. Kandler, *Arch. Mikrobiol.*, 1976, **109**, 247.
[40] D. G. Pitcher, *J. Gen. Microbiol.*, 1976, **94**, 225.
[41] H. Nguyen-Huy, C. Nauciel, and C. G. Wermuth, *European J. Biochem.*, 1976, **66**, 79.

reacts more readily when it is not cross-linked, and the carboxy-terminated portion, *meso*-A_2pm-D-Ala, is immunodominant. The results explain the immunological identity of peptidoglycans in Gram-negative bacteria that possess the same peptide subunit. The antibodies cross-reacted weakly with peptidoglycans of Gram-positive bacteria, where *meso*-diaminopimelic acid is replaced by either lysine or homoserine.

The levels of carboxypeptidase and transpeptidase activities involved in the synthesis of peptidoglycans in a thermosensitive division mutant of *E. coli* have been determined.[42] The inhibitory effects of a number of β-lactam antibiotics were also investigated. Low doses of penicillin or growth at restrictive temperatures – neither of which affected transpeptidation – partly inhibited the carboxypeptidase activity and was accompanied by increased incorporation of newly-synthesized peptidoglycan into the pre-existing cell wall. It is proposed that the carboxypeptidase activity regulates the availability of peptidoglycan precursor(s) for attachment to pre-existing peptidoglycan by transpeptidation.

A lytic enzyme, named murein:murein transglycolase, obtained from *E. coli* W7 degraded pure murein sacculi from *E. coli* almost completely into low-mol.-wt. fragments.[43] The principal mucopeptide fragments in the digests were the disaccharide-tripeptide 2-acetamido-2-deoxy-D-glucosyl-*N*-acetylmuramyl-L-alanyl-D-*iso*-glutamyl-*meso*-diaminopimelic acid and the corresponding disaccharide-tetrapeptide 2-acetamido-2-deoxy-D-glucosyl-*N*-acetylmuramyl-L-alanyl-D-*iso*-glutamyl-*meso*-diaminopimelyl-D-alanine. The unique feature of these mucopeptides is that the disaccharides have no reducing end-groups and the muramic acid residues possess an internal (1 → 6)-anhydro-linkage.

In order to assess the role of D-alanyl residues in the donor phase of the synthesis of a cross-linked glycan by *Gaffkya homari*, six analogues of UDP-*N*-acetylmuramyl pentapeptide (UDP-Mur*N*Ac-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala), in which the D-alanyl residues are replaced singly by either D-(α-aminobutyric acid), D-norvaline, or D-valine, have been compared with the reference pentapeptide as substrates in the nascent (penicillin-sensitive) synthesis of peptidoglycan.[44] The penicillin-sensitive enzyme(s), presumably the transpeptidase(s), has a higher specificity for the penultimate D-alanyl residue in the donor phase than for the terminal D-alanyl residue in the cross-linking stage of peptidoglycan synthesis.

A membrane-bound 2-acetamido-2-deoxy-D-glucosyltransferase isolated from *B. megaterium* transferred 2-acetamido-2-deoxy-D-glucose from UDP-2-acetamido-2-deoxy-D-glucose to an *N*-acetylmuramylpentapeptidyl-lipid intermediate (Mur*N*Ac-L-Ala-D-γ-Glu-*meso*-A_2pm-D-Ala-D-Ala-P-P-lipid).[45]

N-Acetylmuramyldipeptide (*N*-acetylmuramyl-L-alanyl-D-*iso*-glutamine) is the smallest adjuvant-active unit of the cell-wall peptidoglycan of bacteria (*e.g.* norcardia, corynebacteria, and anaerobic coryneforms) in the immune system of guinea pigs, but it is not mitogenic in the normal spleen cells of mice.[46] The cell

[42] D. Mirleman, Y. Yashouv-Gan, and U. Schwarz, *Biochemistry*, 1976, **15**, 1781.
[43] J. V. Höltje, D. Mirelman, N. Sharon, and U. Schwarz, *J. Bacteriol.*, 1975, **124**, 1067.
[44] C. V. Carpenter, S. Goyer, and F. C. Neuhaus, *Biochemistry*, 1976, **15**, 3146.
[45] A. Taku and D. P. Fan, *J. Biol. Chem.*, 1976, **251**, 6154.
[46] I. Azuma, K. Sugimura, T. Taniyama, M. Yamawaki, Y. Yamamura, S. Kusimoto, S. Okada, and T. Shiba, *Infection and Immunity*, 1976, **14**, 18.

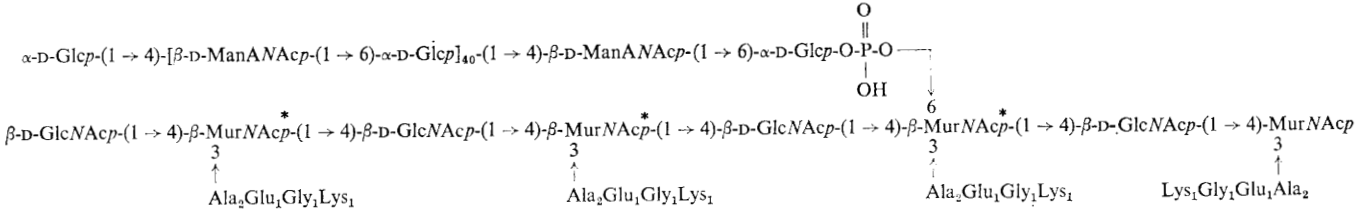
walls of these bacteria, which all contain mucopeptide, have been shown to act as mitogens towards thymus-derived lymphocytes (T cells) and bone-marrow-derived lymphocytes (B cells).[47]

The structures of the soluble and insoluble peptidoglycans obtained from a cell-free preparation from *Micrococcus luteus* in the absence of penicillin have been investigated.[48] The soluble peptidoglycans consisted of a mixture of non-cross-linked polymers of various molecular weights, and peptidoglycans solubilized by trypsin also consisted of a mixture of polymers of various sizes, with a preponderance of non-cross-linked polymers and some bridges with dimer peptides. Peptidoglycans not solubilized by trypsin contained about equal amounts of non-cross-linked and cross-linked peptides; the cross-linkages involve D-alanine and L-lysine or L-alanine residues. Transglycosidase and phosphodiesterase activities have been detected in membrane preparations of *M. luteus*.[49] The transglycosidase activity promoted the *in vitro* synthesis of a non-cross-linked peptidoglycan, which consists of 'soluble' (76%) and 'insoluble' (24%) materials that are completely degraded by lysozyme. Linear strands of the soluble peptidoglycan were found as a single species (mol. wt. 1.9×10^4) containing approximately 20 disaccharide-peptide units. The 'insoluble' peptidoglycan probably represents newly-synthesized material that is incorporated into the existing cell wall. The phosphodiesterase activity cleaved UDP-*N*-acetylmuramylpentapeptide to yield *N*-acetylmuramylpentapeptide, UMP, and inorganic phosphate. A membrane- and cell-wall-derived *N*-acetylmuramyl-L-alanine amidase from *M. luteus*, which is specific for peptidoglycan, functioned in the absence of any detectable transpeptidase activity and may be involved in the formation of cross-bridges between peptidoglycans.[50]

Treatment of cells of *M. luteus* with either D-[^{14}C]- or D-[^{3}H]-glucose in the presence of vancomycin resulted in the accumulation of UDP-2-acetamido-2-deoxy-D-glucose and UDP-*N*-acetylmuramylpentapeptide, which were labelled with ^{14}C or ^{3}H in the amino-sugars.[51]

A fraction from the cell wall of *M. lysodeikticus* obtained by cetylpyridinium precipitation from the non-dialysable portion of the degradation products of egg-white lysozyme has been studied by periodate oxidation and methylation.[52] The fraction (3) consists of a polysaccharide chain composed of about 40 repeating (1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-mannopyranosyluronic acid)-(1 → 6)-*O*-(α-D-glucopyranosyl) residues, with D-glucopyranosyl residues at both ends. The α-D-glucopyranosyl residue at the reducing-end is linked to a phosphate group that is also linked to O-6 of a muramic acid residue of a peptidoglycan chain composed of four repeating (1 → 4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 4)-*O*-[2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranosyl] residues.

[47] I. Azuma, T. Taniyama, K. Sugimura, A. A. Aladin, and Y. Yamamura, *Jap. J. Microbiol.*, 1976, **20**, 263.
[48] G. Pellon, C. Bordet, and G. Michel, *J. Bacteriol.*, 1976, **125**, 509.
[49] S. E. Jensen and J. N. Campbell, *J. Bacteriol.*, 1976, **127**, 309.
[50] S. E. Jensen and J. N. Campbell, *J. Bacteriol.*, 1976, **127**, 319.
[51] D. Mirelman, *Analyt. Biochem.*, 1976, **70**, 424.
[52] Nasir-Ud-Din and R. W. Jeanloz, *Carbohydrate Res.*, 1976, **47**, 245.



(3) D-ManA*NAcp* = 2-acetamido-2-deoxy-D-mannopyranosyluronic acid; the chain composed of D-Glc*p* and D-ManA*NAcp* residues may be linked to any of the three Mur*NAcp* residues marked by an asterisk

The peptidoglycans of all four colonial types of a number of *Neisseria gonorrhoeae* strains have similar structures, but the chain-length of the glycan varies from 80 to 110 disaccharide units.[53]

Penicillin inhibited the synthesis of peptidoglycan by cells of *Nocardia asteroides* and resulted in the accumulation of UDP-*N*-glycolylmuramylpeptide and a mixture of UDP-*N*-acetyl- and UDP-*N*-glycolyl-muramic acids, whereas phosphonomycin inhibited the synthesis of muramic acid and resulted in the accumulation of UDP-2-acetamido-2-deoxy-D-glucose and UDP-2-glycolamido-2-deoxy-D-glucose.[54] However, only muramic acid is *N*-glycolated when the synthesis of peptidoglycans occurs *in vivo* in the absence of an antibiotic.

Although the cell wall of *Spirillum serpens* lost much of its rigidity after infection with *Bdellovibrio bacteriovorus*, the peptidoglycan remained as a discrete cell-wall layer.[55] Comparisons of the abilities of some oligosaccharyl-peptides (obtained from the peptidoglycans of *Staph. aureus* and *Lactobacillus plantarum*) to induce arthritis in rats showed that, at least, one disaccharide unit linked to a peptide is necessary.[56] The presence of teichoic acid did not have any effect.

The ability of specific antibodies to various antigenic determinants of staphylococcal peptidoglycans to neutralize the inhibitory effect of the peptidoglycans on the migration of leucocytes has been investigated.[57] Antibodies to the carboxy-terminal D-alanyl-D-alanine group of the pentapeptides and to the carboxy-terminal amino-acid of the glycine bridge inhibited the migration of leucocytes, whereas antibodies to the tetrapeptide and to the glycan chain had little effect.

Growth of *Staph. aureus* Copenhagen in media deficient in glycine resulted in the accumulation of the nucleotide hexapeptide UDP-Mur*N*Ac*p*-L-Ala-D-Glu-L-Lys-(N^{ϵ}-L-Ala)-D-Ala-D-Ala.[58] The nucleotide hexapeptide was compared with UDP-*N*-acetylmuramylpentapeptide as a substrate in reactions catalysed by phospho-*N*-acetylmuramylpentapeptide translocase and by the peptidoglycan-synthesizing system from *Staph. aureus*.

Data from labelling experiments with the disaccharide-peptide monomers and peptide-cross-linked dimers and trimers found in the peptidoglycan of *Streptococcus faecalis* have shown that the peptide chains of peptidoglycans are cross-linked into dimers and trimers, *etc.*, by a monomer-addition mechanism rather than by random cross-linking.[59] Similarly, the *in vitro* synthesis of a peptide trimer by the extracellular transpeptidase of *Streptomyces* R61 is not a random process.[60] Synthesis occurs preferentially by transpeptidation between a peptide monomer, acting as a donor, and a preformed peptide dimer, acting as an acceptor.

[53] B. H. Hebeler and F. E. Young, *J. Bacteriol.*, 1976, **126**, 1180.
[54] O Gateau, C. Bordet, and G. Michel, *Biochim. Biophys. Acta*, 1976, **421**, 395.
[55] J. E. Snellen and M. P. Starr, *Arch. Mikrobiol.*, 1976, **108**, 55.
[56] O. Kohashi, C. M. Pearson, Y. Watanabe, S. Kotani, and T. Koga, *J. Immunology*, 1976, **116**, 1635.
[57] A. Grov, *Acta Pathol. Microbiol. Scand.*, 1976, **84B**, 315.
[58] J. C. Swenson and F. C. Neuhaus, *J. Bacteriol.*, 1976, **125**, 626.
[59] E. H. Oldmixon, P. Dezélée, M. C. Ziskin, and G. D. Shockman, *European J. Biochem.*, 1976, **68**, 271.
[60] J. M. Frère, J. M. Ghuysen, A. R. Zeiger, and H. R. Perkins, *F.E.B.S. Letters*, 1976, **63**, 112.

A pneumococcal *N*-acetyl-L-alanine amidase has been purified to biochemical homogeneity.[61] Lipoteichoic acid is a non-competitive inhibitor of the enzyme.

Lipopolysaccharides

Improved procedures for the preparation of lipopolysaccharides involve prior treatment of the cells with either lysozyme or H_4edta.[62] The separation of lipopolysaccharides by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate has been shown to depend on the molecular size and not on the intrinsic charge.[63] The heterogeneity of several lipopolysaccharide preparations from rough *E. coli* 08$^-$, semi-rough *Salmonella typhimurium*, and smooth strains of *E. coli* 08 and *Citrobacter* has been demonstrated by gel electrophoresis in the presence of sodium dodecyl sulphate, which is recommended for the molecular characterization of enterobacterial lipopolysaccharides.[64] Treatment with sodium hydroxide in DMSO at *ca.* 100 °C has been used to de-*N*-acetylate lipopolysaccharides,[65] and labile acyl groups in lipopolysaccharides have been replaced with stable methyl ether substituents.[66] Regardless of the chain-length and type of linkages, lipopolysaccharides from various bacterial groups contain D-3-hydroxy-fatty acids that serve as markers for the lipid A.[67] An improved micromethod, which is based on passive haemolysis inhibition, has been described for the quantitative assay of antigenerically active lipopolysaccharides and their immunodeterminant oligosaccharides.[68]

Shigella sonnei lipopolysaccharides or their degradation products immobilized on Sepharose have been used in the purification of homologous antisera.[69] Coupling of the lipopolysaccharides to Sepharose yielded a receptor that specifically adsorbed bacteriophage. When the binding of the bacteriophage is reversible, a significant proportion of the active phage particles can be eluted.

Techniques for reconstituting the lipopolysaccharide–phospholipid transferase complexes in the envelopes of bacterial cells have been described.[70]

The absence of immunogenicity from the enterobacterial common antigen in various *Shigella* and *E. coli* rough mutants has been ascribed to a defective gene that is involved in the synthesis of lipopolysaccharides.[71] *ilv*-Linked *rfe* genes participate in the synthesis of the enterobacterial common antigen and of certain types of O antigens in various *E. coli* serotypes.[72] This and previous work suggest that different mechanisms may exist for the biosynthesis of O-antigenic polysaccharides among the Enterobacteriaceae.

[61] J. V. Höltje and A. Tomasz, *J. Biol. Chem.*, 1976, **251**, 4199.
[62] K. G. Johnson and M. B. Perry, *Canad. J. Microbiol.*, 1976, **22**, 29.
[63] R. R. B. Russell and K. G. Johnson, *Canad. J. Microbiol.*, 1975, **21**, 2013.
[64] B. Jann, K. Reske, and K. Jann, *European J. Biochem.*, 1975, **60**, 234.
[65] C. Erbing, K. Granath, L. Kenne, and B. Lindberg, *Carbohydrate Res.*, 1976, **47**, C5.
[66] G. R. Gray, *Methods in Carbohydrate Chem.*, 1976, **7**, 157.
[67] E. T. Rietschel, *European J. Biochem.*, 1976, **64**, 423.
[68] T. Kontrohr and K. K. Péterffy, *J. Immunol. Methods*, 1976, **13**, 271.
[69] E. Romanowska, C. Lugowski, and M. Mulczyk, *F.E.B.S. Letters*, 1976, **66**, 82.
[70] L. Rothfield and A. Hinkley, *Methods in Enzymology*, 1974, **32B**, 449.
[71] G. Schmidt, D. Männel, H. Mayer, H. Y. Whang, and E. Neter, *J. Bacteriol.*, 1976, **126**, 579.
[72] G. Schmidt, H. Mayer, and P. H. Mäkelä, *J. Bacteriol.*, 1976, **127**, 755.

The lipopolysaccharide of the blue-green bacterium *Agmenellum quadruplicatum* is similar to other Gram-negative lipopolysaccharides in that it contains residues of 2-amino-2-deoxy-D-glucose, 3-deoxy-2-octulosonic acid, and phosphorus in the lipid A region and residues of D-glucose, L-rhamnose, D-mannose, and D-xylose in the polysacccharide region.[73] However, t.l.c. demonstrated that there are differences between this lipopolysaccharide and those of *E. coli* and *Salmonella typhimurium*. The O antigen extracted from whole cells of *Bacteriodes fragilis* with phenol–water has been purified by gel filtration and chromatography on DEAE-cellulose and hydroxyapatite.[74] 1,6-Anhydro-L-*glycero*-D-*manno*-heptopyranose is formed on acidic hydrolysis or methanolysis of an L-*glycero*-D-*manno*-heptose-containing polysaccharide from *Bordetella pertussis* endotoxin.[75]

Since cellular and free lipopolysaccharides obtained from *Neisseria catarrhalis* and *Branhamella catarrhalis* contain identical core oligosaccharides and lipid A components, it is proposed that *N. catarrhalis* should be reclassified under the genus *Branhamella*.[76]

A method based on inhibition of agglutination has been used to differentiate serologically between *Brucella abortus* and *Yersinia enterocolitica*.[77] An antigen isolated from *B. abortus* consists of protein, phosphoglyceride (but no lipid A), and a polysaccharide containing residues of D-glucose, D-mannose, 2-acetamido-2-deoxy-D-glucose, and 3-deoxy-2-octulosonic acid.

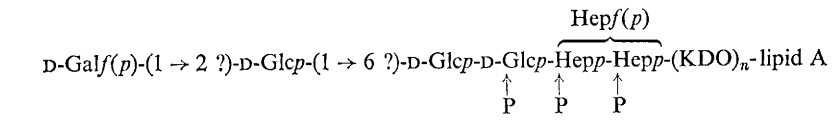
The lipopolysaccharide of *Chromatium vinosum* contains 3-*O*-methyl-D-ribose, D-ribose, L-arabinose, D-glucose, and 2-amino-2-deoxy-D-mannose, and smaller proportions of D-rhamnose, 3-deoxy-2-octulosonic acid, and 2-amino-2,6-dideoxy-D-glucose (quinovosamine).[78] D-*glycero*-D-*manno*-Heptose, but not L-*glycero*-D-*manno*-heptose, was also tentatively identified. The composition of the lipopolysaccharide from *Coxiella burneti* has been reported.[79]

Bacteriophage-resistant mutants of *E. coli* K-12 have been classified into six distinct types based on the compositions of the carbohydrate portion of the lipopolysaccharide core.[80] The composition of the membrane protein is also different in some of the mutants. The lipopolysaccharide of *E. coli* K-12, strain CR34, contains residues of D-galactose, D-glucose, 2-amino-2-deoxy-D-glucose, L-*glycero*-D-*manno*-heptose, 3-deoxy-2-octulosonic acid, and lipid A.[81] The core region, which contains no amino-sugar, is composed of D-galactose, D-glucose, and heptose. Methylation studies, oxidation with periodate, and partial hydrolysis with acid indicate a partial structure (4) for the *E. coli* K-12 core polysaccharide. Two unusual features of the structure (4) are the presence of a phosphated D-glucosyl residue and unsubstituted heptofuranosyl residues.

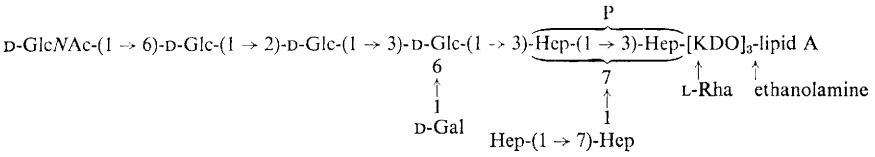
Various core oligosaccharides have been isolated from the lipopolysaccharides of cell-wall-defective mutants of *E. coli* K-12 that are resistant to ampicillin.[82]

[73] T. M. Buttke and L. O. Ingram, *J. Bacteriol.*, 1975, **124**, 1566.
[74] T. Hofstad, *Acta Pathol. Microbiol. Scand.*, 1976, **84B**, 229.
[75] R. Chaby and P. Szabó, *Carbohydrate Res.*, 1976, **49**, 489.
[76] K. G. Johnson, I. J. McDonald, and M. B. Perry, *Canad. J. Microbiol.*, 1976, **22**, 460.
[77] A. Mark, R. Sandulache, A. Pop, and A. Cerbu, *Ann. Microbiol.*, 1975, **126B**, 435.
[78] R. E. Hurlbert, J. Weckesser, H. Mayer, and I. Fromme, *European J. Biochem.*, 1976, **68**, 365
[79] M. L. Chan, J. McChesney, and D. Paretsky, *Infection and Immunity*, 1976, **13**, 1721.
[80] R. E. W. Hancock and P. Reeves, *J. Bacteriol.*, 1976, **127**, 98.
[81] J. P. Benedetto, M. Bruneteau, and G. Michel, *European J. Biochem.*, 1976, **63**, 313.
[82] P. Prehm, S. Stirm, B. Jann, K. Jann, and H. G. Boman, *European J. Biochem.*, 1976, **66**, 369.

Methylation analyses of the oligosaccharides suggested the tentative structure (5) for the lipopolysaccharides. Immunochemical studies of the lipopolysaccharides from wild-type and mutants of *E. coli* K-12 indicated that L-rhamnose, which occurs in the innermost part of the core as a side-chain substituent of 3-deoxy-2-octulosonic acid, is the immunodominant sugar.[83]



(4) KDO = 3-deoxy-2-octulosonic acid; Hep = heptose; P = phosphate



(5)

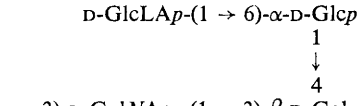
An *E. coli* mutant that lacks UDP-D-galactose 4-epimerase synthesized a lipopolysaccharide containing less carbohydrate than that of the parent organism, unless D-galactose was present in the medium during growth.[84] Measurements on membrane fragments containing carbohydrate-rich and -deficient lipopolysaccharides showed that the lipopolysaccharide is inserted into the outer membrane of the organism in a random manner at 10—22 discrete 'insertion points'.

The O-specific polysaccharide of '*Shigella*-like' *E. coli* 0124 has been characterized by ^{1}H n.m.r. spectroscopy and electrophoresis and by its optical rotation.[85] The polysaccharide contains residues of D-glucose, D-galactose, 2-acetamido-2-deoxy-D-galactose, and 4-*O*-(1-carboxyethyl)-D-glucopyranose (D-glucolactilic acid) in the molecular proportions 1 : 2 : 1 : 1. Fragmentation and methylation analyses of the polysaccharide suggested the repeating structure (6), which is identical to that of the somatic polysaccharide of *Shigella dysenteriae* type 3.[85, 85a] The anomeric configurations assigned to the polysaccharide (6) are tentative, and are based on those found in the polysaccharide from *Sh. dysenteriae* type 3.

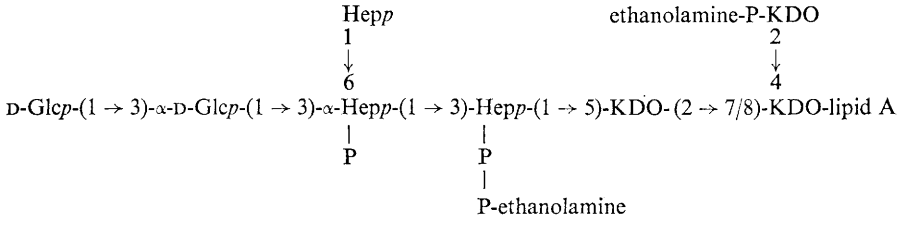
Wheat-germ agglutinin and concanavalin A inhibited the adsorption of bacteriophage T4D to *E. coli* B lipopolysaccharide, but it was undecided whether

[83] H. Mayer, A. M. C. Rapin, G. Schmidt, and H. G. Boman, *European J. Biochem.*, 1976, **66**, 357.
[84] C. F. Kulpa and L. Leive, *J. Bacteriol.*, 1976, **126**, 467.
[85] B. A. Dmitriev, L. V. Lvov, N. K. Kochetkov, B. Jann, and K. Jann, *European J. Biochem.*, 1976, **64**, 491.
[85a] B. A. Dmitriev, L. V. Backinowsky, L. V. Lvov, and N. K. Kochetkov, *European J. Biochem.*, 1975, **50**, 539.

D-glucose or 2-acetamido-2-deoxy-D-galactose is the receptor on the polysaccharide.[86] Mutants of *E. coli* B with increasing structural defects in the cell-wall lipopolysaccharide (7) have been tested as receptors for the bacteriophages T3 and T4.[87] The absence of the terminal D-glucosyl residue or of the residues (*i.e.* heptose, phosphate, and pyrophosphorylethanolamine) on the two chain heptoses had little effect on the susceptibilities to T3 and T4, but mutants lacking both D-glucosyl residues were resistant to these bacteriophages.

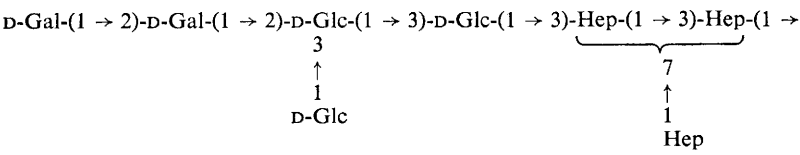


(6) GlcLA*p* = 4-*O*-(1-carboxyethyl)-D-glucopyranose



(7)

Mutants of *E. coli* have been selected for their resistance towards a set of bacteriophages specific for cell-wall lipopolysaccharides.[88] A number of lipopolysaccharides isolated from *E. coli* C and mutants thereof with increasing carbohydrate-deficient core regions were tested for inactivation of bacteriophage ϕX174. Structural analysis of the core oligosaccharides indicated that loss of the terminal D-galactosyl residue from the basic structure (8) produced resistance to adsorption of this bacteriophage.



(8)

The 09 antigen on *E. coli* 09 consists of a mannan, which is bound to the core oligosaccharide, and lipid A.[89] The biosynthesis of 09 mannan occurs by a single-chain mechanism that does not use lipid-bound oligosaccharides, and thus differs from that of other microbial and some plant and mammalian systems.

[86] T. Watanabe, *Canad. J. Microbiol.*, 1976, **22**, 745.
[87] P. Prehm, B. Jann, K. Jann, G. Schmidt, and S. Stirm, *J. Mol. Biol.*, 1976, **101**, 277.
[88] U. Feige and S. Stirm, *Biochem. Biophys. Res. Comm.*, 1976, **71**, 566.
[89] H. J. Kopmann and K. Jann, *European J. Biochem.*, 1975, **60**, 587,

The results of methylation analysis and enzymic and Smith degradations indicated that the mannan consists of the pentasaccharide repeating unit (9) joined by α-(1 → 3)-linkages.[90]

→ 3)-α-D-Man-(1 → 3)-α-D-Man-(1 → 2)-α-D-Man-(1 → 2)-α-D-Man-(1 → 2)-α-D-Man-(1 →
(9)

Esterification of the available hydroxy-groups of the lipopolysaccharide of *E. coli* K235 with *o*-phthalic anhydride markedly altered the spectrum of biological properties, most of which are normally attributed to the lipid A moiety.[91]

The effects of changes in the lipopolysaccharides and of the lipid : protein ratio on the morphology of freeze-etched envelopes of *E. coli* have been studied.[92] There appears to be a morphological correlation between the chemical data relating the amount of outer-membrane protein and the heptose content of the lipopolysaccharide.

The mitogenic properties of *E. coli* are destroyed by mild alkaline treatment of the lipopolysaccharide, whereas the antigenic properties are unchanged.[93] It was also shown that binding of the polysaccharide portion of the lipopolysaccharide to antigenic receptors on the surface of B cells is necessary for triggering off a primary immune response to lipopolysaccharides.

Two rough mutants that lack the capsular and lipopolysaccharide O-antigen polysaccharides have been isolated from *Klebsiella aerogenes* by phage selection.[94] Loss or alteration of the phage-receptor sites through mutation resulted in loss of the galactan portion of the lipopolysaccharide molecule, which is analogous to the Salmonella O-antigen polysaccharides. Studies of the incorporation of sugar nucleotides by wild type *K. aerogenes* and by one of the mutants into lipid intermediates and polymers indicated that transferases are present in all membranes, while polymerases are absent from non-mucoid and rough mutants.

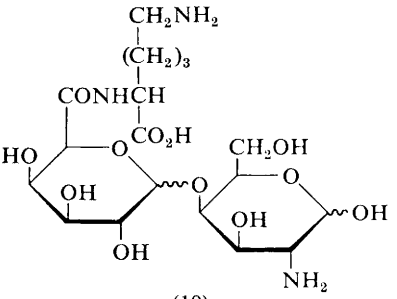
Lipopolysaccharides have been extracted from bacillary forms of several Myxococcus strains, but they could not be extracted from microcysts, which contain mucopeptides and some form of polysaccharide.[95] The preparations did not contain heptose or *O*-methyl-D-xylose, although a methylated pentose has been found in the lipopolysaccharides from other Myxobacteria (I. W. Sutherland and M. L. Smith, *J. Gen. Microbiol.*, 1973, **74**, 259), and the monosaccharides detected in hydrolysates of the lipopolysaccharides revealed differences among the strains.

A study of the lipopolysaccharides isolated from strains of *Neisseria gonorrhoeae* types 1 (T1) and 4(T4) colony forms indicated that strains of T4 cells synthesize a common 'R'-type lipopolysaccharide, whereas T1 cells synthesize 'S'-type lipopolysaccharides having distinct O-polysaccharide structures, which probably account for serologically differentiated strains of *N. gonorrhoeae*.[96]

[90] P Prehm, B. Jann, and K. Jann, *European J. Biochem.*, 1976, **67**, 53.
[91] F. C. McIntire, M. P. Hargie, J. R. Schenck, R. A. Finley, H. W. Sievert, E. T. Rietschel, and D. L. Rosenstreich, *J. Immunology*, 1976, **117**, 674.
[92] M. E. Bayer, J. Koplow, and H. Goldfine, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 5145.
[93] W. J. Poe and J. G. Michael, *Immunology*, 1976, **30**, 241.
[94] I. R. Poxton and I. W. Sutherland, *J. Gen. Microbiol.*, 1976, **96**, 195.
[95] I. W. Sutherland and S. Thomson, *J. Gen. Microbiol.*, 1975, **89**, 124.
[96] M. B. Perry, V. Daoust, B. B. Diena, F. E. Ashton, and R. Wallace, *Canad. J. Biochem.*, 1975, **53**, 623.

More than one type of core oligosaccharide are present in the lipopolysaccharides of non-pathogenic species of *Neisseria*.[97] Whereas types II and III core oligosaccharides are typical of those of the lipopolysaccharides from other bacteria, the type I core oligosaccharide is unusual in that it contains no heptose or amino-sugar and is composed of a branched structure of α-D-glucopyranosyl residues terminated by a reducing end-group of 3-deoxy-D-*manno*-octulosonic acid.

The L-form protoplasts of *Proteus mirabilis* synthesize two types of lipopolysaccharide, which have identical carbohydrate compositions, but differ in the relative proportions of fatty acids in the lipid A moieties.[98] A fragment (10) containing D-galactopyranosyluronic acid, 2-amino-2-deoxy-D-galactose, and lysine has been isolated from the lipopolysaccharide of *P. mirabilis* 1959.[99] Serological studies indicated that structure (10) is an essential part of the serological determinant of *P. mirabilis*. The chemical composition of lipopolysaccharides obtained from twenty serogroups of *Proteus vulgaris* has been used as a basis for classifying the lipopolysaccharides into seven chemotypes.[100]



(10)

2,3-Diamino-2,3-dideoxy-D-glucopyranose has been isolated from the lipopolysaccharide of *Rhodopseudomonas viridis*; it was identified by c.d. spectroscopy.[101]

Cell walls and lipopolysaccharide preparations of *Rhizobium trifolii* have been studied as receptors in order to find the site of attachment of bacteriophage 1P.[102] Destruction of the L-*glycero*-D-*manno*-heptose residues of the lipopolysaccharide was accompanied by inactivation of the bacteriophage.

The physical state of lipopolysaccharides is important in the interaction with complement, and a high molecular weight is essential for the expression of anti-complement activity.[103]

The degradation of lipopolysaccharides by a procedure involving hydrolysis with alkali and acid, reduction with sodium borohydride, hydrazinolysis, and

[97] K. G. Johnson, M. B. Perry, I. J. McDonald, and R. R. B. Russell, *Canad. J. Microbiol.*, 1975, **21**, 1969.
[98] J. Gmeiner and H. H. Martin, *European J. Biochem.*, 1976, **67**, 487.
[99] W. Gromska and H. Mayer, *European J. Biochem.*, 1976, **62**, 391.
[100] Z. Sidorczyk, W. Kaca, and K. Kotelko, *Bull. Acad. polon. Sci., Ser Sci. Biol.*, 1975, **23**, 603.
[101] G. Keilich, J. Roppel, and H. Mayer, *Carbohydrate Res.*, 1976, **51**, 129.
[102] E. Zajac, R. Russa, and Z. Lorkiewicz, *J. Gen. Microbiol.*, 1975, **90**, 365
[103] C. Galanos and O. Lüderitz, *European J. Biochem.*, 1976, **65**, 403.

acetylation has allowed the backbone of the lipid A component to be isolated.[104] The lipid A backbone of all smooth forms and rough mutants of *S. minnesota*, *Sh. flexneri*, *E. coli*, *Xanthomonas sinensis*, and *Rhodopseudomonas gelatinosa* was found to contain 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 6)-2-acetamido-2-deoxy-D-glucosyl units and two phosphate groups, one of which is glycosidically linked and the other is attached to one of the hydroxy-groups of the non-reducing residue.

The O antigen extracted from *S. typhi* with trichloroacetic acid precipitated more antibodies from anti-(*S. typhi*) sera than did the degraded polysaccharide extracted with acetic acid.[105] The heterogeneity of lipopolysaccharides from strains of *S. typhi* isolated from carriers and patients with typhoid fever has been demonstrated by immunoelectrophoresis.[106]

The genetic transfer of *Salmonella* O antigens to *E. coli* 08 has been followed by chemical analysis of the lipopolysaccharide of the hybrid, which contained an *E. coli* 08 lipopolysaccharide (O antigen) and an *S. typhimurium*-specific lipopolysaccharide with only one O-specific repeating unit.[107] The relationship between the enterobacterial common antigen and various mutants of *S. typhimurium* has been discussed.[108] Electron microscopy, in conjunction with compositional studies, has been used to produce a model for the structure of the outer membrane of *S. typhimurium*.[109, 110] The existence of cross-reactive antigenic sites has been demonstrated in the lipid portion of the lipopolysaccharide from *S. typhimurium*.[111] Differences have been detected in the chemical structures, and in the biological effects, of the lipids from a number of Re mutants of Gram-negative bacteria that do not contain heptose residues in the lipopolysaccharides.[112]

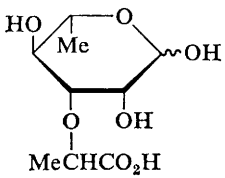
Lipopolysaccharides prepared from strains of *Serratia marcescens* that are resistant to polymyxin have higher carbohydrate and lower protein contents than do those of normal strains, both before, and after, treatment with the antibiotic.[113] The converse is true of lipopolysaccharides obtained from cells sensitive to polymyxin B. Two lipopolysaccharides (one of mol. wt. 1.2×10^4) were detected after polyacrylamide gel electrophoresis of the outer membranes of *S. marcescens*.[114] Staining with a carbocyanine dye was found to be more sensitive and specific for lipopolysaccharides than other staining techniques in general use.

The results of selective cleavage, methylation, and oxidation with chromic oxide have shown that the repeating unit of the polysaccharide chain of the lipopolysaccharide from *Sh. dysenteriae* is 2-acetamido-2-deoxy-β-D-galactosyl-(1 → 3)-α-D-galactopyranosyl-(1 → 6)-α-D-glucopyranose, to which is attached

[104] S. Hase and E. T. Rietschel *European J. Biochem.*, 1976, **63**, 101.
[105] C. Nivet and A. M. Staub, *Immunochemistry*, 1976, **13**, 539.
[106] T. Strýcková and G. Farbakyová, *Folia Microbiol.*, 1976, **21**, 21.
[107] W. Kiefer, G. Schmidt, B. Jann, and K. Jann, *J. Gen. Microbiol.*, 1976, **92**, 311.
[108] P. H. Mäkelä, G. Schmidt, H. Mayer, H. Nikaido, H. Y. Whang, and E. Neter, *J. Bacteriol.*, 1976, **127**, 1141.
[109] J. Smit, Y. Kamio, and H. Nikaido, *J. Bacteriol.*, 1975, **124**, 942.
[110] J. Smit, Y. Kamio, and H. Nikaido, *J. Bacteriol.*, 1976, **125**, 1243.
[111] A. K. Ng, C. H. Chen, C. M. Chang, and A. Nowotny, *J. Gen. Microbiol.*, 1976, **94**, 107.
[112] A. K. Ng, R. C. Butler, C. H. Chen, and A. Nowotny, *J. Bacteriol.*, 1976, **126**, 511.
[113] J. C. Tsang, D. A. Brown, and D. A. Weber, *Microbios*, 1976, **14**, 43.
[114] J. C. Tsang, D. A. Brown, and D. M. Kranz, *Microbios Letters*, 1976, **1**, 209.

an unidentified acidic component at O-4 of the D-galactopyranosyl residue.[115] The corresponding lipopolysaccharide from *Sh. dysenteriae* type 5 contains 3-*O*-[(*R*)-1-carboxyethyl]-L-rhamnose (11), whose structure was established by physical methods and synthesis.[116]

The O-specific polysaccharide of *Sh. flexneri* type 6 contains residues of L-rhamnose and 2-acetamido-2-deoxy-D-galactose and also *O*-acetyl groups, although other reports have claimed that the amino-sugar derivative is



(11)

2-acetamido-2-deoxy-D-glucose.[117] Serological studies indicated that the sequence 2-acetamido-2-deoxy-3-*O*-β-L-rhamnosyl-β-D-galactose is responsible for type 6 specificity, although the presence of *O*- and *N*-acetyl-groups is not required for type 6 specificity.

A number of rough mutants obtained from *Sh. flexneri* and related bacteriophage receptors have been classified into chemotypes based on chemical analysis of the lipopolysaccharides.[118] The results suggest that all *Sh. flexneri* serotypes contain a common core. The receptor sites for several bacteriophages have been found to vary from the entire core structure of the lipopolysaccharide to a single monosaccharide residue.[119]

Endotoxins have been isolated from *Sphaerophorus funduliformis* and *Fusobacterium necrophorum*.[120] The polysaccharide component of the toxin, which contains acid-labile residues of L-fucose and D-galactose, is responsible for the serological activity.

In addition to several unidentified sugars, the lipopolysaccharide from *Spirillum serpens* contains L-*glycero*-D-*manno*-heptose, L-rhamnose, 2-amino-2-deoxy-D-glucose, ethanolamine, and phosphate, but 3-deoxy-2-octulosonic acid is absent.[121]

The O-specific polysaccharide of the lipopolysaccharide from *Yersinia pseudotuberculosis* type IB contains residues of D-mannose, L-fucose, 3,6-dideoxy-D-*ribo*-hexose (paratose), and 2-acetamido-2-deoxy-D-glucose.[122] The repeating unit (12) of the polysaccharide was assigned on the basis of methylation analysis,

[115] B. A. Dmitriev, Y. A. Knirel, I. L. Gofman, and N. K. Kochetkov, *Ivest. Akad. Nauk S.S S.R., Ser. khim.*, 1975, **24**, 2185.
[116] N. K. Kochetkov, B. A. Dmitriev, and L. V. Backinowsky, *Carbohydrate Res.*, 1976, **51**, 229.
[117] E. Katzenellenbogen, M. Mulczyk, and E. Romanowska, *European J. Biochem.*, 1976, **61**, 191.
[118] E. Hannecart-Pokorni, G. Godard, and J. Beumer, *Ann. Microbiol.*, 1976, **127B**, 3.
[119] E. Hannecart-Pokorni, G. Godard, and J. Beumer, *Ann. Microbiol.*, 1976, **127B**, 15.
[120] F. Meisel-Mikolajczyk and E. Kostrzewska, *Bull. Acad. polon. Sci., Ser. Sci. Biol.*, 1975, **23**, 683.
[121] I. R. Chester and R. G. E. Murray, *J. Bacteriol.*, 1975, **124**, 1168.
[122] S. V. Tomshich, R. P. Gorshkova, Y. N. Elkin, and Y. S. Ovodov, *European J. Biochem.*, 1976, **65**, 193.

Par*f*
1
↓
3
→ 2)-D-Man*p*-(1 → 4)-D-Man*p*-(1 → 3)-L-Fuc*p*-(1 → 3)-D-Glc*N*Ac*p*-(1 →

(12) Par = paratose

oxidation with periodate, and partial hydrolysis with acid. The O-specific polysaccharide of *Y. pseudotuberculosis* type 6 contains 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, 3,6-dideoxy-L-*xylo*-hexose (colitose), and an unidentified monosaccharide.[123] The cross-reactivity with *S. greenside* antiserum suggested that colitose is the immunodominant sugar of the lipopolysaccharide.

The cell-wall lipopolysaccharide of *Vibrio cholerae* contains neither 3-deoxy-2-octulosonic acid (as do most other enterobacterial lipopolysaccharides) nor D-galactose.[124]

Capsular Polysaccharides

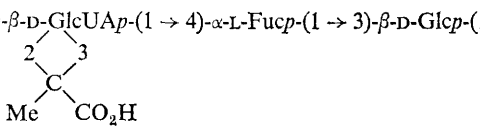
Sugars etherified with lactic acid appear to be more common than was earlier supposed. Virulent strains of *Aerococcus viridans* var. *homari* (formerly *Gaffkya homari*) are heavily encapsulated; an acidic polysaccharide in the capsular material contains 4-*O*-[(*S*)-1-carboxyethyl]-D-glucose, which was isolated following acidic hydrolysis of the polysaccharide.[125] The carboxy-reduced polysaccharide afforded 4-*O*-[(*S*)(1-hydroxy)prop-2-yl]-D-glucose on hydrolysis with acid. A related sugar component of the capsular polysaccharide of *Klebsiella* type 37 has been identified as 4-*O*-[(*S*)-1-carboxyethyl]-D-glucuronic acid.[126]

Pneumococcal polysaccharides have been detected in the sputum of patients with pneumococcal pneumonia by counter-immunoelectrophoresis.[127] D-Galacturonic acid has been identified as a component of pneumococcal type XXV polysaccharide.[128]

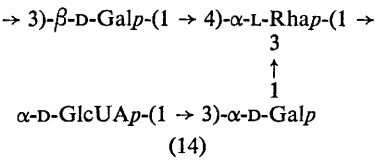
Oligosaccharides isolated from pneumococcal type III polysaccharide have been used as ligands to induce conformational changes in the Fab fragment of homogeneous antibodies to the polysaccharide.[129] Circular polarization of luminescence emitted by the tryptophanyl residues provided a sensitive probe for measuring the ligand-induced changes, and the largest changes were recorded on interaction of the Fab fragments with the intact, soluble type III polysaccharide.[130]

The capsular polysaccharide from *Klebsiella* type 1, which contains equimolar proportions of D-glucose, L-fucose, D-glucuronic acid, and pyruvate, is composed of the trisaccharide repeating unit (13).[131]

[123] R. P. Gorshkova, V. A. Zubkov, and Y. S. Ovodov, *Immunochemistry*, 1976, **13**, 581.
[124] S. Raziuddin and T. Kawasaki, *Biochim. Biophys. Acta*, 1976, **431**, 116.
[125] L. Kenne, B. Lindberg, B. Lindqvist, J. Lönngren, B. Arie, R. G. Brown, and J. E. Stewart, *Carbohydrate Res.*, 1976, **51**, 287.
[126] B. Lindberg, B. Lindqvist, J. Lönngren, and W. Nimmich, *Carbohydrate Res.*, 1976, **49**, 411.
[127] C. A. Perlino and J. A. Shulman, *J. Lab. Clin. Med.*, 1976, **87**, 496.
[128] A. Das, M. Heidelberger, and R. Brown, *Carbohydrate Res.*, 1976, **48**, 304.
[129] J. C. Jaton, H. Huser, Y. Blatt, and I. Pecht, *Biochemistry*, 1975, **14**, 5308.
[130] J. C. Jaton, H. Huser, D. G. Braun, D. Givol, I. Pecht, and J. Schlessinger, *Biochemistry*, 1975, **14**, 5312.
[131] C. Erbing, L. Kenne, B. Lindberg, J. Lönngren, and I. W. Sutherland, *Carbohydrate Res.*, 1976, **50**, 115.



(13)



(14)

Methylation analysis, Smith degradation, and ^{1}H n.m.r. spectroscopy of *Klebsiella* 6412 polysaccharide and of the degraded polysaccharide have identified the tetrasaccharide repeating unit (14).[132] Studies of the cross-reactions between *Klebsiella* K polysaccharides and anti-*Klebsiella* antibodies in sera have been reported.[133]

A high-molecular-weight, partially *O*-acetylated meningococcal polysaccharide of serogroup Y contains equimolar proportions of D-glucose and *N*-acetylneuraminic acid that are linked through O-6 of the D-glucosyl residues and O-4 of the *N*-acetylneuraminic acid residues.[134] Serogroup W135 polysaccharide, which does not contain *O*-acetyl groups, has a structure related to the serogroup Y polysaccharide, except that D-glucose is replaced by D-galactose. G.l.c.–m.s. analysis of the methylated polysaccharides was used to identify (2 → 9)-linked sialic acid residues in the serogroup C polysaccharide and (1 → 4)-linked sialic acid residues in the serogroup Y and W135 polysaccharides.[135] The c.d. spectra of several polysaccharides containing sialic acid isolated from *N. meningitidis* are influenced by the state of ionization of the carboxy-group of the sialic acid residue, the location within the individual sialic acid residues of the inter-saccharide linkages, and changes in the configuration of hydroxy-groups remote from the carboxy-group of the sialic acid.[136]

Ion-exchange chromatography of the capsular polysaccharide of *Staph. aureus*, which contains residues of D-glucose, D-galactose, D-mannose, D-xylose, and uronic acid, has shown that it is heterogeneous.[137]

Extracellular and Intracellular Polysaccharides

The signals in the ^{13}C n.m.r. spectra of two cyclodextrins have been completely assigned.[138] The crystal structure of the complex formed between cyclohexaamylose and 4-iodophenol has been reported.[139] Fifteen products have been

[132] B. Lindberg and W. Nimmich, *Carbohydrate Res.*, 1976, **48**, 81.
[133] M. Heidelberger, W. Nimmich, J. Eriksen, G. G. S. Dutton, S. Stirm, and C. T. Fang, *Acta Pathol. Microbiol. Scand.*, 1975, **83C**, 397.
[134] A. K. Bhattacharjee, H. J. Jennings, C. P. Kenny, A. Martin, and I. C. P. Smith, *Canad. J. Biochem.*, 1976, **54**, 1.
[135] A. K. Bhattacharjee and H. J. Jennings, *Carbohydrate Res.*, 1976, **51**, 253.
[136] H. J. Jennings and R. E. Williams, *Carbohydrate Res.*, 1976, **50**, 257.
[137] G. Seltmann and W. Beer, *Z. allgem. Mikrobiol.*, 1976, **16**, 445.
[138] P. Colson, H. J. Jennings, and I. C. P. Smith, *J. Amer. Chem. Soc.*, 1974, **96**, 8081.
[139] K. Harata, *Carbohydrate Res.*, 1976, **48**, 265.

identified following γ-irradiation of polycrystalline cycloamylose hydrates.[140] Established free-radical mechanisms accounted for the products.

A microchemical method for determining the DP of polysaccharides has been used to study the size of dextran molecules,[141] and the molecular-weight distribution of dextrans has been determined by permeation chromatography on porous glass.[142] A semi-automated method, which uses D-glucose oxidase and anthrone, has been developed for the analysis of dextran in the presence of free D-glucose.[143] A quantitative, highly sensitive method for estimating dextran uses polymer-enhanced immunonephelometry.[144] ^{13}C n.m.r. spectra have been recorded for a series of dextrans of known structures and known degrees of branching.[145] It was shown that the 75—85 p.p.m. region of the spectra can be used to reveal the presence of α-(1 → 2)-, α-(1 → 3)-, and α-(1 → 4)-D-glucopyranosyl linkages.

The microheterogeneity of dextran NRRL B1397 has been demonstrated by precipitation with concanavalin A and by serological studies using homologous rabbit anti-dextran serum.[146] Methylation analyses confirmed these results and showed that the dextrans differ mainly in the number of (1 → 2)-branch points. Fluoresceinylthiocarbamoyl-dextrans have been used to study the diffusion properties of concentrated solutions of dextrans[147] and to show that dextran binds to erythrocyte membranes.[148] One of the neutral polysaccharides isolated during the preparation of soy sauce has been characterized as a dextran that is composed of a main chain of α-(1 → 6)-D-glucosyl residues, to which α-(1 → 3)-D-glucosyl side-chains are attached.[149] The polysaccharide is presumably synthesized by lactic acid bacteria during the fermentation.

Evidence has been presented that the synthesis of α-(1 → 3)-branch linkages in *Leuconostoc mesenteroides* B-512 dextran occurs by nucleophilic attack of a hydroxy-group at C-3 of a free dextran molecule on C-1 of the reducing-end of a dextran–dextransucrase complex; the dextran is displaced from the complex on forming an α-(1 → 3)-linkage.[150] Thus, the synthesis of branched linkages does not require a separate branching enzyme.

α-D-Glucopyranosyl fluoride served as a D-glucopyranosyl donor for a glycosyltransferase complex from *Streptococcus mutans*, producing fluoride ions and a high-molecular weight D-glucan.[151] Since the D-glucan reacted with antisera to dextran, it probably contains α-(1 → 6)-linkages, although the presence of (1 → 3)-branch points was not established. Dextrans synthesized by *S. sanguis* and *S. mutans* bind to the surface of *Actinomyces viscosus*.[152]

[140] P. J. Baugh, J. I. Goodall, G. O. Phillips, C. von Sonntag, and M. Dizdaroglu, *Carbohydrate Res.*, 1976, **49**, 315.
[141] H. Yamaguchi, S. Inamura, and K. Makino, *J. Biochem. (Japan)*, 1976, **79**, 299.
[142] A. M. Basedow, K. H. Ebert, H. Ederer, and H. Hunger, *Makromol. Chem.*, 1976, **177**, 1501.
[143] J. F. Weet, C. A. Cobb, and J. Lebrie, *J. Lab. Clin. Invest.*, 1976, **87**, 898.
[144] K. Hellsing, H. Enström, and W. Richter, *Analyt. Biochem.*, 1976, **76**, 149.
[145] F. R. Seymour, R. D. Knapp, and S. H. Bishop, *Carbohydrate Res.*, 1976, **51**, 179.
[146] M. Torii, K. Sakakibara, B. P. Alberto, and A. Misaki, *Biochem. Biophys. Res. Comm.*, 1976, **72**, 236.
[147] T. C. Laurent, L. O. Sundelöf, K. O. Wik, and B. Wärmegård, *European J. Biochem.*, 1976, **68**, 95.
[148] M. W. Rampling, *Biochem. Pharmcol.*, 1976, **25**, 751.
[149] T. Kikuchi, *J. Agric. Chem. Soc. Japan*, 1975, **49**, 597.
[150] J. F. Robyt and H. Taniguchi, *Arch. Biochem. Biophys.*, 1976, **174**, 129.
[151] W. R. Figures and J. R. Edwards, *Carbohydrate Res.*, 1976, **48**, 245.
[152] G. Bourgeau and B. C. McBride, *Infection and Immunity*, 1976, **13**, 1228.

Purified dextransucrase from *S. mutans* 6715 contains two enzymes that possibly differ in their abilities to synthesize different dextran linkages.[153] The synthesis of dextran by *S. mutans* 6715 proceeds by the addition of D-glucosyl residues to the non-reducing end of primer dextrans, and several enzyme molecules bind simultaneously to a single high-molecular weight dextran molecule.[154] Kinetic data suggest that each molecule of dextransucrase contains only one dextran-binding site. Highly-aggregated forms of dextransucrase are either oligomeric enzyme aggregates, which are formed in cultures grown on D-fructose in the absence of dextrans, or enzyme aggregates induced by dextrans in cultures grown on D-glucose.

Water-soluble D-glucans appear to be synthesized by one of the two principal fractions of the D-glucosyltransferase isolated from *S. mutans* 6715; water-insoluble D-glucans are synthesized by the other fraction.[155]

A purified dextranase from *Spicaria violacea* inhibited the formation of artificial dental plaque on selected surfaces and hydrolysed the dextrans from some strains of *S. mutans* to a limited extent only, probably due to the absence of an α-(1 → 3)-glucanase activity.[156]

The importance of antigenic substances (possibly cell-wall polysaccharides) in the adherence of *S. mutans* serotype *e* to cell surfaces has been reported.[157] In a model representing cell-to-surface adherence, it is suggested that the cell-bound D-glucosyltransferase acts both in the synthesis of adherent D-glucans by the cells and as the only binding-site for the D-glucans on the cell surface. The opposing view is that a protein capable of binding to insoluble D-glucan and soluble dextran mediates cell-to-surface and intercellular adherence in *S. mutans*.[158] Antibodies to the cell-associated D-glucans of *S. mutans* have been raised in monkeys.[159] Absorption experiments showed that the antibodies cross-reacted with Sephadex, and that some reacted with unique determinants on the *S. mutans* D-glucans. Antibodies to D-fructosyl- and D-glucosyl-transferases reduced the activities of the respective enzymes in the synthesis of levans and D-glucans.[160]

A comparison has been made of the methods commonly used to estimate antibodies to the D-glucosyltransferases of *S. mutans* OMZ 176.[161] A radio-enzyme assay showed that the D-glucosyltransferases are composed of many multi-reactive molecules, which enable the enzymes to act as cross-linking reagents. A correlation has been demonstrated between inhibition of the synthesis of dextran by cells of *S. mutans* and inhibition of the adherence by antibodies, which may be brought about by a reduction in the synthesis of dextran.[162] The synthesis of soluble D-glucans by a number of serotypes of

[153] A. M. Chludzinski, G. R. Germaine, and C. F. Schachtele, *J. Dental Res.*, 1976, **55**, C75.
[154] G. R. Germaine and C. F. Schachtele, *Infection and Immunity*, 1976, **13**, 365.
[155] J. E. Cardi, G. J. Hageage, and C. L. Wittenberger, *J. Dental Res.*, 1976, **55**, C87.
[156] S. Hamada, J. Mizuno, Y. Murayama, T. Ooshima, N. Masuda, and S. Sobue, *Infection and Immunity*, 1975, **12**, 1415.
[157] S. Hamada and H. D. Slade, *J. Dental Res.*, 1976, **55**, C65.
[158] M. M. McCabe, *J. Dental Res.*, 1976, **55**, C226.
[159] R. J. Genco, F. G. Emmings, R. T. Evans, and M. Apicella, *J. Dental Res.*, 1976, **55** C115.
[160] A. N. Bahn, S. G. Cummings, and J. A. Hayashi, *J. Dental Res.*, 1976, **55**, C134.
[161] J. J. Burckhardt and B. Guggenheim, *Infection and Immunity*, 1976, **13**, 1009.
[162] R. T. Evans, R. J. Genco, and F. G. Emmings, *J. Dental Res.*, 1976, **55**, C127.

S. mutans was not inhibited by a preparation of anti-D-glucosyltransferase, although that of insoluble D-glucans in some of the serotypes was inhibited by a crude preparation of the enzyme.[163]

Antibodies have been raised against the activities in *S. mutans* serotype c that synthesize soluble and insoluble D-glucans.[164] Antibodies directed against the D-glucosyltransferase involved in the synthesis of the insoluble D-glucan may not inhibit to the same extent the sucrose-mediated attachment to smooth surfaces of organisms from the four main serotypes of *S. mutans*.

A low-molecular-weight form of *S. mutans* dextransucrase free from any D-glucan primer has been isolated after growth of the organism on a synthetic medium.[165] Regulation of the synthesis of the extracellular D-glucosyltransferase and the relationship between the extracellular and cell-associated activities of *S. mutans* have been discussed.[166] Dextran, secretory immunoglobulin A (SIgA), and secretory component (SC) stimulated the dextransucrase activity of *S. mutans* to an equal extent,[167] although previous reports have claimed a seven-fold stimulation of the activity by both secretory products.[168] The extracellular dextrans synthesized from sucrose by two strains of *S. mutans* did not prevent either the synthesis or the release of active bacteriocins.[169]

Hollow-fibre dialysis has been used to remove ampholines from dextransucrase after isoelectric focusing.[170]

The kinetic properties of an ADP-D-glucose pyrophosphorylase isolated from a mutant of *E. coli* B, which synthesizes large amounts of glycogen, have been studied.[171] The enzyme from the mutant appears to have a greater affinity for its activator (D-fructose 1,6-diphosphate) and less affinity for the inhibitor AMP than does the parent enzyme. Glycogen synthase (ADP:1,4-α-D-glucan 4-α-glucosyltransferase) from *E. coli* B has been obtained in a purified form, and the reaction catalysed by the enzyme was shown to be reversible.[172] Microchemical techniques have been used to investigate the extent of localization of glycogen synthase in developing spore and stalk cells during differentiation of *Dictyostelium discoideum*.[173] Two enzymes, an α-1,4-glucan phosphorylase (α-1,4-glucan:orthophosphate glucosyltransferase; E.C. 2.4.1.1) and a soluble maltodextrin phosphorylase, have been prepared from *S. mitior*.[174] It is likely that only a single phosphorylase exists, the specificity being determined by how it binds in the cell. Thus, the enzyme acts as a glycogen phosphorylase when in the particulate state and as a maltodextrin phosphorylase when soluble; the equilibrium between the two forms depends on the glycogen content of the cells.

[163] R. Linzer and H. D. Slade, *Infection and Immunity*, 1976, **13**, 494.
[164] H. Kuramitsu and L. Ingersoll, *Infection and Immunity*, 1976, **14**, 636.
[165] C. F. Schachtele, S. K. Harlander, and G. R. Germaine, *Infection and Immunity*, 1976, **13**, 1522.
[166] W. M. Janda and H. K. Kuramitsu, *Infection and Immunity*, 1976, **14**, 191.
[167] J. S. Cole, G. E. Clark, and R. Wistar, *J. Bacteriol.*, 1976, **127**, 1595.
[168] Y. Fukui, K. Fukui, and T. Moriyama, *J. Bacteriol.*, 1974, **118**, 805.
[169] A. L. Delisle, *Infection and Immunity*, 1976, **13**, 619.
[170] M. F. Callaham, W. E. Poe, and J. R. Heitz, *Analyt. Biochem.*, 1976, **70**, 542.
[171] J. Preiss, C. Lammel, and E. Greenberg, *Arch. Biochem. Biophys.*, 1976, **174**, 105.
[172] J. Fox, K. Kawaguchi, E. Greenberg, and J. Preiss, *Biochemistry*, 1976, **15**, 849.
[173] J. F. Harris and C. L. Rutherford, *J. Bacteriol.*, 1976, **127**, 84.
[174] A. Pulkownik and G. J. Walker, *J. Bacteriol.*, 1976, **127**, 281.

Methylation analysis and digestion with isoamylase have shown that a reserve D-glucan from the rumen bacterium *Megasphaera elsdenii* belongs to the amylopectin–glycogen class of polysaccharides.[175]

Light scattering, sedimentation analysis, and other physical measurements on *S. salivarius* levan have revealed that the polysaccharide behaves as a compact coil having spherical symmetry.[176] Kinetic measurements have shown that the acid hydrolysis of *S. salivarius* levan occurs by two first-order reactions: a fast reaction in which linkages at branch points are broken and a slower reaction in which linkages in the main chain are broken.[177] Thus, the hydrolysis of this levan differs fundamentally from the completely random hydrolysis of such branched polysaccharides as dextran, glycogen, and amylopectin. Physical parameters obtained from light-scattering measurements have been reported for *S. salivarius* levan in DMSO.[178] Chemical-ionization m.s. has been used to assign structures to acetylated *O*-methylalditols formed in the methylation analysis, *etc.*, of *S. salivarius* levan.[179] The levan synthesized by cell-free extracts of *S. mutans* contains (2 → 1)-β-D-fructofuranosyl residues, whereas the polysaccharide elaborated by cell-free extracts of *S. salivarius* has a branched structure composed of (2 → 6)-β-D-fructofuranosyl residues.[180]

The production of commercially important bacterial polysaccharides of the alginate type by *Azotobacter vinelandii* has been reviewed.[181] The sequence of reactions used by *A. vinelandii* to synthesize alginic acid from sucrose has been elucidated by studies of the incorporation of labelled intermediates into polysaccharides by cell-free extracts and of the individual enzymes involved in the synthesis.[182] Poly(D-mannuronic acid) is synthesized first and then some of the D-mannuronic acid residues are converted into L-guluronic acid at the polymer level; L-guluronic acid does not appear to be formed either as the free acid or in combination with GDP. Slime polysaccharides resembling the alginic acids, but which contain *O*-acetyl groups and high contents of D-mannuronic acid, are elaborated by some Pseudomonads.[183] A polysaccharide that contains residues of D-glucose, D-mannose, L-rhamnose, acetate, and pyruvate has been isolated from non-proliferating suspensions of *Pseudomonas* PB1.[184]

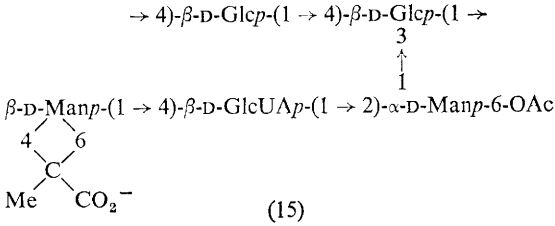
Compositional studies on the extracellular polysaccharides of *Rhizobium japonicum* indicated that a number of strains contain 4-*O*-methyl-D-galactose and D-galacturonic acid, in addition to D-glucose, D-galactose, and D-mannose.[185] 4-*O*-Methyl-D-glucose has been identified as a component of the extracellular polysaccharide of another strain of *Rhizobia*.[186] Previous doubts about the occurrence of D-mannose in rhizobial polysaccharides have been removed by the

[175] R. G. Brown, B. Lindberg, and K. J. Cheng, *Canad. J. Microbiol.*, 1975, **21**, 1657.
[176] S. S. Stivala, W. S. Bahary, L. W. Long, J. Ehrlich, and E. Newbrun, *Biopolymers*, 1975, **14**, 1283.
[177] M. D. Laureen, S. S. Stivala, W. S. Bahary, and L. N. Long, *Biopolymers*, 1975, **14**, 2373.
[178] N. S. Bahary, S. S. Stivala, E. Newbrun, and J. Ehrlich, *Biopolymers*, 1975, **14**, 2467.
[179] R. A. Hancock, K. Marshall, and H. Weigel, *Carbohydrate Res.*, 1976, **49**, 351.
[180] S. Ebisu, K. Kato, S. Kotani, and A. Misaki, *J. Biochem. (Japan)*, 1975, **78**, 879.
[181] C. Bucke, L. Deavin, C. J. Lawson, and D. F. Pindar, *Biochem. Soc. Trans.*, 1975, **3**, 844.
[182] D. F. Pindar and C. Bucke, *Biochem. J.*, 1975, **152**, 617.
[183] A. Linker and L. R. Evans, *Carbohydrate Res.*, 1976, **47**, 179.
[184] A. G. Williams and J. W. T. Wimpenny, *Biochem. Soc. Trans.*, 1975, **3**, 983.
[185] W. F. Dudman, *Carbohydrate Res.*, 1976, **46**, 97.
[186] L. D. Kennedy and R. W. Bailey, *Carbohydrate Res.*, 1976, **49**, 451.

isolation of D-mannose-containing polysaccharides from a number of rhizobial strains grown on a D-mannose-free medium.

One of the extracellular polysaccharides synthesized by *Streptococcus bovis* is a viscous dextran.[187] A linear polysaccharide synthesized by a *Streptomyces* sp. is composed of α-(1 → 3)- and α-(1 → 4)-linked D-glucopyranosyl residues in roughly equal amounts.[188]

The polysaccharide synthesized by a small-colony variant of *Xanthomonas campestris* differs from that synthesized by the large-colony parental type in that it contains less pyruvic acid and *O*-acetyl substituents and has adverse solution properties.[189] The structure of xanthan gum has been reinvestigated by two groups, who suggest the structure (15) for the pentasaccharide repeating unit.[190, 191]



(15)

Potentiometric titration and other physical data indicated that the native polysaccharide possesses an ordered secondary structure that is stabilized by non-ionic interactions, which outweigh the repulsion between adjacent carboxy-groups.[192] An acidic heteropolysaccharide from *Xanthomonas* S19 is composed of residues of D-glucuronic acid, D-glucose, D-galactose, D-mannose, and acetate.[193] Although methylation analysis showed that this polysaccharide possesses many structural features similar to those of other *Xanthomonas* polysaccharides, it is unusual in that D-mannosyl and D-galactosyl residues occur in the structure.

Miscellaneous Bacterial Polysaccharides

The chemical compositions of cell-wall polysaccharides of mycobacteria have been ably reviewed.[194]

Acidic polysaccharides can be detected by a combination of electrophoresis in an agar gel and precipitation with Cetavlon.[195]

Flocculating, but not non-flocculating, strains of *Rhizobium* species have been found to produce microfibrils; the microfibrils isolated from *R. trifolii* are composed of cellulose.[196] D-[^{14}C]Glucose from UDP-D-[^{14}C]glucose was

[187] K. J. Cheng, R. Hironaka, G. A. Jones, T. Nicas, and J. W. Costerton, *Canad. J. Microbiol.*, 1976, **22**, 450.
[188] T. Miyazaki, H. Yamada, J. Awaya, and S. Omura, *J. Gen. Microbiol.*, 1976, **95**, 31.
[189] M. C. Cadmus, S. P. Rogovin, K. A. Burton, J. E. Pittsley, C. A. Knutson, and A. Jeanes, *Canad. J. Microbiol.*, 1976, **22**, 942.
[190] P. E. Jansson, L. Kenne, and B. Lindberg, *Carbohydrate Res.*, 1975, **45**, 275.
[191] L. D. Melton, L. Mindt, D. A. Rees, and G. R. Sanderson, *Carbohydrate Res.*, 1976, **46**, 245.
[192] G. Holzwarth, *Biochemistry*, 1976, **15**, 4333.
[193] V. A. Fareed and E. Percival, *Carbohydrate Res.*, 1976, **49**, 427.
[194] C. Ratledge, *Adv. Microbial Physiol.*, 1976, **13**, 116.
[195] F. Ørskov, *Acta Pathol. Microbiol. Scand.*, 1976, **84B**, 319.
[196] C. Napoli, F. Dazzo, and D. Hubell, *Appl. Microbiol.*, 1975, **30**, 123.

incorporated into both alkali-soluble and -insoluble β-(1 → 4)-D-glucans by a particulate enzyme system isolated from encysting cells of *Acanthamoeba castellanii*.[197] The alkali-soluble D-glucan appears to be a precursor of the insoluble D-glucan (cellulose). The chain-length distributions of the celluloses obtained from different generations of *Acetobacter xylinum* showed that the polydispersity and the number of maxima increase with increasing numbers of generations.[198] The results support the view – based on kinetic investigations – that each generation of the bacterium initiates the synthesis of a certain number of chains, which grow without a termination step. I.r. spectroscopy and electron microscopy have indicated that *A. xylinum* cellulose exists in disordered, entangled web structures.[199]

A high-molecular-weight heteropolysaccharide composed of residues of D-glucose, 2-acetamido-2-deoxy-D-glucose, and D-mannose has been isolated from lysates of cells of *Aerobacter aerogenes* infected with the bacteriophage K2.[200] The polysaccharide also occurs as an integral part of the structure of mature bacteriophage particles, and its synthesis is totally dependent upon infection of the host cells with the bacteriophage.

A smooth strain of *E. coli* synthesizes a polysaccharide exhibiting blood-group H activity.[201] Enzymic and chemical investigations indicated that the side-chain of the polysaccharide contains a tetrasaccharide unit (16), to which a

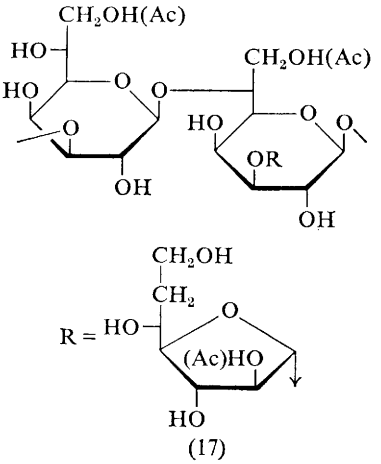
β-D-Gal*p*-(1 → 3)-D-Gal*NAc*p-(1 → 3)-D-Gal*NAc*p-L-Fuc*p*-(1 →
(16)

D-glucopyranosyl residue is attached. Removal of some of the L-fucosyl residues from the polysaccharide with α-L-fucosidase resulted in loss of blood-group activity.

A combination of methylation analysis, oxidation with periodate, and partial hydrolysis with acid has shown that the polysaccharide antigen of *Eubacterium saburreum* 49 consists of a chain of (1 → 3)- and (1 → 6)-linked β-D-*glycero*-D-*galacto*-heptopyranosyl residues; the residues linked at O-6 are substituted at O-3 with 6-deoxy-α-D-*altro*-heptofuranosyl residues.[202] Acetyl groups are present at O-7 of some of the heptopyranosyl residues and, possibly, at O-2 of some of the 6-deoxyheptofuranosyl residues. The simplest oligosaccharide repeating unit appears to be the trisaccharide (17), although chemical analysis suggests that the repeating unit may be more complex.

An antigen complex from *Leptospira biflexa*, serotype *patoc*, which reacted with antibodies prepared against a number of serogroups, contains polysaccharides composed of residues of D-mannose, D-glucose, L-arabinose, and 2-amino-2-deoxy-D-glucose.[203] Two antigens having distinct properties were isolated by gel chromatography of the complex; one is a polysaccharide composed of L-arabinose and 2-amino-2-deoxy-D-glucose, and carries *patoc* specificity,

[197] J. L. Potter and R. A. Weisman, *Biochim. Biophys. Acta* 1976, **428**, 240.
[198] M. Marx-Figini and B. Pion, *Makromol. Chem.*, 1976 **177**, 1013.
[199] E. Correns and H. J. Purz, *Cellulose Chem. Technol.*, 1975, **9**, 449.
[200] T. N. Oeltmann and E. C. Heath, *J. Biol. Chem.*, 1975, **250**, 8696.
[201] K. Kishi and S. Iseki, *Jap. J. Microbiol.*, 1976, **20**, 109.
[202] J. Hoffman, B. Lindberg, J. Lönngren, and T. Hofstad, *Carbohydrate Res.*, 1976, **47**, 261.
[203] R. Tinelli and J. Gabay, *Compt. rend.*, 1976, **283**, *D*, 215.



(17)

whereas the other is probably a lipoglycoprotein that carries a group specificity shared by many *Leptospira*.[204] A previously unidentified sugar found in acid hydrolysates of the polysaccharide isolated from *L. biflexa* has been identified as 3-*O*-methyl-D-mannose, which also occurs in other *Leptospira*.[205] 3-*O*-Methyl-D-glucose and diaminopimelic acid are considered to be important in distinguishing the genus *Leptospira* from other genera of Spirochaetes.

Polysaccharides possessing different affinities for concanavalin A have been isolated from *Mycobacterium tuberculosis*; they were identified as an arabinogalactan and an arabinomannan.[206] No variation in the chemical composition of an arabinogalactan was detected when cells of *M. lepraemurium* grow subcutaneously in mice or in an egg-yolk medium.[207] A cell-free, particulate preparation from *M. smegmatis* incorporated D-[^{14}C]mannose from GDP-D-[^{14}C]mannose into endogenous acceptors, producing a series of labelled, neutral D-manno-oligosaccharides; the principal component of the D-manno-oligosaccharides was identified as the trisaccharide α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 2)-D-mannose, while some of the higher oligosaccharides may contain α-(1 → 2)- or α-(1 → 6)-linkages or both.[208]

Immunological studies have confirmed that *Bacillus pumilus* Sh-17 polysaccharide cross-reacts with meningococcal group A polysaccharide.[209] The cross-reaction is ascribed to the presence of residues of 2-acetamido-2-deoxy-D-mannose 1-phosphate in both polysaccharides. Two polysaccharides isolated from two strains of *Neisseria meningitidis* group C are identical to the group C antigen, but they differ in the extent of hydrolysis by neuraminidase.[210]

[204] J. Gabay and R. Tinelli, *Biochimie*, 1976, **50**, 827.
[205] I. Azuma, K. Takeda, Y. Yamamura, Y. Yanagihara, and I. Mifuchi, *J. Bacteriol.*, 1976, **128**, 492.
[206] T. M. Daniel and A. Misaki, *Amer. Rev. Respiratory Diseases*, 1976, **113**, 705.
[207] I. Azuma, Y. Yamamura, and T. Mori, *Jap. J. Microbiol.*, 1975, **19**, 333.
[208] J. C. Schultz and K. Takayama, *Arch. Biochem. Biophys.*, 1976, **177**, 62.
[209] W. F. Vann, T. Y. Liu, and J. B. Robbins, *Infection and Immunity*, 1976, **13**, 1654.
[210] M. A. Apicella, *Infection and Immunity*, 1976, **14**, 106.

A sensitive, passive haemagglutination test for antibodies to group A streptococcal polysaccharide has been developed; it involves coating erythrocytes with a conjugate of the polysaccharide and human immunoglobulin IgG.[211] A polysaccharide (mol. wt. 1×10^6) isolated from group B Streptococci has been degraded to a fragment that retained the immunological properties of the group B antigen.[212] Residues of D-galactose, D-glucose, D-mannose, heptose, sialic acid, and 2-amino-2-deoxy-D-glucose were detected in the native polysaccharide, which does not share antigenic determinants with either the capsular type K antigen of *E. coli* or the group B polysaccharide antigen of *N. meningitidis.*

The structure of a cell-wall heteroglycan isolated from *S. bovis* strain C3, a member of the group D Streptococci, has been reinvestigated.[213] In addition to residues of D-galactose, L-rhamnose, and 6-deoxy-L-talose (J. H. Pazur, J. A. Kane, D. J. Dropkin, and L. M. Jackman, *Arch. Biochem. Biophys.*, 1972, **150**, 382), D-glucuronic acid residues have been shown to form an integral part of the structure. Methylation analysis and oxidation with periodate showed that the heteroglycan is composed of the repeating unit (18). The D-glucuronic acid

$$\begin{array}{l}\rightarrow 3)\text{-L-Rha}p\text{-}(1 \rightarrow 3)\text{-D-Gal}p\text{-}(1 \rightarrow 2)\text{-L-Rha}p\text{-}(1 \rightarrow 3)\text{-6-deoxy-L-Tal}p\text{-}(1 \rightarrow \\ \quad\quad 4 \\ \quad\quad \uparrow \\ \quad\quad 1 \\ \quad \text{D-GlcUA}p\end{array} \qquad (18)$$

residue is the principal immunodeterminant group of the glycan, and inhibition studies with haptens suggested that the antigen binds to the antibody at the hydroxy-groups at C-2 and C-3 and the carboxy-group of the hexuronic acid residues. The type-specific antigen of *S. mutans* serotype *e* has been purified and identified as a polysaccharide composed of residues of D-glucose and L-rhamnose, although it also contains small proportions of protein and phosphorus.[214] A type-specific, cell-wall antigen isolated from two strains of *S. mutans* serotype *f* also contains these sugars.[215] Immunological studies revealed the presence of α-(1 → 6)-linked D-glucopyranosyl residues in the antigen, thereby demonstrating a similarity of structure between the type *f* polysaccharide and the dextrans of *S. mutans.* A correlation has been made, with respect to the sugars and proteins, between the serogroup and the composition of the cell walls of strains of *S. mutans.*[216]

Fungal Polysaccharides

The secretion of polysaccharides by growing hyphae of *Achyla bisexualis* has been studied.[217] The cell walls of *Agaricus bisporus* contain chitin and a D-glucan; cellobiose octa-acetate was isolated from acetolysates of the D-glucan, indicating

[211] A. A. Hirata, S. J. Ronspies, J. C. Petruska, M. Hargie, J. R. Schenck, and W. T. Stall, *J. Immunol. Methods*, 1976, **13**, 167.
[212] C. J. Baker, D. L. Kasper and C. E. Davis, *J. Exp. Med.*, 1976, **143**, 258.
[213] J. H. Pazur, D. J. Dropkin, K. L. Dreher, L. S. Forsberg, and C. S. Lowman, *Arch. Biochem. Biophys.*, 1976, **176**, 257.
[214] S. Hamada and H. D. Slade, *Infection and Immunity*, 1976, **14**, 68.
[215] S. Hamada, K. Gill, and H. D. Slade, *Infection and Immunity*, 1976, **14**, 203.
[216] D. C. Ellwood, J. K. Baird, J. R. Hunter, and V. M. C. Longyear, *J. Dental Res.*, 1976, **55**, C42.
[217] R. Dargent, *Compt. rend.*, 1975, **280**, *D*, 1445.

that it is composed, in part, of (1 → 4)-linked β-D-glucopyranosyl residues.[218] The architecture of the cell wall of *Agaricus bisporus* has been investigated by enzymic digestion, in conjunction with electron microscopy.[219] A cell-wall fraction could be degraded completely by extraction with alkali and digestion in turn with a mixed β-D-glucanase and chitinase. The outer layer of the cell wall is composed of an alkali-soluble D-glucan. β-D-Glucan does not constitute a separate wall-layer, but is associated with the matrix of fibrillar chitin.

The principal cell-wall constituents of an *Apodachlya* sp. are (1 → 3)- and (1 → 6)-linked D-glucans, although chitin and cellulose are also present.[220] A (1 → 3)-linked β-D-glucan, a (1 → 6)-linked β-D-glucan, and chitin are among the polysaccharides identified in the mycelial cell wall of *Penicillium charlesii* NRRL 1887.[221] Electron microscopy, *X*-ray diffraction, and chemical investigations on the network of microfibrils produced by protoplasts of *Saccharomyces cerevisiae* have confirmed the presence of an alkali-soluble (1 → 3)-linked β-D-glucan and chitin.[222] Cell walls isolated from vegetative yeast cells and from hormone-induced conjugation tubes of the basidiomycete *Tremella mesenterica* contain identical polysaccharides, namely a (1 → 3)-linked α-D-glucan, a (1 → 3 and 6)-linked β-D-glucan, and chitin, but in different proportions.[223]

Glucans.—Three methods for isolating glycogen from fungal tissues have been assessed.[224] The synthesis of pullulan is associated with the deceleration and early stationary phases of the growth cycle of *Aureobasidium pullulans*.[225] A gel-forming D-glucan isolated from the tree fungus *Cyttaria harioti* is composed of a highly-branched backbone of (1 → 3)-linked β-D-glucopyranosyl residues having every second or third residue substituted at O-6.[226]

Cellulose has not been confirmed as a cell-wall component of *Cephaloascus fragrans*.[227] The absence of L-rhamnose and cellulose and the presence of D-mannose in cell-wall components from *Cephaloascus* have been used in distinguishing this fungus from *Ophiostoma* and *Europhium*. D-[1-^{14}C]Mannitol was incorporated into an α-(1 → 4)-linked D-glucan, which is probably glycogen, by the mycelium of *Dendryphiella salina*.[228] The structure of lentinan, an anti-tumour polysaccharide from *Lentinus edodes*, has been investigated by methylation analysis, oxidation with periodate, and Smith degradation.[229] This highly-branched polysaccharide contains a main chain of (1 → 3)-linked β-D-glucopyranosyl residues, with branch points at O-6 bearing chains of (1 → 6)- and (1 → 3)-linked β-D-glucopyranosyl residues. The cell wall of a morphological mutant of *Paracoccidiodes brasiliensis* differs from that of the parent strain, principally in the replacement of a (1 → 3)-linked α-D-glucan by an amorphous

[218] A. Temeriusz, *Roczniki Chem.*, 1975, **49**, 1803.
[219] G. O. Michalenko, H. R. Hohl, and D. Rast, *J. Gen. Microbiol.*, 1976, **92**, 251.
[220] C. C. Lin, R. S. Sicher, and J. M. Aronson, *Arch. Mikrobiol.*, 1976, **108**, 85.
[221] R. A. Bulman and G. J. F. Chittenden, *Biochim. Biophys. Acta*, 1976, **444**, 202.
[222] D. R. Kreger and M. Kopecká, *J. Gen. Microbiol.*, 1976, **92**, 207.
[223] I. D. Reid and S. Bartnicki-Garcia, *J. Gen. Microbiol.*, 1976, **96**, 35.
[224] J. B. W. Hammond and R. Nichols, *Trans. British Mycol. Soc.*, 1976, **66**, 325.
[225] B. J. Catley and P. J. Kelly, *Biochem. Soc. Trans.*, 1975, **3** 1079.
[226] A. F. Cirelli and R. M. de Lederkremer, *Carbohydrate Res.*, 1976, **48**, 217.
[227] A. C. M. Weijman, *J. Microbiol. Serol.*, 1976, **42**, 315.
[228] J. C. B. McDermott and D. H. Jennings, *J. Gen. Microbiol.*, 1976, **97**, 193.
[229] T. Sasaki and N. Takasuka, *Carbohydrate Res.*, 1976, **47**, 99.

(1 → 3)-linked D-mannan.[230] *Phytophthora cinnamomi* has been grown at a high concentration of D-glucose in order to depress the intracellular level of protease, thereby attaining and maintaining a high level of β-D-glucan synthase activity in the cell-free extracts.[231] Microfibrils of a linear (1 → 3)-linked β-D-glucan were synthesized *in vitro* by cell-free extracts of *P. cinnamomi* that contain a high level of β-(1 → 3)-D-glucan synthase and a low level of protease. However, the microfibrils are not of the type found in the native cell wall in which the principal microfibrillar D-glucan contains both (1 → 3)- and (1 → 6)-linked D-glucosyl residues.

Methylation analysis and ^{13}C n.m.r. spectroscopy have indicated that a gel-forming β-D-glucan from *Pleurotus ostreatus* consists of a (1 → 3)-linked chain, to which side-chains of α-(1 → 4)-D-glucosyl residues are attached.[232] A multiple-strand helix is considered to be the most likely conformation of this β-D-glucan. Chemical analysis and studies of the ultrastructure of *Pythium debaryanum* have indicated that the cell wall is constructed of cellulosic microfibrils, which exist both as a framework and as a finer network.[233] The microfibrils are covered by matrix material composed of a mixture of amorphous (1 → 3)- and (1 → 6)-linked, branched β-D-glucans.

Examination of an alkali-soluble D-glucan isolated from *Saccharomyces cerevisiae* has shown that it contains mainly β-(1 → 3)-linked residues.[234] In this respect and in molecular size (mol. wt. 2.4×10^5), it is closely similar to the alkali-insoluble D-glucan that forms the rigid component of the cell walls; it differs in that it contains a significant proportion of (1 → 6)-linked β-D-glucopyranosyl residues, which probably account for the different solubilities in alkali. An enzyme system from *S. cerevisiae* catalysed the transfer of D-glucosyl units from GDP- and UDP-D-[U-^{14}C]glucose into polysaccharides tentatively identified as β-D-glucans containing both (1 → 3)- and (1 → 6)-linked residues.[235] Preliminary investigations suggest that the polysaccharide synthesized from UDP-D-[U-^{14}C]glucose is a (1 → 3)-linked β-D-glucan that contains a few (1 → 6)-linkages in the side-chains, whereas the polysaccharide synthesized from GDP-D-[U-^{14}C]glucose is a (1 → 6)-linked β-D-glucan that contains a few (1 → 3)-linked residues in the side-chains. Both polysaccharides appear to be heterogeneous, containing different proportions of D-glucosyl residues linked covalently to peptide.

The uptake and incorporation of ^{14}C-labelled D-glucose, maltose, and maltotriose into polysaccharides have been studied during the fermentation of wort by a synchronous culture of brewer's yeast.[236] The label was incorporated into glycogen in a stepwise manner in which brief periods of glycogen synthesis, which coincide with the budding process, alternate with longer periods of stability. By contrast, the incorporation of sugars into the cell-wall D-glucan is a continuous process that is not associated with any particular phase.

[230] F. San-Blas, G. San-Blas, and L. J. Cova, *J. Gen. Microbiol.*, 1976, **93**, 209.
[231] M. C. Wang and S. Bartnicki-Garcia, *Arch. Biochem. Biophys.*, 1976, **175**, 351.
[232] H. Saito, T. Ohki, Y. Yoshioka, and F. Fukuoka, *F.E.B.S. Letters*, 1976, **68**, 15.
[233] M. Yamada and T. Miyazaki, *Jap. J. Microbiol.*, 1976, **20**, 83.
[234] G. H. Fleet and D. J. Manners, *J. Gen. Microbiol.*, 1976, **94**, 180.
[235] Š. Bálint, V. Farkas, and Š. Bauer, *F.E.B.S. Letters*, 1976, **64**, 44.
[236] J. A. Budd, *J. Gen. Microbiol.*, 1975, **90**, 293.

A polysaccharide solubilized from the cell walls of *Schizophyllum commune* has been identified, on the basis of chemical and physical studies, as a linear (1 → 3)-linked α-D-glucan containing occasional (1 → 6)-linked D-glucosyl residues.[237] The polysaccharides remaining in the cell wall are chitin and a highly-branched D-glucan composed of linear segments of (1 → 6)- and (1 → 3)-linked β-D-glucopyranosyl residues and β-(1 → 6)-D-glucopyranosyl branch-points. Light stimulated the accumulation of a (1 → 3)-linked D-glucan in *Sclerotium rolfsii*.[238]

The cell walls of spores of *Trichoderme viride* contain (1 → 3)- and (1 → 6)-β-D-glucans similar to those of the mycelial cell wall, but chitin, which is always present in the mycelium, does not occur in the spores.[239] The preparation of protoplasts from *Ustilago maydis* has been studied using commercially available glycosidases, which indicated the presence of a (1 → 3)-linked α-D-glucan in the conidial walls.[240] Protoplasts have been prepared from a large number of fungi by lysis of the cell walls with various β-(1 → 3)-glucanases, and a taxonomic correlation of the fungi was achieved.[241]

The association of chitin, D-glucan, and D-mannan in the cell walls of *Candida albicans* and the spatial distribution of these polysaccharides have been found to vary appreciably between two dimorphs and with the age of the culture.[242]

Mannans.—A β-(1 → 3)-glucanase has been used to prepare protoplasts from *C. utilis* and *S. cerevisiae*.[243] Scanning electron microscopy of prefixed protoplasts, using colloidal gold labelled with concanavalin A, showed that the D-mannan is distributed randomly at the surface of the protoplasts.

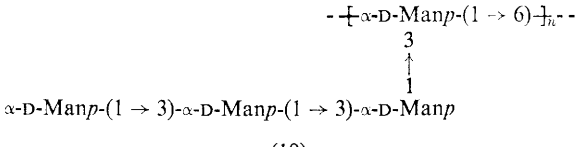
The synthesis of a D-glucan by *C. guilliermondii* was inhibited by diphenylamine, which, at the same time, stimulated the synthesis of a D-mannan.[244]

Various strains of yeasts that produce characteristic phosphono-D-mannans also synthesize characteristic extracellular α-D-mannans, which appear to differ in the degree of branching and in the type of glycosidic linkages.[245] Methylation analysis showed that the mannans contain a (1 → 6)-linked α-D-mannosyl backbone, to which (1 → 3)-linked [*e.g.* (19) in *Pichia mucosa*] or (1 → 3)- and/or (1 → 2)-linked [(20) in *Pichia* sp. and (21) in *Pachysolen tannophilus*] or (1 → 2)- and (1 → 6)-linked α-D-mannosyl side-chains [(22) in *Torulopis pinus*] are attached.

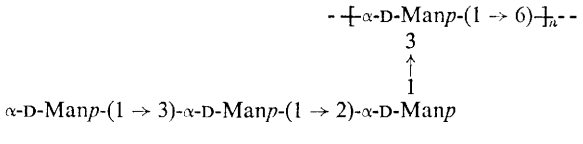
D-Glucono-1,4-lactone inhibited the synthesis of the D-mannan and the β-(1 → 3)-D-glucan in living cells of *P. polymorpha*, whereas the synthesis of the β-(1 → 6)-D-glucan was unaffected.[246]

Binding sites for concanavalin A have been detected on the plasmalemma and the vacuolar membrane of yeast protoplasts.[247] Concanavalin A is probably

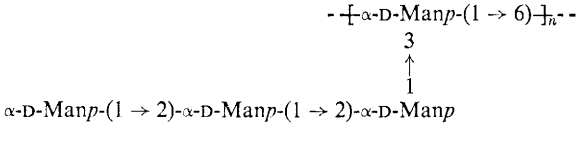
[237] D. J. Siehr, *Canad. J. Biochem.*, 1976, **54**, 130.
[238] R. M. Miller and A. E. Liberta, *Canad. J. Microbiol.*, 1976, **22**, 967.
[239] T. Benitez, T. G. Villa, and I. Garcia Acha, *Canad. J. Microbiol.*, 1976, **22**, 318.
[240] M. A. de Waard, *J. Microbiol. Serol.*, 1976, **42**, 211.
[241] M. Bastide, E. H. Hadibi, J. M. Bastide, and S. Jouvert, *Compt. rend.* 1976, **283**, *D*, 1555.
[242] F. W. Chattaway, S. Shenolikar, J. O'Reilly, and A. J. E. Barlow, *J. Gen. Microbiol.*, 1976, **95**, 335.
[243] M. Horisberger, J. Rosset, and H. Bauer, *Arch. Mikrobiol.*, 1976, **109**, 9.
[244] L. D. Phai and G. Reuter, *Z. allgem. Mikrobiol.*, 1976, **16**, 197.
[245] F. R. Seymour, M. E. Slodki, R. D. Plattner, and R. M. Stodola, *Carbohydrate Res.*, 1976, **48**, 225.
[246] T. G. Villa, V. Notario, T. Benitez, and J. R. Villanueva, *Arch. Mikrobiol.*, 1976, **109**, 157.
[247] T. Boller, M. Dürr, and A. Wiemken, *Arch. Mikrobiol.*, 1976, **109**, 115.



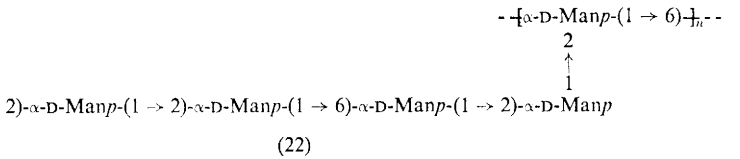
(19)



(20)



(21)



(22)

bound by the D-mannan, which has been detected in vacuoles and is also known to be a principal constituent of plasmalemmas. A lipid-bound D-manno-trisaccharide is formed in yeast microsomes by a mechanism similar to that in other organisms, and the carbohydrate residues are incorporated directly into proteins without further D-mannosylation.[248]

Selective radio-labelling of the cell-wall D-mannan in growing *S. cerevisiae* showed that it is not metabolized during exponential growth, since, once inserted into the wall, it is not subject to turnover or release into the growth medium.[249] A D-mannan that was isolated from the growth medium is either non-structural or synthesized *de novo*, but not trapped in the structure of the growing cell wall.

The antibiotic tunicamycin prevented the incorporation of 2-amino-2-deoxy D-[^{3}H]glucose into the mannan-peptides of protoplasts of a strain of *Saccharomyces*, although the same concentration of the antibiotic did not inhibit the synthesis of chitin.[250] In addition to inhibiting the synthesis of glycoproteins, the antibiotic interacted with the components of lipophilic membranes.

Chitin.—The distribution of chitin synthase was shown to be similar in protoplasts and material from the mycelia of *Aspergillus flavus*; the enzyme requires

[248] K. Nakayama, Y. Araki, and E. Ito, *F.E.B.S. Letters*, 1976, **72**, 287.
[249] Z. Krátký, P. Biely, and Š. Bauer, *Biochim. Biophys. Acta*, 1975, **404**, 1.
[250] S. C. Kuo and J. O. Lampen, *Arch. Biochem. Biophys.*, 1976, **172**, 574.

2-acetamido-2-deoxy-D-glucose and Mg^{2+} ions, and is activated by soluble chitodextrins.[251] Polyoxin D competitively inhibited the enzyme, which is activated by fungal proteases and which is inactivated by animal and plant proteases.[252]

Germination of the zoospores of *Blastocladiella emersonii* is accompanied by the synthesis of chitin in the cell wall in a way that is not inhibited by cycloheximide.[253] UDP-2-acetamido-2-deoxy-D-glucose, a substrate for chitin synthase and also the end-product of amino-sugar synthesis, acts as a powerful negative regulator of L-glutamine:D-fructose 6-phosphate amidotransferase, a key enzyme of amino-sugar synthesis. The concentration of UDP-2-acetamido-2-deoxy-D-glucose in the zoospores is sufficient to act as a feed-back regulator for the amidotransferase.

The principal polysaccharides of the cell wall of *Choanephora cucurbitarum* have been identified as chitin and chitosan.[254] Examination of the cell walls by electron microscopy revealed that there are microfibrillar and amorphous regions. The microfibrils, which are composed of chitin, are organized into two distinct layers. Polyoxin D affected the synthesis of chitin in *Coprinus cinereus* by inhibiting the chitin synthase in stripe tissues and by preventing the elongation of stripes in fruiting bodies of the fungus.[255]

The pigments associated with chitin in the cell walls of *Helminthosporium spiciferum* increased the resistance of chitin to solubilization by chitinases.[256]

The maximum specific activity of the chitin synthase in *Mortierella vinacea* is associated with cessation of exponential growth.[257] Mg^{2+} ions stimulated the enzymic activity, whereas soluble chitodextrins are inhibitors, in contrast to their effect on the corresponding enzyme from *A. flavus*.[251] Chitin synthase has been detected in yeast forms of preparations of *Mucor rouxii*, chiefly in a latent state that is activated by proteases.[258] It is initially present as an active form in mycelial preparations, but is rapidly degraded by endogenous proteases. The different behaviours of chitin synthase in crude extracts of mycelium and yeast cells is consistent with, and perhaps responsible for, differences in the structures of the cell walls. The activating factor for the chitin synthase of *S. cerevisiae* has been purified and shown to be identical with an enzyme previously designated as protease B (E.C. 3.4.22.9).[259]

The receptor sites for wheat-germ agglutinin on the surface of *S. cerevisiae* have been located on the bud scars, the mother-cell bud junction, and the bud, but not on the mother cell.[260] The lectin has a combining site complementary to sequences of (1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucopyranosyl residues,

[251] P. M. Moore and J. F. Peberdy, *Canad. J. Microbiol.*, 1976, **22**, 915.
[252] E. López-Romero and J. Ruiz-Herrera, *J. Microbiol. Serol.*, 1976, **42**, 261.
[253] C. P. Selitrennikoff, D. Allin, and D. R. Sonneborn, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 534.
[254] D. R. Letourneau, J. M. Deven, and M. S. Manocha, *Canad. J. Microbiol.*, 1976, **22**, 486.
[255] G. W. Gooday, A. De Rousset-Hall, and D. Hunsley, *Trans British Mycol. Soc.*, 1976, **67**, 193.
[256] M. C. Berthe, R. Bonaly, and O. Reisinger, *Canad. J. Microbiol.*, 1976, **22**, 929.
[257] J. F. Peberdy and P. M. Moore, *J. Gen. Microbiol.*, 1975, **90**, 228.
[258] J. Ruiz-Herrera and S. Barnticki-Garcia, *J. Gen. Microbiol.*, 1976, **97**, 241.
[259] R. E. Ulane and E. Cabib, *J. Biol. Chem.*, 1976, **251**, 3367.
[260] M. Horisberger and J. Rosset, *Experientia*, 1976, **32**, 998.

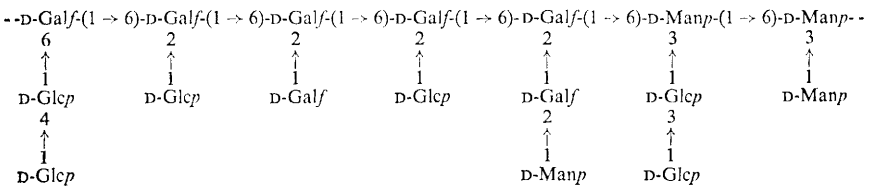
which are found predominantly in chitin of the bud scars in budding yeasts. A temperature-sensitive mutant of *S. cerevisiae* has been used to study the synthesis of chitin during the cell cycle and the involvement of the enzyme in growth and septum formation.[261]

The hyphal cell walls of three mycobionts isolated from the lichens *Xanthoria parientina*, *Tornabenia intricata*, and *Sarcogyne* sp. are composed of chitin and glucan.[262] Fluorescein-labelled lectins were used to locate the polysaccharides in the cell wall.

Miscellaneous Fungal Polysaccharides

A procedure for the selective *N*-acylation of chitosan, using aqueous methanolic acetic acid in the presence of carboxylic anhydrides, has been reported.[263] A study of the ^{13}C and ^{1}H n.m.r. spectra of methyl aldofuranosides and their *O*-alkyl derivatives has permitted signals in the spectra of furanose-containing oligo- and poly-saccharides, including the galactomannan of *Penicillium charlesii*, to be assigned.[264] The hyphal cell-wall of *A. niger* contains an alkali-soluble galactosaminogalactan having a DP of *ca.* 100.[265, 266] Methylation analysis, Smith degradation, deamination, and the optical rotation indicated that the polysaccharide is composed of a linear chain of D-galactopyranosyl and 2-amino-2-deoxy-D-galactopyranosyl residues joined by α-(1 → 4)-linkages. The amino-sugar residues, which account for roughly 20% of the polysaccharide, are distributed randomly along the chain.

The presence of *O*-acetyl groups in the peptidogalactomannan isolated from *Cladosporium werneckii* has been revealed by chemical analysis and ^{13}C n.m.r. spectroscopy.[267] The principal cell-wall polysaccharide of *C. tricoides* has been purified and shown to contain residues of D-galactose, D-glucose, and D-mannose.[268] A branched structure (23) having a backbone of D-galacto-furanosyl and D-mannopyranosyl residues, bearing side-chains of D-gluco-pyranosyl, D-galactofuranosyl, and D-mannopyranosyl residues, was proposed for the polysaccharide.



(23)

[261] E. Cabib and B. Bowers, *J. Bacteriol.*, 1975, **124**, 1586.
[262] M. Galun, A. Braun, A. Frensdorff, and E. Galun, *Arch. Mikrobiol.*, 1976, **108**, 9.
[263] S. Hirano, Y. Ohe, and H. Ono, *Carbohydrate Res.*, 1976, **47**, 315.
[264] P. A. J. Gorin and M. Mazurek, *Carbohydrate Res.*, 1976, **48**, 171.
[265] P. C. Bardalaye and J. H. Nordin, *J. Bacteriol.*, 1976, **125**, 655.
[266] P. C. Bardalaye and J. H. Nordin, *J. Bacteriol.*, 1976, **126**, 561.
[267] W. L. Lee and K. O. Lloyd, *Arch. Biochem. Biophys.*, 1975, **171**, 613.
[268] T. Miyazaki and Y. Naoi, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 1718.

The toxic mushroom *Lampteromyces japonicus* contains a highly dextrorotatory polysaccharide that is composed of residues of D-glucose, D-mannose, and D-galactose; the polysaccharide showed anti-tumour activity towards implanted sarcoma 180 in mice.[269] Structural studies indicated that the purified polysaccharide contains mainly (1 → 6)- and (1 → 2)-linked D-mannopyranosyl, (1 → 3)-linked D-glucopyranosyl, and (1 → 6)-linked D-galactopyranosyl residues, with branching at O-6 of some of the D-glucopyranosyl residues.[270]

An extracellular polysaccharide isolated from *Lipomyces tetrasporus* contains D-galactopyranosyl, D-mannopyranosyl, and D-glucopyranosyluronic acid residues.[271] The results of methylation analysis, selective decomposition of the derived uronamide, and selective oxidation of the β-glycosidic linkages indicated that the polysaccharide is composed of the basic structural unit (24).

$$\begin{array}{l}\beta\text{-D-GlcUA}p\text{-}(1 \rightarrow 2)\text{-D-GlcUA}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Man}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad 3 \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad \uparrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad 1 \\ \qquad\qquad\qquad\qquad\qquad\qquad \alpha\text{-D-Gal}p\end{array}$$

(24)

The surface of the oocytes of starfish is thought to carry receptors for 1-methyladenine, which induces maturation by a mechanism as yet unexplained.[272] A heterogalactan from *Lentinus edodes*, which contains an α-(1 → 6)-linked D-galactopyranosyl backbone with single-unit L-fucopyranosyl and D-mannopyranosyl side-chains, completely inhibited maturation of the oocytes, due to the presence of either α-(1 → 6)-galactan or α-(1 → 6)-D-galacto-oligosaccharide sequences in the polysaccharide.[273]

A polysaccharide released with alkali from the cell walls of *Penicillium charlesii* is composed mainly of D-galactofuranosyl and D-glucopyranosyl residues.[274] The absence of detectable quantities of D-mannosyl residues in this polysaccharide suggests that it differs from the peptidophosphogalactomannan, which is the principal extracellular product of the fungus. The results conflict with other work in which no evidence was presented for the presence of D-galactofuranosyl residues in the cell-wall polysaccharides of another strain of *P. charlesii* grown under different conditions.[221] Acidic fractions having anti-tumour activity have been isolated from *Pleurotus ostreatus*.[275] Three components were isolated in a chromatographically pure form from one of the fractions, and preliminary investigations of their structures were reported; the main structure of the component of highest molecular weight, which is the only one retaining anti-tumour activity, consists of β-(1 → 3)-linked D-glucosyl residues, probably having branches of D-galactosyl and D-mannosyl residues, and also containing acidic sugars.

[269] K. Fukuda, T. Uematsu, A. Hamada, S. Akiya, N. Komatsu, and S. Okubo, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1955.
[270] K. Fukada and A. Hamada, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 1133.
[271] N. K. Kochetkov, O. S. Chizov, A. F. Sviridov, S. E. Gorin, and I. P. Bab'eva, *Bull. Acad. Sci. U.S.S.R.*, 1975, **24**, 2660.
[272] M. Dorée and P. Guerrier, *Exp. Cell Res.*, 1975, **96**, 296.
[273] H. Shida and M. Shida, *Nature*, 1976, **263**, 77.
[274] J. E. Gander and F. Fang, *Biochem. Biophys. Res. Comm.*, 1976, **71**, 719.
[275] Y. Yoshioka, M. Emori, T. Ikekawa, and F. Fukuoka, *Carbohydrate Res.*, 1975, **43**, 305.

The black yeast fungus NRRL YB-4163 (now tentatively identified as *Rhinocladiella elatior*) synthesizes an extracellular microbial polysaccharide composed mainly of either (1 → 3)- or (1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucuronic acid residues.[276] This acidic polysaccharide appears to have a structure related to the antigenic polysaccharide from *Staphylococcus aureus*, which is also composed entirely of 2-amino-2-deoxy-D-glucuronic acid with the amino-groups substituted equally with acetyl and *N*-acetylalanyl groups.

The preferential synthesis of different polysaccharides by specific morphological types of *Sporothrix schenckii* has been reported.[277] Thus, yeasts synthesize rhamnomannans containing monorhamnosyl side-chains, conidia or conidia-forming mycelia synthesize rhamnomannans with dirhamnosyl side-chains, and unsporulated mycelia synthesize galactomannans or mannans that are readily excreted into the culture medium. A combination of methylation analysis, Smith degradation, and ^{13}C n.m.r. spectroscopy was used to deduce the structure (25) for the D-mannan core of *S. schenckii* galactomannan.

α-D-Man*p*
1
↓
2
→ 6)-α-D-Man*p*-(1 → 6)-α-D-Man*p*-(1 →

(25)

The immunoadjuvant activities of the cell walls of a number of fungi have been examined.[278] Some fungal preparations showed definite adjuvant activity, but fungal cell walls generally seem to be less potent than bacterial cell walls in stimulating either tumoral or cellular immune responses.

[276] P. R. Watson, P. A. Sandford, K. A. Burton, M. C. Cadmus, and A. Jeanes, *Carbohydrate Res.*, 1976, **46**, 259.
[277] L. Mendonca, P. A. J. Gorin, K. O. Lloyd, and L. R. Travassos, *Biochemistry*, 1976, **15**, 2423.
[278] S. Kotani, Y. Watanabe, T. Narita, T. Shimono, D. E. S. Stewart-Tull, S. Iwata, H. Yamaguchi, K. Iwata, T. Shimizu, I. Mifuchi, Y. Nozawa, Y. Ito, F. Kanetsuna, K. Yano, A. Misaki, T. Matsuoka, and K. Fukui, *Biken. J.*, 1975, **18**, 135.

5

Glycoproteins, Glycopeptides, and Animal Polysaccharides

By B. J. CATLEY

Microbial Glycoproteins

The glycoproteins of Sendai virus which are responsible for the adsorption to cell surfaces are present as oligopeptides bridged by disulphide linkages.[1] Cleavage of these linkages confers a haemagglutinating ability on the virus, but the significance of this is not known. A glycoprotein (mol. wt. 8×10^4) has been extracted from rabies virus that had been grown in primary foetal bovine-kidney cells.[2] The principal glycoprotein of avian myeloblastosis virus has been purified by gel filtration in the presence of guanidinium hydrochloride; it has a molecular weight of 7.7×10^4.[3] The carbohydrate moiety (40% by weight) contains 2-amino-2-deoxy-D-glucose, as the principal component, and D-mannose, D-galactose, L-fucose, and sialic acid. The principal oncornavirus glycoprotein in mice has been studied, using quantitative radioimmunoassays and immunofluorescence to locate the main sites of expression.[4] Despite considerable variations in the strains of mice examined, the glycoprotein appears to be restricted to lymphoid and epithelial cells.

An L-fucose-deficient, precursor glycoprotein of Rauscher leukaemia virus glycoprotein (69/71) has been reported; it is synthesized in virus-infected cells from a glycoprotein (mol. wt. 9×10^4) which can be labelled with radioactive 2-amino-2-deoxy-D-glucose, but not with L-fucose, whereas the Rauscher glycoprotein can be labelled with both.[5] The principal glycoprotein in three strains of Rous sarcoma virus has been purified, and the structures (1) and (2) were proposed for the carbohydrate moieties of the subgroups A and B, respectively.[6]

α-Neu*N*Ac*p*-β-D-Gal*p*-β-D-Glc*N*Ac*p*-α-D-Man*p*
↓
[β-D-Glc*N*Ac*p*]-β-D-Man*p*-β-D-Glc*N*Ac*p*-β-D-Glc*N*Ac*p*-Asn
↑
[α-Neu*N*Ac*p*]-β-D-Gal*p*-β-D-Glc*N*Ac*p*-α-D-Man*p*

(1)

Residues enclosed in [ ] are not present in some cases

[1] M. Ozawa, A. Asano, and Y. Okada, *F.E.B.S. Letters*, 1976, **70**, 145.
[2] P. Atanasiu, P. Perrin, H. Tsiang, and S. Favre, *Ann. de Microbiologie*, 1976, **127B**, 91.
[3] W. H. Porter and R. J. Winzler, *Arch. Biochem. Biophys.*, 1975, **166**, 152.
[4] R. A. Lerner, C. B. Wilson, B. C. Del Villano, P. J. McConahey, and F. J. Dixon, *J. Exp. Med.*, 1976, **143**, 151.
[5] R. B. Naso, L. J. Arcement, W. L. Karshin, G. A. Jamjoom, and R. B. Arlinghaus, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 2326.
[6] M. J. Krantz, Y. C. Lee, and P. P. Hung, *Arch. Biochem. Biophys.*, 1976, **174**, 66.

$(\alpha\text{-D-Man}p)_{4-6}\text{-}\beta\text{-D-Man}p\text{-}\beta\text{-D-Glc}N\text{Ac}p\text{-}\beta\text{-D-Glc}N\text{Ac}p\text{-Asn}$

(2)

The oligosaccharides are probably present in the ratio of 3 : 1, suggesting the presence of thirteen oligosaccharide chains for each monomer of molecular weight 7.5×10^4. The three envelope glycoproteins of the Semliki Forest virus do not contain 2-amino-2-deoxy-D-galactose, which was previously thought to be present.[7] A study of the assembly of vesicular stomatitis proteins into HeLa cell plasma membranes has shown that, although the two protein components take less than 5 min to appear, the glycoprotein takes 20 min.[8] A similar delay is observed in the incorporation of the glycoprotein into the released virus. Pulse-chase experiments showed that it takes 20 min for the glycoprotein to pass from the site where its synthesis is completed to the plasma membrane. Specific rabbit antisera to the principal glycoproteins of Friend leukaemia virus (gp 71) and mouse mammary tumour virus (gp 52) have been used in studies of the surfaces of transformed and normal cells.[9] The virus gp 71 is expressed by a wide variety of normal and transformed cells, whereas gp 52 is expressed mainly by murine cells with a high incidence of mammary tumours.

The sequences of peptides derived from influenza-virus haemagglutinin have been determined, but there is no information about the location of the carbohydrate moieties.[10] The genes of the influenza-virus genome coding for the haemagglutinin and for neuraminidase proteins have been identified.[11] The HA_1 heavy-chain (mol. wt. 4.7×10^4) of the haemagglutinin derived from a variant of Hong Kong influenza virus has been shown to contain most of the carbohydrate residues and to be of much lower molecular weight than that estimated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate.[12] Both the HA_1- and HA_2-chains are glycosylated; the former contains a carbohydrate moiety (mol. wt. 1.14×10^4) consisting of 2-acetamido-2-deoxy-D-glucose (19), D-galactose (10), D-mannose (33), and L-fucose (5 residues), whereas the light-chain appears to contain only 2-acetamido-2-deoxy-D-glucose (7 residues).

Sialic acid, D-galactose, and 2-amino-2-deoxy-D-glucose have been identified as components of the type-specific antigen of group B *Streptococcus*.[13] Exposure of the cell envelopes isolated from purified elementary bodies of *Chlamydia psittaci* to alkaline H_4edta resulted in the release of spherical and rod-like particles, which are thought to be components of the cell surface.[14] Preliminary analysis of the particles indicated that some of them are probably complexes of protein, carbohydrate, and lipid. The glycoprotein accounting for half of the protein and all of the non-lipid-linked carbohydrate in the cell envelope of *Halobacterium salinarium* has been purified; it has a molecular weight of

[7] K. Mattila, A. Luukkonen, and O. Renkonen, *Biochim. Biophys. Acta*, 1976, **419**, 435.
[8] P. H. Atkinson, S. A. Moyer, and D. F. Summers, *J. Mol. Biol.*, 1976, **102**, 613.
[9] W. D. Holder, G. W. Peer, D. P. Bolognesi, and S. A. Wells, *Cancer Res.*, 1976, **36**, 3217.
[10] D. J. Bucher, S. S.-L. Li, J. M. Kehoe, and E. D. Kilbourne, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 238.
[11] P. Palese and J. L. Schulman, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 2142.
[12] C. W. Ward and T. A. A. Dopheide, *F.E.B.S. Letters*, 1976, **65**, 365.
[13] C. J. Baker and D. L. Kasper, *Infection and Immunity*, 1976, **13**, 284.
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2.0×10^5, is extremely acidic, and contains 10—12% of carbohydrate, which consists of a single *N*-linked oligosaccharide, 22—24 *O*-linked disaccharides, and 12—14 *O*-linked trisaccharides per molecule.[15] This glycoprotein is thought to be chiefly responsible for maintaining the shape of the bacterial cell.[16]

Protease II – an enzyme of trypsin-like activity present in crude extracts of *Escherichia coli* harvested in the late log phase – has been separated into two glycoproteins that exhibit different kinetic properties.[17] Only one of these enzymes is present in extracts of the bacteria harvested during the mid-exponential phase. The macromolecules arrayed on the outer cell walls of *Clostridium thermosaccharolyticum* and *C. thermohydrosulphuricum* have been shown to be acidic glycoproteins (mol. wt. 1.4×10^5) containing D-glucose, D-galactose, D-mannose, and L-rhamnose.[18] Glycoproteins in the intact sheath of *Volvox rousselatii* have been located by means of various histochemical techniques.[19] Glycopeptidic antigens prepared from surface-grown cultures of *Trichophyton mentagrophytes* var. *granulosum* have been shown to have higher molecular weights than, and different carbohydrate compositions from, those previously reported.[20] An extracellular glycoprotein (mol. wt. 2.0×10^5) isolated from cell-free culture fluids of *Neisseria meningitidis* (serogroup A CN4937) was found to contain 60—65% of carbohydrate; the principal amino-sugar was identified as 2-amino-2-deoxy-D-mannose.[21]

Treatment of the cell walls of *Rhodotorula rubra* with ethylenediamine afforded a water-soluble fraction containing three glycoproteins; the principal component (mol. wt. 6.4×10^4) in this fraction contained D-mannose, D-glucose, and 2-amino-2-deoxy-D-glucose, and the carbohydrate—peptide linkage is thought to involve asparagine.[22] Methylation studies indicated that the carbohydrate chain is branched at positions 3 or 6 or both of the D-mannosyl residues, and that some D-glucosyl residues occur at non-reducing termini. Following reports of the occurrence of antitumour activities in the fruiting bodies of the Basidiomycete *Armillaria mellea*, the structure of the associated glycoprotein has been investigated.[23] The D-glucan moiety of the glycoprotein consists of β-(1 → 3)- and β-(1 → 6)-linked residues, and is covalently linked to threonine. The acid proteases of *Mucor miehei* – already shown to be glycoproteins – bind concanavalin A.[24]

A carbohydrate-binding protein, whose appearance is regulated during development, has been located on the outer surface of the plasma membrane of *Dictyostelium discoideum*.[25] An examination of mutants showed that the amount

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[16] M. F. Mescher and J. L. Strominger, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 2687.
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[20] M. T. Arnold, S. F. Grappel, A. V. Lerro, and F. Blank, *Infection and Immunity*, 1976, **14**, 376.
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[25] C-H. Siu, R. A. Lerner, G. Ma, R. A. Firtel, and W. F. Loomis, *J. Mol. Biol.*, 1976, **100**, 157.

of this protein present is related to the cohesiveness of the cells. An α-D-galactosidase that participates in the turnover of mucopolysaccharides in *D. discoideum* has been examined.[26] The enzymic activity is highest during interphase, and the subcellular distribution of the enzyme appears to differ from that of other glycosidases, since most of it is soluble. The excretion of this enzyme by *D. discoideum* during development of the cells is little understood. Immunological characterization of the β-glucosidases of *D. discoideum* revealed that they are glycosylated after translation.[27] The binding of concanavalin A to the surfaces of amoebae produced different reactions that depended on the concentration of the lectin.[28] Low concentrations of the lectin produced little differentiation and an increased level of phosphodiesterase, whereas both processes were inhibited at high concentrations of the lectin.

Plant Glycoproteins

The properties of extracellular macromolecules, including extensin, found in plants, and the role of the Golgi apparatus in the synthesis of these macromolecules have been reviewed.[29] Sunflower heads contain several glycoproteins composed of D-galacturonic acid, D-galactose, D-glucose, L-arabinose, D-xylose, and L-rhamnose.[30] An *O*-glycosyl linkage between L-arabinose and hydroxyproline has been found in rice-bran proteoglycan.[31] Legumin and vicilin, the reserve globulins in cotyledons of *Pisum sativum*, contain $\leqslant 1\%$ of D-mannose, D-glucose, and 2-amino-2-deoxy-D-glucose, but changes in the extent of glycosylation were noted during the development and germination of the seeds.[32] A linear relationship has been established between the rate of elongation of the cell wall and the concentration of an hydroxyproline-rich glycoprotein in the cell wall of *P. sativum*.[33] It is suggested that the glycoprotein stiffens the cell wall during extension growth, thereby reducing the rate of elongation. The cell-wall glycoproteins of carrots contain D-galactosyl → serine linkages, and the carbohydrate consists mostly of D-galactose.[34] The carbohydrate residues of horseradish peroxidase isoenzyme C are attached to the protein at six or seven sites; the sequence containing the asparagine linkage and the proportions of L-fucose, L-arabinose, D-xylose, D-mannose, D-glucose, and 2-amino-2-deoxy-D-glucose were determined for several oligoglycopeptides.[35] β-(1 → 4)-Linked D-xylopyranosyl and (1 → 2)-, (1 → 3)-, and (1 → 5)-linked L-arabinofuranosyl residues have been found in the glycoprotein of the primary leaves of *Phaseolus vulgaris* cv. 'Pinto'.[36] Differences in the structure of this glycoprotein were observed after inoculation of the leaves with tobacco necrosis virus. Glycoproteins

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[36] R. G. Brown, W. C. Kimmins, and B. Lindberg, *Acta Chem. Scand.* (*B*), 1975, **29**, 843.

isolated from *P. vulgaris* cv. 'Black Valentine' are similar to those from cv. 'Pinto'. A procedure for the isolation and purification of stem bromelain has been described.[37]

Lectins

The structure–function relationship, the effects of chemical modification, and the interaction with glycoproteins (especially those associated with cell surfaces) of concanavalin A have been comprehensively reviewed.[38] Current investigations of this non-glycosylated lectin include the preparation of univalent dimers by a combination of succinylation and photoaffinity labelling with 4-azidophenyl α-D-mannopyranoside.[39, 40] The dimers failed to agglutinate sheep erythrocytes and also showed saturated dose–response curves in the mitogenic stimulation of mouse-spleen lymphocytes. Admixture of native concanavalin A and its dimeric succinylated derivative yielded a new species containing one protomer of each.[41] Similar exchanges were also observed with acetyl- and succinyl-concanavalin A. The preparation of these derivatives increases the range of reagents available for studies of receptor–receptor and receptor–cytoplasm interactions in the proliferation and differentiation of cells. Fluorometric studies have indicated that the main conformational changes that result when methyl α-D-mannopyranoside binds to concanavalin A do not affect surface-located tryptophan residues, so that conformational changes previously described must be ascribed to internal tryptophan residues.[42] Longitudinal nuclear relaxation times of the glycosidic methoxy-group have been used to locate the binding site for methyl α-D-mannopyranoside on concanavalin A.[43] This inhibitor appears to bind to the cavity of the saccharide-binding site, but alternative sites cannot be discounted. The effects of temperature and pH on the binding of methyl α-D-mannopyranoside to concanavalin A, in conjunction with the results of similar studies with the lectins from *Phaseolus vulgaris* and with the agglutinins of wheat germ and soybean, suggest that conformational changes may be induced in the proteins on binding the inhibitor.[44] The binding of Mg^{2+} and Ca^{2+} ions to carboxy-groups at the *N*-terminus of the chain appears to contribute to the stabilization of the lectin to heat and to guanidinium hydrochloride.[45] Removal of the metal ions, followed by trypsinolysis, afforded a univalent lectin that was able to bind Mg^{2+} and Ca^{2+} ions and 4-nitrophenyl α-D-mannopyranoside, but not glycogen.[46] Although this lectin could not agglutinate *Bacillus subtilis* cells, it inhibited the agglutination of the cells by unmodified concanavalin A. The binding to Sephadex G-75 and elution with

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a solution of methyl α-D-mannopyranoside have been used to assess the binding properties of derivatized forms of the lectin.[47]

Three forms of agglutinin have been found in the hexaploid wheat-germ *Triticum aestivum* L., whereas the tetraploid wheat-germ *T. turgidum* L. (durum group) contains only two; this emphasizes the advisability of conducting experiments with genetically identified varieties of plants.[48] Oxidation of wheat-germ agglutinin with *N*-bromosuccinimide modified two of the three tryptophan residues.[49] The oxidation of one tryptophan residue in each subunit in the presence of acetic acid and urea resulted in almost complete loss of the haemagglutinating activity, a decrease in the affinity for tri-*N*-acetylchitotriose, and the failure of the dissociated subunits to reassemble. The changes in luminescence produced on binding a series of chito-oligosaccharides to wheat-germ agglutinin have been used to probe protein–substrate interactions.[50]

Two agglutinating activities have been detected in the seeds of the pea tree *Caragana arborescens*.[51] The principal activity is composed of two types of non-identical subunits (each of mol. wt. 3×10^4), which in the native molecule are cross-linked by disulphide bonds to form dimers. The dimers can combine to form tetramers. 2-Amino-2-deoxy-D-galactose and D-galactose are bound by this lectin. Two polypeptide chains (mol. wts. 3.2×10^4 and 3.3×10^4) have been separated from phaetohaemagglutinin M from *Phaseolus vulgaris*.[52] The hexose content (16%) is greater than that of the associated phaetohaemagglutinin L, but the molecular weights of the subunits are similar. A number of glycopeptides have been isolated following proteolysis of the 7S protein of soybean globulins; they all contained an asparagine–sugar linkage and 2-amino-2-deoxy-D-glucose (2) and D-mannose (7—9 residues).[53] Studies of the aggregation of soybean lectin showed that it has at least four saccharide-binding sites.[54]

Specific interactions between the O-antigen-containing lipopolysaccharides of *Rhizobium japonicum*, *R. leguminosarum*, and *R. phaseoli* and the lectins from Jack bean, lima bean, and other legume hosts have been investigated.[55] In each case, the carbohydrate from a *Rhizobium* interacted with the lectin of its symbiont, but not with the other lectins. The synthesis of myeloma protein by plasma cytoma (MOPC-315) cells was inhibited by ricin, supporting the views that a component of the lectin penetrates the membrane and inhibits protein synthesis, and that less accessible, free polyribosomes are inhibited after more accessible, membrane-bound polyribosomes.[56] Other work has indicated that the elongation factor EF-2 involved in protein synthesis competes for the target site for abrin and ricin A when it binds to ribosomes.[57] Purified peanut agglutinin (mol. wt.

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1.1×10^5) has been shown not to be a glycoprotein.[58] The agglutination of lymphocytes by this lectin is accomplished only with neuraminidase-treated cells.[59] A lectin with blood-group N specificity is present in *Vicia graminea* seeds.[60] Its activity was not inhibited by any of the monosaccharides present in the blood-group substances, but inhibition was achieved with the principal glycoprotein present in the erythrocyte membrane and with tryptic fragments obtained from it. The lectin contains four subunits (mol. wt. 2.5×10^4) and carbohydrate (7.3%).

Sulphenylation has demonstrated an homology in the amino-acid sequences near the sugar-binding sites of pea lectins and concanavalin A.[61] A preliminary *X*-ray analysis of the mitogenic lectin from pea seeds has been published.[62] The lectin from broad beans has been purified by affinity chromatography on Sepharose to which 2-amino-2-deoxy-3-*O*-methyl-D-glucose was covalently attached.[63] D-Mannose and 2-amino-2-deoxy-D-glucose are components of the carbohydrate portion of the lectin, which consists of two identical subunits (mol. wt. 2.4×10^4). 3-*O*-Substituted monosaccharides are efficient inhibitors of the lectins from broad beans, peas, and lentils, but not of concanavalin A. However, (1 → 3)-linked disaccharides are not inhibitors. The α-D-galactose-binding lectin from the seeds from *Bandeiraea simplicifolia* preferentially agglutinated blood-group B red cells, and it could also distinguish between blood-group A_1 and A_2 erythrocytes.[64] Chemical modification of the lectin showed that carboxy-groups play a role in the binding of sugars, whereas amino- and sulphydryl groups do not.[65] Reduction of the lectin from *Wistaria floribunda* seeds with 2-mercaptoethanol afforded two inactive subunits, each of molecular weight 3.2×10^4.[66] Re-oxidation with air restored the activity. The carbohydrate moiety (3.2%) of this lectin, which contains D-mannose, D-galactose and 2-amino-2-deoxy-D-glucose, is disposed as a single chain on each subunit.

Extracts of the marine polychaetous annelid *Amphitrite ornata* contain a glycoprotein (mol. wt. 3.2×10^4) that possesses haemagglutinating activity towards human ABO erythrocytes.[67]

Lectins from soybean, *Wistaria floribunda*, *Bauhinia purpurea* var. *alba*, and *Sophora japonica* were bound by acid-treated Sepharose 6B, whereas those from lima bean, *Dolichos biflorus*, and kidney bean were not.[68] The binding of lectins by acid-treated Sepharose was affected by temperature and pH.

Anti-galactan lectins from the clam *Tridacna maxima* and the sponge *Axinella polypoides* have been used in studies of murine myeloma protein J 539.[69] Three

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lectins were separated from the sponge; the two principal fractions (mol. wts. 2.1×10^4 and 1.5×10^4), which contain 0.5% of carbohydrate, precipitated A_1, A_2, B, Le^a, and precursor I blood-group substances to different extents.[70] The mucus of the Pacific hagfish, *Eptatretus stoutii*, is a source of haemagglutinin.[71]

Extracts from cohesive cells of four species of cellular slime mould (*Dictyostelium mucoroides*, *D. purpureum*, *D. rosarium*, and *Polysphondylium violaceum*) agglutinated erythrocytes in a manner akin to *D. discoideum*.[72] The lectin from *Polysphondylium pallidum* has been purified to homogeneity by adsorption onto formalinized erythrocytes; it has a subunit of molecular weight 2.4×10^4 and appears to differ from the lectins isolated from other slime moulds.[73]

An agglutinin specific for the non-reducing, terminal sequence β-D-Gal*p*-(1 → 4)-β-D-Glc*NAc*p-(1 → 3)-D-Gal*p* has been isolated from the serum of a patient with Waldenstrom's macroglobulinaemia.[74] The involvement of the β-D-galactose-specific lectin in cultured chicken-muscle cells in myoblast fusion has been questioned, since the potent inhibitors 4-*O*-β-D-galactopyranosyl-1-thio-β-D-galactopyranose and lactose were without effect on the interaction with the cells.[75] Anti-(B + HP) specificity towards human erythrocytes has been reported for a heterohaemagglutinin isolated from toad (*Bufo regularis*) serum.[76]

Blood-group Substances

Acetylation of red blood cells bearing the B antigen restored the A_1 specificity, with concomitant loss of agglutinability with anti-B sera.[77] The reverse transformation was achieved by a deacetylase from *Clostridium tertium*. The α-D-galactosyltransferases in the blood and serum of the Japanese tortoise transferred D-galactose from UDP-D-galactose to human O cells, converting them into B cells, but not into Bombay O_h cells.[78] The relationship between serum α-D-galactosyltransferase activity and the B phenotype has been found to be only partially correlated in subjects from various ethnic groups.[79] Blood-group substances isolated from an ovarian cyst fluid, by extraction with phenol and fractional precipitation with ethanol, possessed remarkably diverse biological activities.[80] Progressive decreases in the proportions of L-fucose and D-galactose and of B activity were accompanied by increases in I and i activities. Some fractions were precipitated by concanavalin A and may contain non-reducing, terminal 2-acetamido-2-deoxy-α-D-glucopyranosyl residues. Two of the fractions showing the greatest difference in their biological activities were analysed.[81, 82]

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Weak blood-group A activity has been found in a glycolipid-free glycoprotein fraction extracted with lithium di-iodosalicylate from the membranes of blood-group A erythrocytes.[83] A blood-group A substance has been identified in eggs of the prosobranch snails *Pomacea canaliculata* and *P. insularum*.[84] Affinity chromatography using the L-fucose-binding lectins from *Lotus tetragonolobus* and *Dolichos biflorus* has separated the blood-group substances in human and porcine gastric mucosa.[85] Fractions possessing A or H activity only were readily obtained. Human blood-group A_1 and A_2 substances were also examined using this chromatographic procedure, and the fractionation of blood-group A_2 substance into two components was reported.

Optimal expression of H antigens on the surface of HeLa cells has been shown to depend on a growth pattern similar to that of monolayer cells during exponential growth.[86] Changes in the method of culture may produce transient cellular and enzymic alterations, which result in a temporary loss of phenotypic H activity. 2′-*O*-L-Fucosyl-*N*-acetyl-lactosamine and 2-*O*-L-fucosyl-D-galactose have been examined as acceptors in reactions catalysed by glycosyltransferases specified by blood-group A and B genes.[87] There appeared to be little to choose between the two acceptors. A 2-acetamido-2-deoxy-α-D-galactosyltransferase in human milk has been used in the detection and characterization of a glycoprotein possessing H determinants present on O-erythrocyte membranes.[88] Structural studies of group A glycoproteins obtained by this reaction suggested that all erythrocyte H glycoproteins contain only the type-2 antigenic determinant. Six reduced oligosaccharides have been obtained by treatment of the blood-group substances isolated from hog-stomach linings with sodium borohydride.[89] Structural determinations revealed that they contain a common tetrasaccharide core, to which terminal α-L-fucopyranosyl and 2-acetamido-2-deoxy-α-D-glucopyranosyl residues are attached. The transfer of bovine blood-group J activity from the plasma to J(−) erythrocytes has been examined.[90] It was shown that the J activity of the cells is present in the lipid fraction of the membrane. Biochemical studies of J activity in cattle have been reported.[91]

The isolation, composition, and blood-group activity of neutral glycoproteins from human meconiums have been investigated.[92] The ABH substances have sedimentation coefficients of *ca.* 10S—11S, whereas those of Le^a preparations have a value of 7S. The substances are thought to have a common peptide core, and the relative proportions of the constituent sugars, excepting L-fucose, show little variation. Enzymes secreted by enteric bacteria, which are able to hydrolyse blood-group substances, have been isolated from faecal cultures.[93] Evidence that the ABO serotype and secretor status of the host affects the specificity of the hydrolases has been presented.[94]

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Collagens

Two reviews containing references to the glycosylation of collagens have appeared: one describes the normal metabolism of collagen and comments on the lysylhydroxylase deficiency in the Ehlers–Danlos syndrome type VI,[95] while the other highlights recent developments in studies of articular cartilage collagen.[96]

Rabbit corneal scar-tissue has been shown to contain a high content of glycosylated dihydroxylysylnorleucine cross-linkages.[97] The formation of such cross-linkages may regulate the diameter of the fibril, which, in turn, may be a factor in the loss of transparency of the cornea. A cross-linked peptide obtained by treatment of human skin with cyanogen bromide has been shown to be completely glycosylated; it was compared with similar fragments from rat skin.[98] The presence of D-mannose in a glycopeptide isolated from procollagen α_1(II) infers that a portion of the precursor molecule is not present in collagen α_1(II).[99] The normal complement of hydroxylysine-bearing disaccharides in $[\alpha_1(II)]_3$ collagen has been detected in fractions isolated at autopsy from human nucleus pulposus and annulus of patients 7—30 years old.[100] The addition of α_1-acid glycoprotein to dialysed solutions of collagen resulted in a significant decrease in the intensity of the c.d. spectrum of collagen.[101] Removal of all the sialic acid residues from α_1-acid glycoprotein prevented collagen from forming fibrous long-spacing fibres, whereas removal of only 35% of these residues had no effect on the interaction between the proteins. Partial oxidation of monomeric and polymeric collagens with periodate delayed the polymerization of the monomer and inhibited the ability of collagen to interact with platelets, suggesting that the carbohydrate residues are located at, or near, the site of platelet adhesion.[102] The composition of rat glomerular basement membrane, unlike that of the human membrane, did not appear to alter on prolonged exposure to streptozotocin.[103] D-Galactosylhydroxylysine has been isolated from marine sponges.[104]

The carbohydrate contents of the glycopeptides of newly synthesized procollagen have been examined.[105] The resistance of the glycopeptides to mild alkaline hydrolysis suggested that >90% of the oligosaccharide chains are not linked to serine or threonine residues. The glycosylation of hydroxylysine during the synthesis of procollagen in embryonic chicken tendon and cartilage has been examined.[106] Measurement of the incorporated hydroxylysine and carbohydrate showed that hydroxylation took place only on membrane-bound ribosomes and that some glycosylation of the nascent peptide occurred.

The D-glucosyltransferase present in Triton X-100 extracts of whole chicken embryos has been purified 2000-fold, and that from chicken-embryo cartilage

[95] J. Uitto and J. R. Lichtenstein, *J. Invest. Dermatol.*, 1976, **66**, 59.
[96] J. M. Lane and C. Weiss, *Arthritis and Rheumatism*, 1975, **18**, 553.
[97] D. J. Cannon and C. Cintron, *Biochim. Biophys. Acta*, 1975, **412**, 18.
[98] W. Henkel, J. Rauterberg, and T. Stirtz, *European J. Biochem.*, 1976, **69**, 223.
[99] A. Oohira, A. Kusakabe, and S. Suzuki, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 1086.
[100] W. R. Osebold and V. Pedrini, *Biochim. Biophys. Acta*, 1976, **434**, 390.
[101] C. Franzblau, K. Schmid, B. Faris, J. Beldekas, P. Garvin, M. H. Kagan, and B. J. Baum, *Biochim. Biophys. Acta*, 1976, **427**, 302.
[102] L. F. Brass and H. B. Bensusan, *Biochim. Biophys. Acta*, 1976, **444**, 43.
[103] P. J. Beisswenger, *J. Clin. Invest.*, 1976, **58**, 844.
[104] D. F. Smith, *Analyt. Biochem.*, 1976, **71**, 106.
[105] C. C. Clark and N. A. Kefalides, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 34.
[106] R. Harwood, M. E. Grant, and D. S. Jackson, *Biochem. J.*, 1975, **152**, 291.

has been purified 160-fold.[107] The transferase from these tissues has a molecular weight of 5.3×10^4, although an activity of molecular weight 1.3×10^5 was sometimes detected. Mg^{2+} was the most effective bivalent cation in stimulating enzymic activity, which was also stimulated by 1,4-dithiothreitol. After 40 days, the amount of this transferase in the renal cortex of genetically diabetic mice is significantly increased, and can be regarded as an early marker in the diagnosis of diabetic microangiopathy.[108]

Lysylphosphatidylcholine stimulated the D-galactosyl- and D-glucosyl-transferase activities present in particulate fractions of chicken-embryo extracts, whereas no enhancement of the enzymic activities was noted in soluble preparations; this may be significant in regulating these transferases *in vivo*.[109] D-Galactosyltransferase (purified 150-fold) from chicken-embryo extracts has been fractionated into three forms of molecular weights 5×10^4, 2×10^5, and 4.5×10^5.[110] It was suggested that the triple-helix conformation of collagen may prevent D-galactosylation of the hydroxylysine residues. Control of the synthesis of collagen in rat liver has been examined.[111] Hydroxylase activity increases to a greater extent than glycosylating activity during hepatic injury, whereas ageing produces a decrease in both enzymic activities. A study of the glycosylation of hydroxylysine in the endothelial cells of Descemet's membrane from rabbit cornea showed that 98% of the residues are substituted, principally with D-glucosyl-D-galactose.[112] Hydroxylation and glycosylation of lysine during the synthesis of collagen have been studied in isolated chicken-embryo cartilage.[113] Both reactions continue after the completed polypeptide chain is released into the endoplasmic reticulum, whereas they are probably discontinued after the formation of the triple helix and the transfer of collagen to the Golgi vesicles. β-D-Xylopyranosides depressed the synthesis of collagen, whereas they stimulated that of glycosaminoglycans, by cultured fibroblasts.[114]

The carbohydrate content of collagens isolated from patients with idiopathic pulmonary fibrosis appears to be normal.[115]

Glycogen

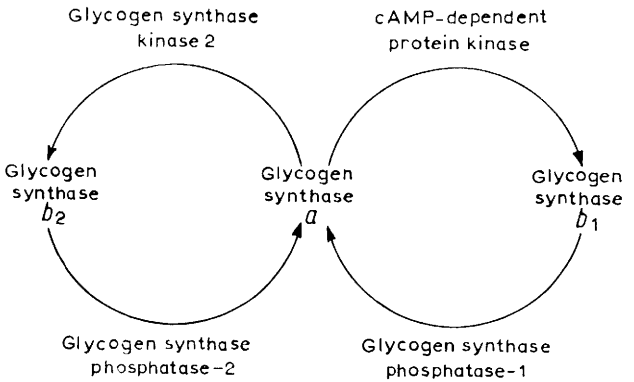
The control of the metabolism of glycogen in liver has been reviewed: the properties of the various enzymes are described, and the processes used by hormones and D-glucose to control the synthesis and breakdown of glycogen in liver are discussed.[116] Reference is also made to the metabolism of glycogen in muscle, where the events are better understood.

Elucidation of the molecular basis for the hormonal control of glycogen levels has received a good deal of attention, but the site of insulin action is still not clear.

[107] R. Myllylä, L. Risteli, and K. I. Kivirikko, *European J. Biochem.*, 1976, **61**, 59.
[108] A. S. Reddi, W. Oppermann, M. P. Reddy, C. A. Velasco, and R. A. Camerini-Davalos, *Experientia*, 1976, **32**, 1237.
[109] H. Anttinen, *Biochem. J.*, 1976, **160**, 29.
[110] L. Risteli, R. Myllylä, and K. I. Kivirikko, *Biochem. J.*, 1976, **155**, 145.
[111] L. Risteli and K. I. Kivirikko, *Biochem. J.*, 1976, **158**, 361.
[112] N. A. Kefalides, J. D. Cameron, E. A. Tomichek, and M. Yanoff, *J. Biol. Chem.*, 1976, **251**, 730.
[113] A. Oikarinen, H. Anttinen, and K. I. Kivirikko, *Biochem. J.*, 1976, **156**, 545.
[114] N. B. Schwartz and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 1108.
[115] J. M. Seyer, E. T. Hutcheson, and A. H. Kang, *J. Clin. Invest.*, 1976, **57**, 1498.
[116] H. G. Hers, *Ann. Rev. Biochem.*, 1976, **45**, 167.

The autoactivation of phosphorylase kinase – as distinct from phosphorylation mediated by protein kinase – has been shown to afford a high level of activity, which is greater than that produced by the protein kinase.[117] Autoactivation may be regulated by Ca^{2+} ions, glycogen, and phosphate.

An investigation of the molecular basis of phosphorylase kinase deficiency in mice has shown that neither of the normal subunits of the enzyme is present.[118] The genetics of the deficiency indicate that a single gene defect on the X-chromosome is involved. Further investigation of the phosphorylase kinase has shown that dephosphorylation of the α- and β-subunits is not catalysed by a single enzyme, and that rabbit skeletal muscle contains two distinct phosphorylase kinase phosphatases, with essentially absolute specificities towards the α- and β-subunits.[119] The enzymes associated with the α- and β-subunits have molecular weights of 1.7—1.8 × 10^5 and 7.5—8.0 × 10^4, respectively. Since the activity of phosphorylase kinase correlates with the degree of phosphorylation of the β-subunit, β-phosphorylase kinase phosphatase is responsible for reversing the activation of phosphorylase kinase. α-Phosphorylase kinase phosphatase may be considered to inhibit the reversal of the activation of phosphorylase kinase, since the extent of phosphorylation of the α-subunit of the kinase determines the rate of dephosphorylation of the β-subunit. A reappraisal of the interconversion of the *a* and *b* forms of rabbit skeletal muscle glycogen synthase has led to the characterization of a new glycogen synthase kinase.[120, 121] This kinase is not activated by adenosine cyclic 3′,5′-phosphate (cAMP), nor is it inhibited by the protein inhibitor of the cAMP-dependent enzyme. Moreover, it appears to phosphorylate different sites of the glycogen synthase molecule. It is suggested that glycogen synthase activity *in vivo* is controlled by means of two distinct phosphorylation–dephosphorylation cycles (Scheme 1). The phosphorylated



Scheme 1 *Regulation of glycogen synthase by two phosphorylation–dephosphorylation cycles*

[117] J. H. Wang, J. T. Stull, T.-S. Huang, and E. G. Krebs, *J. Biol. Chem.*, 1976, **251**, 4521.
[118] P. T. W. Cohen, A. Burchell, and P. Cohen, *European J. Biochem.*, 1976, **66**, 347.
[119] J. F. Antoniw and P. Cohen, *European J. Biochem.*, 1976, **68**, 45.
[120] H. G. Nimmo, C. G. Proud, and P. Cohen, *European J. Biochem.*, 1976, **68**, 21.
[121] H. G. Nimmo, C. G. Proud, and P. Cohen, *European J. Biochem.*, 1976, **68**, 31.

synthases are designated b_1 and b_2, and there must also be corresponding phosphatases to dephosphorylate the phosphorylated sites. The identification of a second synthase kinase, which is independent of cAMP and which does not act on phosphorylase kinase, may explain why glycogen synthase is largely phosphorylated in resting muscle, whereas phosphorylase and phosphorylase kinase are almost completely dephosphorylated. It also poses the question: 'Which phosphorylation–dephosphorylation step is affected by insulin?' Regulation of the functions of proteins by multisite phosphorylation has been reviewed.[122]

The nature of the primer in the reactions of glycogen and starch synthases has been reviewed.[123] UDP-2-amino-2-deoxy-D-glucose has been used as a substrate for rat-liver glycogen synthase, the amino-sugar being transferred to the terminal D-glucosyl residues of glycogen.[124]

A parallel and opposing mechanism to that mediated by cAMP-dependent kinase has been suggested for phosphorylase phosphatase.[125] A homogeneous rabbit-liver enzyme also dephosphorylated glycogen synthase *b*, thus providing co-ordinated control of phosphorylase inactivation and synthase activation. This regulation by the action of phosphatase has been discussed further, and the enzyme was suggested as an alternative target, rather than the kinase (*vide supra*), for insulin.[126] Another report has described the purification of two phosphoprotein phosphatases in rabbit liver by fractionation on Sepharose–histone.[127] These enzymes (mol. wts. 3.0×10^5 and 3.4×10^5) appear to have the same specificity, differing only in their K_m values.

The relations between hormonal action, the phosphorylation of proteins, and the kinetic properties of the enzymes involved in the synthesis and degradation of glycogen have been reviewed.[128] The control of glycogen synthase has also been discussed.[129]

The relation between the degree of phosphorylation of rabbit skeletal muscle glycogen synthase and its kinetic properties has been examined using samples of the purified enzyme containing 0.27—3.49 residues of phosphate per subunit of molecular weight 8.5×10^4.[130] It appears that phosphate cannot be introduced without altering the kinetic properties of the enzyme, and that either the sites of phosphorylation are not equivalent or identical sites interact in determining the kinetic properties. The ratio of effector concentrations and the degree of phosphorylation of the synthase have also been investigated: small changes only in the level of *a* activity bring about large changes in the entire synthase activity.[131] The compositions of the *a* and *b* forms of the rabbit muscle enzyme have been studied.[132] Two subunits of glycogen synthase *a* (mol. wts.

[122] P. Cohen, *Trends in Biochemical Sciences*, 1976, **1**, 38.
[123] W. J. Whelan, *Trends in Biochemical Sciences*, 1976, **1**, 13.
[124] A. L. Tarentino and F. Maley, *F.E.B.S. Letters*, 1976, **69**, 175.
[125] S. D. Killilea, H. Brandt, E. Y. C. Lee, and W. J. Whelan, *J. Biol. Chem.*, 1976, **251**, 2363.
[126] S. D. Killilea, H. Brandt, and E. Y. C. Lee, *Trends in Biochemical Sciences*, 1976, **1**, 30.
[127] R. L. Khandelwal, J. R. Vandenheede, and E. G. Krebs, *J. Biol. Chem.*, 1976, **251**, 4850.
[128] P. Cohen, J. F. Antoniw, H. G. Nimmo, and C. G. Proud, *Biochem. Soc. Trans.*, 1975, **3**, 849.
[129] P. J. Roach and J. Larner, *Trends in Biochemical Sciences*, 1976, **1**, 110.
[130] P. J. Roach, Y. Takeda, and J. Larner, *J. Biol. Chem.*, 1976, **251**, 1913.
[131] P. J. Roach and J. Larner, *J. Biol. Chem.*, 1976, **251**, 1920.
[132] Y. Takeda, H. B. Brewer, jun., and J. Larner, *J. Biol. Chem.*, 1975, **250**, 8943.

8.1×10^4 and 8.5×10^4) and one subunit of glycogen synthase *b* (mol. wt. 8.5×10^4) were found, although the smaller subunit of synthase *a* appears to be a product of proteolysis. On gel electrophoresis in the absence of a detergent, the purified *a* and *b* enzymes each showed two active protein bands (mol. wts. 1.5×10^5 and 3.4×10^5 for synthase *a*; 2.8×10^5 and 3.5×10^5 for synthase *b*), indicating that they exist as dimeric and tetrameric forms, and trimeric and tetrameric forms, respectively. Another purification of glycogen synthase *b* afforded an enzyme (mol. wt. 1.8×10^5) with a subunit molecular weight of 8.5×10^4.[133] On conversion of the *a* form into the *b* form, 1.13 molar equivalents of phosphate were incorporated per 8.5×10^4 g of protein, and the degree of phosphorylation correlated with the loss of enzymic activity. A single, unique phosphorylation of each molecule is required to convert the enzyme into a form that is wholly dependent on D-glucose 6-phosphate for activity. Limited trypsinolysis of either synthase *a* or *b* afforded a new D-glucose 6-phosphate-dependent enzyme composed of subunits of molecular weight 7.5×10^4.[134] Proteolysis is thought to occur near the carboxy-terminus, where there is probably a site susceptible to phosphorylation. Related investigations have suggested that one of the two sites in the subunit susceptible to phosphorylation is removed on treatment with trypsin.[135] The kinetic properties of glycogen synthase in the adipose tissues of fed, fasted, and re-fed rats, and of diabetic rats suggested that there may be as many as four interconvertible forms of the enzyme.[136]

The properties and the control of phosphorylase have been extensively reviewed.[137]

X-Ray analyses of glycogen phosphorylase *a* at 6 and 3 Å resolution, as well as the identification of the ligand-binding sites at 6 Å resolution, have been reported.[138, 139] The principal sites of contact between the subunits were identified, and the active site, of which there are two per dimer, is shared between two subunits at the interface. Within this region are found the binding sites for D-glucose 1-phosphate, arsenate, UDP-D-glucose, and AMP. The site of metabolic control (*i.e.* serine-14) was also identified. Maltoheptaose binds in another region, and its binding is sufficiently tight that the closest D-glucosyl residue is 25 Å from the binding site for D-glucose 1-phosphate. It is suggested that this poly(oligo)saccharide-binding site represents a storage site whereat phosphorylase is bound to glycogen particles in muscle cells. A spin-labelled derivative of AMP [N^6-(2,2,6,6-tetramethylpiperidin-4-yl-1-oxide)adenosine 5′-phosphate] and its diamagnetic analogue have been used to obtain information on the nature of the sites on phosphorylases that bind nucleotides, and also to detect conformational changes at these sites when other ligands are bound.[140]

[133] S. D. Killilea and W. J. Whelan, *Biochemistry*, 1976, **15**, 1349.
[134] Y. Takeda and J. Larner, *J. Biol. Chem.*, 1975, **250**, 8951.
[135] T. R. Soderling, *J. Biol. Chem.*, 1976, **251**, 4359.
[136] R. D. Eichner, *J. Biol. Chem.*, 1976, **251**, 2316.
[137] S. J. W. Busby and G. K. Radda, in 'Current Topics in Cellular Regulation', Vol. 10, ed. B. L. Horecker and E. R. Stadtman, Academic Press, New York, San Francisco, and London, 1976, p. 89.
[138] R. J. Fletterick, J. Sygusch, N. Murray, N. B. Madsen, and L. N. Johnson, *J. Mol. Biol.*, 1976, **103**, 1.
[139] R. J. Fletterick, J. Sygusch, H. Semple, and N. B. Madsen, *J. Biol. Chem.*, 1976, **251**, 6142.
[140] S. J. W. Busby, M. A. Hemminga, G. K. Radda, W. E. Trommer, and H. Wenzel, *European J. Biochem.*, 1976, **63**, 33.

Phosphorylases *a* and *b* were labelled with this group at essentially one sulphydryl group per subunit; the labelled enzyme is fully active and exhibits all the properties of the original enzyme.[141] Changes in the e.s.r. spectra of phosphorylases have been used to study conformational changes that result from the binding of activators or inhibitors or substrates. In most cases, the derived dissociation constants were in agreement with accepted values, except that a much lower value was found for the interaction between D-glucose 6-phosphate and phosphorylase *b*. The heterotropic interactions of ligands with phosphorylase *b* were also studied by e.s.r. spectroscopy.[142] The results suggest that at least four binding sites (for D-glucose 1- and 6-phosphates, nucleotides, and glycogen) are likely to be involved in regulating the enzyme.

Processes accompanying the contraction of skeletal muscle, which result in Ca^{2+}-dependent, transient phosphorylation of phosphorylase *b*, have been monitored in a glycogen particulate fraction from rabbit muscle by ^{31}P n.m.r. spectroscopy.[143] Changes in the concentrations of small phosphate-containing metabolites associated with muscle contraction were also measured. In addition, the conformation of phosphorylase *b* has been measured during transient activation by observing changes in the e.s.r. signal from added spin-labelled phosphorylase. Transient activation involves a loss of D-glucose 6-phosphate from phosphorylase *b*, and the newly-formed phosphorylase *a* binds to the nucleotides ADP, AMP, and IMP; because of fast interconversion of these nucleotides, the species bound to phosphorylase *a* change throughout the process. Analogues of AMP have been used to gain a better understanding of the structures required for the efficient binding of activators and of their mode of action.[144] It was shown that the 5′-phosphate group is essential for binding. One group of mononucleotides examined produced strong activation, released two molecules of D-glucose 6-phosphate, protected a crucial cysteine residue from attack by 5,5′-dithiobis(2-nitrobenzoic acid), and enhanced the conversion of the dimer into the tetramer. Another group of poor activators released one molecule of D-glucose 6-phosphate, protected two cysteine residues from attack by 5,5′-dithiobis(2-nitrobenzoic acid), and dissociated the small amount of the tetramer present to the dimer. Confirmation has been obtained that pyridoxal phosphate reacts with phosphorylase *b* at neutral pH to form a Schiff-base structure that is embedded in a hydrophobic pocket.[145]

The glycogen phosphorylase of *Dictyostelium discoideum* has been purified over 200-fold from cells at the culmination stage of development.[146] The enzyme (mol. wt. 2.1×10^5; subunit mol. wt. 9.5×10^4) exhibited normal Michaelis–Menten kinetics, and its activity was not stimulated by AMP; it could not be detected during the first five hours of the cell's cycle, but its rate of synthesis increased six-fold between aggregation and culmination, and then decreased to

[141] J. R. Griffiths, R. A. Dwek, and G. K. Radda, *European J. Biochem.*, 1976, **61**, 237.
[142] J. R. Griffiths, R. A. Dwek, and G. K. Radda, *European J. Biochem.*, 1976, **61**, 243.
[143] S. J. W. Busby, D. G. Gadian, J. R. Griffiths, G. K. Radda, and R. E. Richards, *European J. Biochem.*, 1976, **63**, 23.
[144] M. Morange, F. García-Blanco, B. Vandenbunder, and H. Buc, *European J. Biochem.*, 1976, **65**, 553.
[145] M. Cortijo, J. Llor, J. S. Jimenez, and F. García-Blanco, *European J. Biochem.*, 1976, **65**, 521.
[146] D. A. Thomas and B. E. Wright, *J. Biol. Chem.*, 1976, **251**, 1253.

an insignificant amount in young sorocarps.[147] The rate of degradation of the enzyme was negligible during maximum synthesis, and reached its highest value (40%) after culmination.

The isoenzyme pattern of the phosphorylase in the white blood cells and cultured fibroblasts of a patient with Type VI glycogen-storage disease showed only two bands, instead of the three bands normally found.[148] The liver-type phosphorylase was not produced by cultured fibroblasts.

Activity could be induced in monomeric rabbit skeletal muscle phosphorylase covalently bound to Sepharose by analogues of pyridoxal 5′-phosphate and by salicylaldehyde.[149]

The glycogen phosphorylase activity in *Bombyx mori* has been studied in connection with diapause initiation.[150] Differentiation of the larvae was followed by a breakdown of glycogen and an increase in the level of phosphorylase activity. Glycogen levels have been shown to fluctuate between 0.3 and 2.3% of the dry weight during development of the embryo of the Taser silkworm, *Antheraea mylitta*.[151]

It is noteworthy in passing that the branching enzyme involved in the synthesis of potato amylopectin acts on a complex of two chains, each containing 40 D-glucosyl residues.[152] Branching is suggested to take place between two adjacent chains of 1,4-α-glucan that are covalently attached to the protein primer.

There is little glycogen synthesis in isolated hepatocytes below concentrations of 20 mM D-glucose or L-lactate, but substantial synthesis occurs at concentrations of 60 mmol l^{-1}.[153] The addition of glutamine, alanine, or asparagine stimulated glycogenesis. Histochemical methods have been used to detect the sites of glycogen synthesis in the cytoplasm of mammalian cells.[154] Perfused livers of adrenalectomized rats were unable to synthesize glycogen, owing to a deficiency of insulin.[155] The level of brain glycogen in Mongolian gerbils (*Meriones unguiculatus*) dropped following experimental cerebral ischaemia; the accumulation of glycogen afterwards depended on the intensity of the ischaemic insult.[156]

Orotic acid significantly increased the levels of glycogen in the livers of rats, mice, and catfish, whereas it depressed that in the livers of frogs.[157]

Evidence supporting the view that the ratio of glucagon to insulin controls the metabolism of carbohydrates in hepatic tissues has been produced.[158] The availability of cultured astrocytoma and neuroblastoma cells has allowed the

[147] D. A. Thomas and B. E. Wright, *J. Biol. Chem.*, 1976, **251**, 1258.
[148] J. F. Koster, R. G. Slee, D. Daegelen, M.-C. Meienhofer, J.-C. Dreyfus, M. F. Niermeyer, and J. Fernandes, *Clinica Chim. Acta*, 1976, **69**, 121.
[149] K. Feldmann, H. J. Zeisel, and E. J. M. Helmreich, *European J. Biochem.*, 1976, **65**, 285.
[150] O. Yamashita, K. Suzuki, and K. Hasegawa, *Insect Biochem.*, 1975, **5**, 707.
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[152] W. J. Whelan, *J. Biochem. (Japan)*, 1976, **79**, 42P.
[153] J. Katz, S. Golden, and P. A. Wals, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 3433.
[154] T. Takeuchi, M. Sasaki, H. Miyayama, M. Ohyumi, and H. Miyajima, *J. Histochem. Cytochem.*, 1975, **23**, 945.
[155] P. D. Whitton and D. A. Hems, *Biochem. J.*, 1976, **156**, 585.
[156] B. B. Mrsulja, W. D. Lust, B. J. Mrsulja, J. V. Passonneau, and I. Klatzo, *Experientia*, 1976, **32**, 732.
[157] I. Fekete and G. Tóth, *Experientia*, 1976, **32**, 332.
[158] S. R. Wagle, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 1019.

metabolism of glycogen by neuronal and glial cells to be studied independently. Both cell lines responded to D-glucose, insulin, and cAMP.[159] Isobutyl methylxanthene effectively promoted glycogenolysis in both cell lines, whereas norepinephrine was effective in astrocytoma cells and prostaglandin E_1 in neuroblastoma cells. Hydrocortisone has been shown to increase, and glucagon to decrease slightly, the *in vivo* incorporation of D-[^{14}C]glucose into foetal rat-liver glycogen.[160] The steroid increased the total synthase activities, but was without effect on the phosphorylase, whereas glucagon increased the activity of the phosphorylase. The effects of insulin and glucagon on the metabolism of glycogen in transplanted foetal hepatocytes from 15-day-old foetuses have been examined.[161] Marked differences were found between the onset of developmental responses towards glucagon and insulin. The glucagon-response system appears at an early stage, whereas that to insulin seems to be induced. Processes controlling the activation of phosphorylase may be sensitive to the nutrient.[162] Exposure of rat diaphragm to adrenergic stimulants, such as terbutaline, appeared to affect the activity of phosphorylase kinase in fed, rather than fasted, rats. The effects of epinephrine and phenylephrine on the metabolism of carbohydrates in rat-liver parenchymal cells are mediated predominantly by α-adrenergic receptors.[163, 164] Stimulation of these receptors by epinephrine or phenylephrine resulted in the activation of phosphorylase and gluconeogenesis and in the inactivation of glycogen synthase by mechanisms that do not involve an increase in cellular [cAMP]. The activation of β-adrenergic receptors by epinephrine led to the accumulation of cAMP, with minimal activation of phosphorylase or inactivation of glycogen synthase. Thus, it appears that α-adrenergic activation of glycogenolysis and gluconeogenesis in isolated rat-liver parenchymal cells occurs by processes that involve neither an increase in the total level of cAMP in the cells nor the activation of the cAMP-dependent kinase.

Cholinergic muscarinic agonists and α-adrenergic agonists stimulated the secretion of amylase in rabbit parotid gland by processes involving guanosine cyclic 3′,5′-phosphate.[165] 8-Arginine[vasopressin] stimulated the breakdown of glycogen in perfused livers of fed mice.[166]

The α-glucosidase in the kidneys of patients with the infantile form of Pompe's disease has been shown to be less stable to heat than, and immunologically distinct from, that in the normal tissue.[167] 2-Methoxyethanol has been used to increase the solubility of 4-methylumbelliferyl α-D-glucopyranoside in assays of α-D-glucosidase, thereby giving increased sensitivity.[168] Two methods are

[159] J. V. Passonneau and S. K. Crites, *J. Biol. Chem.*, 1976, **251**, 2015.
[160] M. Pines, N. Bashan, and S. W. Moses, *Biochim. Biophys. Acta*, 1975, **411**, 369.
[161] C. Plas and J. Nunez, *J. Biol. Chem.*, 1976, **251**, 1431.
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[163] N. J. Hutson, F. T. Brumley, F. D. Assimacopoulos, S. C. Harper, and J. H. Exton, *J. Biol. Chem.*, 1976, **251**, 5200.
[164] A. D. Cherrington, F. D. Assimacopoulos, S. C. Harper, J. D. Corbin, C. R. Park, and J. H. Exton, *J. Biol. Chem.*, 1976, **251**, 5209.
[165] J. D. Wojcik, R. J. Grand, and D. V. Kimberg, *Biochim. Biophys. Acta*, 1975, **411**, 250.
[166] G. Y. Ma and D. A. Hems, *Biochem. J.*, 1975, **152**, 389.
[167] J. F. Koster, R. G. Slee, J. M. Van der Klei-Van Moorsel, P. J. G. M. Rietra, and C. J. Lucas, *Clinica Chim. Acta*, 1976, **68**, 49.
[168] A. Fujimoto, A. L. Fluharty, R. L. Stevens, H. Kihara, and M. G. Wilson, *Clinica Chim. Acta*, 1976, **68**, 177.

now available for the assay of lysosomal α-1,4-glucosidase in single fibroblasts: one uses an enzyme-cycling procedure ($0.5—10 \times 10^{-13}$ mol of D-glucose released per hour can be assessed) and the other is based on the fluorescence of a 4-methylumbelliferyl substrate.[169] The patterns of amylase isoenzymes in the sera and urine of healthy people have been measured by electrophoresis on a thin layer of polyacrylamide gel.[170] The usefulness of this method of electrophoresis in clinical diagnosis was then evaluated. The synthesis of porcine pancreatic α-amylase was increased up to 20-fold following the addition of cAMP, prostaglandin E_1, isoproterenol, and pancreozymin.[171] The normal endogenous metabolism of sliced rat spleen has been shown to use lipids and amino-acids rather than glycogen.[172] A glyconeogenic pathway has been identified in rat skin.[173] Variations in the amount of glycogen in the livers of mice subjected to stress have been examined.[174]

UDP-D-glucose pyrophosphorylase isolated from calf liver was extensively inhibited by the products of its action.[175]

Glycosaminoglycuronans and Glycosaminoglycans

Analytical Methods.—The latest volume of *Methods in Carbohydrate Chemistry* contains details of the application of n.m.r., c.d., and o.r.d. to structural studies of glycosaminoglycans, microanalysis, the characterization of glycosaminoglycans isolated from human tissues, and the determination of molecular weights by gel electrophoresis, *etc.*[176] Gel filtration has been used to fractionate the glycosaminoglycans in urine[177] and in cultured chondrocytes from chicken embryos;[178] the latter separation was conducted on glass beads. Quaternary ammonium complexes of glycosaminoglycans have been partitioned using hexadecylpyridinium chloride.[179] Electrophoresis of glycosaminoglycans on agarose gel in the presence of diamine buffers, notably propylenediamine acetate and barbital, affords an effective method of separation, without a preliminary purification,[180] and Acridine Orange and Toluidine Blue have been used together to visualize glycosaminoglycans during electrophoresis.[181] An ion-exchange step, which removes glycoproteins, has improved the determination of dermatan sulphate in urine by Di Ferrante's method.[182] A sensitive assay for hyaluronate is based on the formation of high-molecular-weight aggregates with proteoglycans.[183] The extent of *N*-sulphation of heparin and its analogues can be estimated by a

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[170] M. Otsuki, S. Saeki, H. Yuu, M. Maeda, and S. Baba, *Clinical Chem.*, 1976, **22**, 439.
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[176] 'Methods in Carbohydrate Chemistry', Vol. 7, ed. R. L. Whistler and J. N. BeMiller, Academic Press, New York, San Francisco, and London, 1976.
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[178] P. L. Lever and P. F. Goetinck, *Analyt. Biochem.*, 1976, **75**, 67.
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[183] T. E. Hardingham and P. Adams, *Biochem. J.*, 1976, **159**, 143.

procedure based on turbidimetric determination of the inorganic sulphate released by the 2-deoxy-2-sulphoamino-D-glucopyranosyl residues with nitrous acid in acetic acid.[184]

A chondroitinase has been isolated from *Flavobacterium heparinum*; it acted on chondroitin 6-sulphate to give a tetrasaccharide and an unsaturated, sulphated disaccharide, and on hyaluronic acid to give an unsaturated disaccharide.[185] An *endo*-β-galactosidase from *Escherichia freundii* hydrolyses β-D-galactopyranosyl linkages, provided that the residues are not sulphated.[186]

Occurrence, Isolation, and Structure.—The radial distribution of hyaluronate closely follows the distribution of proteoglycans in human intervertebral-disc tissues.[183] A study of bovine arterial tissue has suggested that distinct compartments exist for the synthesis of hybrid chondroitin sulphates.[187] Foetal lung tissue from rabbits contains a relatively high proportion of chondroitin 4-sulphate, which is replaced by dermatan sulphate, heparan sulphate, and heparin at a later stage of growth.[188] The heterogeneity of proteoglycans from bovine nasal and pig articular cartilages has been compared.[189]

The proteoglycan complexes resulting from digestion of bovine heart valves with collagenase have been extracted with guanidinium hydrochloride, after which they were purified by ion-exchange chromatography and fractionated by density-gradient centrifugation under dissociative conditions.[190] In addition to hyaluronic acid, dermatan sulphate, and the chondroitin sulphates, a gel-like substance, which appeared to be a proteoglycan aggregate, was isolated. The isolation and purification of beef-lung heparan sulphate have been described.[191] An active form of heparin has been separated from an inactive form; this was achieved by binding the active form to antithrombin-heparin cofactor during sucrose density-gradient centrifugation.[192] A method for preparing hyaluronic acid from cultures of a haemolytic *Streptococcus* has been reported.[193]

The proteoglycan isolated from normal human intervertebral discs consists of chains of chondroitin sulphate and keratan sulphate, having molecular weights in the region of 2×10^5 and 1×10^5, respectively, attached to a common peptide core.[194] Neither chondroitin 4-sulphate nor hyaluronic acid was detected, although both have been reported to be present in the disc.

Theoretical investigations of the conformations of glycosaminoglycans have focused on the conformational properties associated with the β-D-(1 → 3)-glycosidic linkage; the structure has a bond angle, a torsional potential, and a general steric map similar to those of the β-D-(1 → 4)-glycosidic linkage.[195]

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[190] A. Honda, Y. Kanke, and Y. Mori, *J. Biochem. (Japan)*, 1976, **79**, 17.
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Desulphated keratan sulphate has been shown to interact with wheat-germ agglutinin, supporting the view that non-consecutive (1 → 4)-2-acetamido-2-deoxy-β-D-glucopyranosyl residues interact specifically with the lectin to precipitate the complex.[196] The distribution and chemical composition of keratan sulphate in various tissues, including those from a patient with Marfan syndrome, have been related to ageing.[197]

The interactions of dermatan sulphate, covalently bound to agarose, with soluble galactosaminoglycans have been examined, and determan sulphate itself was fractionated into aggregating and non-aggregating species.[198] Chondroitin 4-sulphate, heparan sulphate, and heparin also interacted with gels substituted with copolymeric galactosaminoglycans, whereas chondroitin 6-sulphate, hyaluronate, and keratan sulphate did not. The structures of ^{35}S-labelled dermatan sulphate–chondroitin sulphate copolymers synthesized and secreted by cultured fibroblasts displayed significant differences with regard to the distribution of such repeating units as L-IdUA-D-Gal*N*AcSO$_4$, D-GlcUA-D-Gal*N*AcSO$_4$, and L-IdUASO$_4$-D-Gal*N*Ac.[199]

Nearest-neighbour analysis has been used to determine the sequences of heparin and heparan sulphates.[200] Structures have been proposed for heparan sulphates A, B, and D based on analysis of the products released by the actions of heparinitase and heparinase from *Flavobacterium heparinum*.[201]

C.d. studies of vitreous hyaluronic acid have indicated that there is a long-range order in the conformation of the polymer,[202] and a regular, left-handed, three-fold helix stabilized by specific intramolecular hydrogen-bonds has been suggested for hyaluronic acid.[203] N.m.r. studies of sodium hyaluronate in solution suggested that the conformation contains alternate stiff and flexible regions.[204] The proteins in pig laryngeal cartilage have been shown to be heterogeneous with respect to their capacities to bind hyaluronate.[205] Selective chemical modification of the proteins confirmed that binding depends on the presence of disulphide bridges, arginine, tryptophan, and the ε-amino-group of lysine residues. The role of link proteins in stabilizing the hyaluronic acid–proteoglycan complex has been questioned.[206] The pattern of polydispersity in the proteoglycan core subunit from bovine articular cartilage has been examined.[207] The core protein is suggested to have a hyaluronic-acid-binding region of constant size and composition, and a region of variable length and composition, which consists of repeating peptide sequences containing equimolar amounts of serine and glycine, where chains of chondroitin sulphate are attached.

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The interaction of proteoglycans with poly(L-arginine) has been examined.[208] Three distinct proteoglycans, each with a constant number of chondroitin sulphate chains of different average molecular weight, have been suggested, to account for the heterogeneity of bovine nasal cartilage.[209]

Biosynthesis.—Hyaluronic acid depressed the synthesis of the proteoglycan in cultures of chondrocytes; this effect appears to originate in the chain-elongation process.[210] Hyaluronic acid is the principal glycosaminoglycan synthesized by rabbit corneal endothelial and stromal cells, although appreciably less hyaluronic acid is produced by the stromal cells.[211] Sulphated proteoglycans synthesized by definitive chondroblasts have been shown to differ qualitatively from those synthesized by the mother cells (*i.e.* the presumptive chondroblasts), which could not be distinguished from sulphated proteoglycans synthesized by non-chondrogenic cells, de-differentiated cells, or cells suppressed with 5-bromo-2′-deoxyuridine.[212] Changes in the acidic glycosaminoglycans have been investigated in embryonic chicken skin at various stages of development.[213] A sharp decrease in the chondroitin sulphate content was observed between the eleventh and fourteenth days, while the dermatan sulphate content increased four-fold and the hyaluronic acid content slowly diminished. The synthesis of proteoglycans was significantly higher in explants taken from eroded human articular cartilage than in those from normal tissue.[214] This increase in metabolic activity could well be a protective phenomenon. The glycosaminoglycans present in cultures of chicken limb-bud mesenchymal cells have been shown to be similar to those present in other hyaline cartilages.[215] The rate of incorporation of D-[^{14}C]glucose into heparin and heparan sulphate in the skin of new-born rats was found to be 4—5 times faster than the rate of assimilation into chondroitin sulphate.[216] Puromycin immediately inhibited the synthesis of sulphated polysaccharides, whereas it had much less effect on the synthesis of hyaluronic acid. A rise in pH from 7 to 8 produced a three-fold increase in the rate of synthesis of sulphated glycosaminoglycans in cultured human chondrocytes.[217] Although the fibroblasts and the epithelial and endothelial cells of cornea all synthesized sulphated glycosaminoglycans, the pattern of sulphated glycosaminoglycans in the fibroblasts differs from those in the other cells.[218] The hexosaminyl- and D-glucuronyl-transferases present in porcine aortic tissue required Mg^{2+} or Mn^{2+} ions for optimum activity.[219] The uptake of sugars catalysed by the enzymes was greatest into hyaluronic acid and heparan sulphate and least into chondroitin sulphate. Examination of the cell cycle in hamster fibroblasts

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revealed that hyaluronic acid, chondroitin sulphate, and heparan sulphate are produced during the G1 and S phases, and that heparan sulphate is either absent or altered during the G2/M phase.[220]

Slices from the uterus of oestrogen-treated rabbits have been incubated *in vitro* with 2-acetamido-2-deoxy-D-[1-^{3}H]glucose and D-[*U*-^{14}C]glucose; the specific activity of the incorporated hexosamines was shown to decrease in the order: hyaluronic acid, sulphated glycopeptides, heparan sulphate, chondroitin 4-sulphate, chondroitin 6-sulphate, and dermatan sulphate.[221] Calcitonin and parathyroid hormone stimulated the uptake of sulphate into the glycosaminoglycans of cultured chondrocytes from rat ribs.[222] The synthesis of glycosaminoglycans in chicken-embryo cartilage was stimulated by 3,3′,5-tri-iodothyroxine, but only under conditions that are optimal for synthesis.[223] Actinomycin D and reagents that interfere with the formation of microtubules attenuated this stimulation, which resulted in increases in the amounts of chondroitin sulphate and hyaluronic acid.[224] Bleomycin stimulated the synthesis of glycosaminoglycans in fibroblasts obtained from carrageenan-induced granulomas in rats.[225] Colchicine had little effect on the synthesis of the matrix of fibroblasts and osteoblasts; this observation casts doubt on the role played by microtubules in the secretion of glycosaminoglycans.[226] N^{6},$O^{2'}$-Dibutyryladenosine cyclic 3′,5′-phosphate stimulated the release of proteoglycans from immature rabbit-ear cartilage.[227] Analysis of the released proteoglycans suggested that the action of the nucleotide may be mediated by the proteolytic action of neutral proteases.

As many as sixteen residues of D-[^{14}C]glucuronic acid and 2-acetamido-2-deoxy-D-[^{3}H]galactose were added alternately to sulphated penta- or hexasaccharides derived from chondroitin 6-sulphate when the oligosaccharides were incubated with the appropriate UDP-nucleotide and an enzyme preparation from chicken-cartilage microsomes.[228] However, the absence of longer polysaccharide chains indicated the absence of a suitable primer. It has been shown that the kinetic rate of incorporation of counts from ^{35}S-sulphate into the chondroitin sulphates of cultured chicken-embryo chondrocytes differs from that of the incorporation of 2-amino-2-deoxy-D-[^{14}C]glucose. These differences can be traced to the fact that the specific activity of the UDP-2-acetamido-2-deoxyhexose pool, which is generated in part from the added 2-amino-2-deoxy-D-[^{14}C]glucose and in part from D-glucose in the medium, increases continuously as the concentration of D-glucose in the medium is depleted.[229] The effect of phospholipids on the activity of UDP-D-galactose : D-xylose galactosyltransferase in embryonic chicken cartilage has been studied.[230] The activities of this enzyme,

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[230] N. B. Schwartz, *J. Biol. Chem.*, 1976, **251**, 285.

UDP-D-xylose : core protein xylosyltransferase, and UDP-2-acetamido-2-deoxy-D-galactose : (D-GlcUA-D-Gal*N*Ac 4-sulphate)$_3$-2-acetamido-2-deoxy-D-galactosyltransferase have been measured during the development of chondrocytes.[231] The highest levels of these activities occurred on the eighth day, preceding the maximum rate of appearance of chondroitin sulphate by two days. β-D-Xylopyranosides have been shown to inhibit the addition of chains of chondroitin sulphate to the core protein, but they did not affect the synthesis of the core protein.[232] Other evidence indicated that neither the synthesis nor the deposition of collagen is affected by these glycosides.[233] Whether epimerization of D-glucopyranosyluronic acid to L-idopyranosyluronic acid takes place at the polymer level has been questioned, following an investigation of the specific labelling of glycosaminoglycan residues by D-[^{14}C]glucose.[234] Other investigations have shown that H-5 is lost during the course of this epimerization.[235] Pulse-chase experiments with [^{35}S]sulphate in murine mastocytoma cells have demonstrated that the molecular weight of heparin initially synthesized in subcellular particles is considerably higher than that of commercial heparins.[236] Thus, subcellular heparin must be degraded, possibly by lysosomal enzymes, to a form of lower molecular weight.

Degradation.—Three metallo-proteases from human articular cartilage have been shown to digest the protein component of cartilage-matrix proteoglycan, affording fragments containing 5—12 chains of chondroitin sulphate.[237] The link proteins were not digested. A model for joint injury has been proposed: polymorphonuclear leucocytes are considered to release neutral proteases, which penetrate the cartilage and fragment the proteoglycan complex, giving products that are trapped in the collagen mesh.[238, 239] An acid β-D-glucuronidase in human synovial fluid has been characterized; an inhibitor of the enzyme appears to be present in the same preparation.[240] Lysozymes from humans were without effect on either hyaluronate or aggregated proteoglycans.[241] Murine macrophages showed higher desulphatase activities than either neutrophils or lymphocytes.[242]

The ability of heavy-metal ions, riboflavin, or ascorbate to lower the viscosity of solutions of hyaluronate could be prevented by catalase, suggesting that hydrogen peroxide is produced by these compounds; the resulting changes in the conformation of hyaluronate may be brought about by attack by free radicals.[243] Depolymerization of heparin with nitrous acid yielded 2,5-anhydrohexuronic acids.[244]

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[244] J. E. Shively and H. E. Conrad, *Biochemistry*, 1976, **15**, 3932.

Function.—The extent of *N*-sulphation of heparin has been related to the anticoagulant activity.[245] The binding of heparin to antithrombin probably requires a specific sequence of residues,[246] and recent work has supported the suggestion that thrombin undergoes a conformational change on binding to heparin, thus facilitating the formation of a complex between thrombin and antithrombin.[247] Heparin is also thought to initiate inflammatory reactions in the presence of protamine by the activation of the complement system.[248]

An examination of cartilage indicated that chondroitin 4-sulphate is involved in calcification, whereas chondroitin 6-sulphate is important in maintaining the integrity of articular surfaces.[249] The interactions of proteoglycans with tropoelastin have been implicated in elastogenesis.[250]

Proteoglycans acted as competitive inhibitors of DNA-dependent DNA and RNA polymerases and of RNA-dependent DNA polymerase,[251] and also induced changes in the structure of chromatin.[252]

Dermatan sulphate in porcine cartilage occurs in tissues containing coarse fibres of type I collagen, whereas it is absent from tissues containing type II collagen.[253] Proteoglycan subunits have been shown to bind preferentially to the β-chains of type II collagen, indicating that they have a higher affinity for cross-linked collagen than for α-chains, and that cross-linking of collagen is important in binding with the proteoglycan matrix.[254] Immunological studies have suggested that link proteins may be derived from the region of the proteoglycan that binds hyaluronic acid.[255]

An assay for soluble collagen has been based on its precipitation with embryonic proteoglycans.[256] The sulphated proteoglycans in the tissues and mitochondria of rats appear to have similar compositions, implying that they may be involved in cellular differentiation or maturation, or both.[257]

Pathology.—A useful review of mucopolysaccharidoses, which deals with the defective or missing enzymes, prenatal diagnoses, and enzyme-replacement therapy, has appeared.[258] Other reviews have discussed the ageing of connective tissues[259] and the diagnosis of mucopolysaccharidoses;[260] it was pointed out in the latter review that screening tests using cetylpyridinium chloride are unreliable

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for the determination of glycosaminoglycans in urine and should be abandoned. Preparative gel filtration of untreated samples of urine permits mucopolysaccharidoses types I, II, and III to be distinguished.[261]

The contents, compositions, and molecular weights of glycosaminoglycans in grey and white cerebral matter, livers, and spleens of patients with mucopolysaccharidoses have been examined and compared with those from normal tissues.[262] Changes in the contents of glycosaminoglycans in the brain of a patient with type V mucopolysaccharidosis were not significant, whereas a three- to four-fold increase in glycosaminoglycans was found in the brains of patients with types I, II, or IIIA mucopolysaccharidosis. A partially degraded form of dermatan sulphate accounted for most of the increase in types I, II, and V mucopolysaccharidoses, whereas a degraded form of heparan sulphate is the principal component in type IIIA mucopolysaccharidosis.

The screening of heterozygotes for Hurler's syndrome is now possible, since the specific activity of the leucocyte-derived α-L-iduronidase in carriers is reduced by roughly 50%.[263] The molecular size of dermatan sulphate excreted in the urine of patients with Hurler's syndrome can be monitored by gel-filtration chromatography on Sephadex G-75.[264] The *in vitro* correction of the defect in Hurler's fibroblasts with bovine testicular hyaluronidase has produced encouraging results, and such treatment is also effective with Hunter's cells.[265] A sulphated tetrasaccharide, which was obtained on digestion of chondroitin 6-sulphate with testicular hyaluronidase, has been used as the substrate in the determination of 2-acetamido-2-deoxy-D-galactose 6-sulphate sulphatase, an enzyme that is absent in patients with Morquio's disease.[266]

The reduction (to roughly one-sixth) in the molecular weight of the intracellular heparan sulphate in the fibroblasts of patients with Sanfilippo B or Scheie's syndromes has been attributed to the actions of an *endo*-glycosaminidase and an *endo*-hexuronidase.[267] Immunochemical evidence has been obtained for the presence of a defective 2-acetamido-2-deoxy-α-D-glucosidase in the tissues of patients with Sanfilippo B disease,[268] and that, in addition to arylsulphatase B, other enzymes are present in the corrective factor for Maroteaux–Lamy disease.[269] Arylsulphatases A and B have been separated by chromatography on DEAE-cellulose.[270]

The keratan sulphate stored in macular dystrophy of the cornea can be dispersed using preparations from rabbit keratocytes or homogenates of porcine corneal stroma.[271] The glycosaminoglycans in the skins of patients with systemic

[261] R. E. Hurst, J. M. Settine, and A. E. Lorincz, *Clinica Chim. Acta*, 1976, **70**, 427.
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scleroderma appear to be more resistant to the actions of hyaluronidase and chondroitinase.[272] A re-examination of the glycosaminoglycans and glycopeptides excreted in the urine of patients with spondyloepiphyseal dysplasia congenita indicated that the sulphate content, rather than the uronic acid or sialic acid contents, of the polymers is reduced.[273] Analyses of the proteoglycans and glycosaminoglycans in human chondrosarcoma have shown that large amounts of hyaluronic acid are present.[274] Appreciable amounts of hyaluronic acid have also been identified in the myxomatous stroma of intracanalicular fibroadenoma, which also contain high contents of chondroitin 4- and 6-sulphates.[275]

The inability of proteoglycans to aggregate is significant in the molecular organization of osteoarthritic cartilage.[276] Non-aggregated proteoglycans are present in amounts larger than normal; consequently there are fewer aggregates. The failure of the proteoglycans to interact with hyaluronic acid may be responsible, in part, for the decrease in aggregation.[277] A local depletion in the content of glycosaminoglycans is associated with fibrillated tissues in the patella.[278]

Changes in the levels of glycosaminoglycans in the intima and media of human aorta during atherosclerosis have been examined; the media showed little change, except for a decrease in the level of chondroitin 6-sulphate, whereas pronounced decreases in the levels of chondroitin 6-sulphate and heparan sulphate occurred in the intima.[279] The levels of hyaluronic acid in both tissues remained constant, whereas those of dermatan sulphate increased. Hyaluronidase has been used to reduce the extent of myocardial infarction following occlusion of the coronary artery in rats.[280] Hepatic tissues damaged with carbon tetrachloride produced a nine-fold increase in the low-sulphate fraction of dermatan sulphate, but only a two-fold increase in the corresponding high-sulphate fraction.[281] Changes also occurred in heparan sulphate fractions.

Mammalian Cell and Tissue Glycoproteins

References to macromolecules present in cell surfaces are grouped together in a separate section (see p. 307).

Two classes of carbohydrate-protein are thought to be associated with the isolated genome chromatin of $HeLaS_3$ cells; one consists of glycoproteins (mol. wts. 1×10^4—1.9×10^5) and the other of glycosaminoglycans of much

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higher molecular weight.[282] Removal of 80% of the carbohydrate from interferons obtained from human leucocytes and fibroblasts failed to alter the antiviral or antibody-binding properties.[283] Granuloma glycoproteins that inhibit macrophage phagocytosis have been suggested to act on the complement system by inactivating chemotactic factors.[284] One such glycoprotein (mol. wt. 4.8×10^4) contains 5.6% of carbohydrate consisting of D-glucose (4.1),D-galactose (3.0), D-mannose (3.3), 2-amino-2-deoxy-D-glucose (1.0), and sialic acid (1.2 residues per molecule).[285]

The implications of heterogeneity in carcinoembryonic antigen and the role of carcinoembryonic antigen as a tumour marker have been discussed.[286] The presence of carcinoembryonic antigen in tumours of non-entodermal origin can be detected by affinity chromatography on concanavalin A and by using the appropriate antiserum.[287] The immunodominant group has been shown to be more complex than a simple, terminal carbohydrate residue.[288] An antigen possessing some of the molecular and immunochemical properties of carcinoembryonic antigen, but which is absent from cancerous tissues, has been found in the gastric juices of normal individuals and patients with gastric cancer.[289] Blood-group H specificity has been detected on the purified antigen of a hepatic metastasis obtained from a blood-group O patient with cancer of the rectum; the H specificity could be changed to either A or B specificity by the action of appropriate glycosyltransferases.[290] The biology and chemistry of carcinoembryonic antigen and carcinofoetal serum proteins have been ably reviewed.[291]

Glycoproteins found in perchloric acid extracts of carcinoembryonic antigen have been partially characterized by immunochemical methods.[292] Multiple Smith degradations of carcinoembryonic antigen and its asialo derivative progressively removed *ca.* 90% of the carbohydrate moiety, with loss of the antigenic activity; there was also a small amount of proteolysis.[293] Methylation analysis and oxidation with periodate have established that carcinoembryonic antigen has a core composed of residues of D-mannose and 2-acetamido-2-deoxy-D-glucose, to which are attached residues of L-fucose.[294] Electron microscopy showed the antigen in the form of twisted, rod-shaped particles.

The hydrodynamic properties and molecular weights of the F, M, and S glycoproteins present in the perchloric-acid-soluble fraction of the ascitic fluid

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from Ehrlich ascites tumour cells have been reported.[295] Conformational changes that occur on heating Yoshida glycoprotein do not involve regions of the protein responsible for the binding of antibodies.[296]

A purified glycoprotein isolated from human liver stimulated the enzymic hydrolysis of sphingolipids; it has a molecular weight of 2.2×10^4 and contains 2-acetamido-2-deoxy-D-glucose (2), D-mannose (5), and sialic acid (1 residue(s) per molecule).[297] The molecular weight and amino-acid and carbohydrate compositions of pregnancy-specific β_1-glycoproteins from the placenta of humans and rhesus monkeys are closely similar.[298] Similarities also exist between the antigenic properties of the β_1-glycoproteins obtained from chimpanzees, rhesus monkeys, cynomolguses, and baboons. A glycoprotein (mol. wt. 3.6×10^4) isolated from the alveoli of patients with alveolar proteinosis was shown to contain hydroxyproline (5), sialic acid (3), D-mannose (4), D-galactose (4), 2-amino-2-deoxy-D-glucose (6), and L-fucose [1 residue(s) per molecule].[299] The carbohydrate moieties appear to be linked to asparagine in the protein, which has regions possessing a collagen-like structure. Cultured skin fibroblasts released a non-collagenous, L-fucosylated glycoprotein, which normally occurs as an aggregate linked by disulphide bonds, but which has a molecular weight of 2.5×10^5 under reducing conditions.[300] An *N*-aspartyl-β-glucosaminidase from the livers of patients with aspartylglucosaminuria contains only 10% of the normal activity, although its composition appears to be little changed.[301]

Porcine kidney α-mannosidase successfully hydrolysed the D-mannose-containing material that accumulates in the skin fibroblasts of patients with mannosidosis.[302] The acid α-mannosidase (pH optimum 5.5) is not a reliable indicator of the mannosidosis heterozygote.[303]

Standard procedures for the preparation of pancreatic ribonuclease B have been described.[304] The composition of the carbohydrate moiety and the amino-acid sequence in the linkage region of pancreatic ribonuclease B have been compared in a variety of domestic and wild animals.[305] Marked differences were found between carbohydrate chains attached to identical sites in different species and between those attached to different sites in the same species. The attachment of complex oligosaccharides to a protein does not appear to have a significant effect on the circulating half-life of the protein (*cf.* ribonucleases C or D with ribonuclease A).[306] The mechanism in rats for clearing glycoproteins is based on specific recognition of exposed α-D-mannosyl residues (*cf.* ribonuclease B to the other isoenzymes and to α-mannosidase-treated ribonuclease B).

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Revised values of D-mannose (9) and 2-amino-2-deoxy-D-glucose (5 residues) have been reported for the proportions of sugars in bovine rhodopsin.[307] Bovine aorta glycoprotein has been shown to contain D-galactose (17.4), D-glucose (3.0), L-fucose (2.9), hexosamine (12.4), and sialic acid (6.1 residues per molecule); each of the two carbohydrate residues has sialic acid at the non-reducing termini and sequences of D-Gal*p*-D-Glc*N*Ac*p* and L-Fuc*p*-D-Glc*p*-D-Gal*p*.[308] A branched structure has been tentatively proposed for one of the carbohydrate moieties of glycoproteins isolated from the intima of porcine aorta.[309] Two of the four principal glycoproteins from the tissues of mitral valves have been characterized: one (mol. wt. 1.2×10^5) contains a large proportion of acidic amino-acids and L-fucose (5), D-mannose (10), D-galactose (15), D-glucose (12), 2-amino-2-deoxy-D-glucose (7), and 2-amino-2-deoxy-D-galactose (2 residues per molecule), while the other (mol. wt. 7.2×10^4) contains D-glucose (3), D-galactose (3), D-mannose (2), L-fucose (1), 2-amino-2-deoxy-D-glucose (4), and sialic acid (1 residue(s) per molecule).[310]

Extraction with urea of the insoluble material obtained following the digestion of rabbit uterus with collagenase yielded a structural glycoprotein (mol. wt. 1.6×10^4) containing 5.1% of carbohydrate.[311] A sulphated glycoprotein has been obtained from endometrial scrapings of rabbit uterus after treatment with oestrogen.[312]

The three principal glycoproteins excreted by C-1300 murine neuroblastoma cells have molecular weights 8.7×10^4, 6.6×10^4, and 5.5×10^4.[313] Only the glycoprotein of molecular weight 6.6×10^4 was excreted when cell differentiation was induced with N^6,$O^{2'}$-dibutyryl cAMP. The increase in the molecular weights of glycoproteins that often accompanies cell differentiation is now thought to result from the attachment of carbohydrate residues in addition to sialic acid.[314]

A purified glycoprotein (gp 70) from mouse ascites fluid appears to be related to the principal glycoprotein found in murine leukaemia viruses (Scripps, Moloney, and Rauscher).[315]

Of four glycoproteins (40—50% of carbohydrate) isolated from guinea-pig testes, two (mol. wts. 4.7×10^4 and 1.8×10^4) are aspermatogenic and two [mol. wts. 1.05×10^5, and 4.1 and 2.3×10^4 (two bands)] are inactive;[316] they all appeared to be homogeneous on examination by gel electrophoresis and immunoelectrophoresis.[317] A sialoglycoprotein having trypsin-like specificity occurs in the acrosomes of spermatozoa; it has been named acrosin.[318–320]

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Immunochemical and histological investigations of tissues from the oligodendrocytes of human brain have revealed the presence of glycoproteins,[321] and L-fucose-containing glycoproteins are present in the perikaryal fraction of neurons.[322] Comparisons of the substrate specificity, isoelectric point, enzymic activity, and optimum pH have shown that hexosaminidase C is identical with the principal residual activity in Sandhoff's fibroblasts.[323]

Sialoglycopeptides (mol. wts. 1.04—1.25 × 10^4) obtained by digestion of heifer brain with protease contained carbohydrate moieties linked both to asparagine and serine or threonine residues.[324] Sulphate groups and L-fucose are located in alkali-stable chains of these sialoglycoproteins, while D-mannose occurs both in alkali-labile and -stable chains. The arylsulphatase B (mol. wt. 4.5 × 10^4) in ovine brain is a glycoprotein containing neutral sugars (11.7%), consisting of D-glucose, D-mannose, and D-galactose, and sialic acid (0.4%).[325]

Five oligosaccharides (3)—(7) obtained by treating rat-brain sialoglycoproteins with alkaline sodium borohydride have been characterized by a combination of

α-D-Gal*p*-(1 → 3)-D-Gal*N*Acol
(3)

β-D-Gal*p*-(1 → 3)-D-Gal*N*Acol
(4)

α-Neu*N*Ac-[β-D-Gal*p*-(1 → 3)-D-Gal*N*Acol]
(5)

α-Neu*N*Ac-(2 → 3)-β-D-Gal*p*-(1 → 3)-D-Gal*N*Acol
(6)

α-Neu*N*Ac-(2 → 3)-β-D-Gal*p*-(1 → 3)-[α-Neu*N*Ac-(2 → 6)]-Gal*N*Acol
(7)

oxidation with periodate, methylation analysis, oxidation with chromium trioxide, and g.l.c.–m.s.[326] A phosphoglycoprotein having phosphate groups located on the carbohydrate residue has also been isolated from defatted rat brain.[327]

The glycoprotein component of an adenosine triphosphatase which is activated by Na^+ and K^+ ions has been shown to be responsible for binding these cations.[328]

Cell-surface Glycoproteins

A number of reviews on cell-surface glycoproteins have appeared during the past year. The biochemistry and chemistry of glycoprotein components of mammalian membranes have been extensively reviewed in a book that deals

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mainly with the literature published during the past ten years.[329] A text for advanced undergraduate students has outlined many features of biological membranes, including the cell-surface specificity and the nature of the carbohydrate components.[330] More advanced texts to be published include Volume 3 of the series 'Biological Membranes', which contains a short section on cell recognition in membrane–membrane interactions,[331] and Parts A and B of 'Methods in Receptor Research'.[332] A review of cell-surface proteins and malignant transformations deals with large, external, transformation-sensitive (LETS) glycoproteins.[333] The physiological detection and assay of receptors, especially in connection with the actions of hormones, have been discussed.[334] The proceedings of the Ninth Leucocyte Culture Conference have given extensive coverage to immune-recognition phenomena,[335] and those of the Ninth Miami Winter Symposia on 'Molecular Approaches to Immunology' deal with cell-surface antigens and receptors at a molecular level.[336] A review of the functions of cell-surface glycosyltransferases has also appeared.[337]

The resistance of cells to lysis by antibodies and complement can be removed by treating the cells with neuraminidase.[338] Glycoproteins have been shown to participate in binding Ca^{2+} ions to, and in transporting Ca^{2+} ions across, mitochondrial membranes.[339] Removal of sialic acid residues from the surface of cultured heart cells increased the ability of the cells to exchange Ca^{2+} ions, but that of K^+ ions was unaffected.[340] Glycoproteins on the surface of human KB cells are involved in binding adenovirus.[341]

A colloquium on cell-surface antigens has discussed the biological significance and structures of histocompatibility antigens,[342] tumour antigens,[343] and lymphocyte antigens.[344] The binding of concanavalin A to plasma-membrane-enriched fractions of lactating mammary gland activated the Mg^{2+}-ATPase, whereas the 5′-nucleotidases were inactivated.[345] Both effects can be inhibited by methyl α-D-mannopyranoside.

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A low-molecular-weight effector protein from the spleen of a patient with Gaucher's disease specifically bound α-D-glucocerebroside β-D-glucosidase, but it did not bind β-D-galactosidase or β-D-2-acetamido-2-deoxyglucosidase.[346] The increase in the surface area of platelets induced by either ADP or 5-hydroxytryptamine is accompanied by an increase in the number of sialic acid residues susceptible to enzymic removal.[347] It appears that sialic acid residues stimulate the binding of thyroid-stimulating hormone to beef thyroid membranes.[348] α-L-Fucose has been shown to have an important role as a component of the receptors for the migration inhibitory factor on human monocytes.[349] Two polypeptide subunits (mol. wts. 3.5×10^4 and 1.5×10^5) comprise the cytosol retinol-binding lipoglycoprotein (mol. wt. 1.5×10^6) present in the pigment epithelial cells of bovine retina.[350] Lipoglycoproteins in outer rod segments contain four polypeptides (mol. wts. 5×10^4, 7.5×10^4, 1.2×10^5, and 2.0×10^5).

The antigenic products of murine H-2K and H-2D genes are glycoproteins (mol. wt. 4.5×10^4) which release a glycopeptide fragment (mol. wt. 3.7×10^4) and the associated putative β_2-microglobulin (mol. wt. 1.2×10^4) on treatment with papain.[351] The glycoproteinaceous nature of rat-liver microsomes and Golgi membranes has been examined; the ratio of D-mannose to D-galactose falls in passing from the rough microsomes to the smooth microsomes, and some of the glycoproteins are bound more tightly than others.[352] Cell agglutination and fluorescent-lectin binding have been used to study changes in the structures of oligosaccharides of the zona pellucida and plasma membranes of hamster eggs during maturation and development of the eggs.[353]

The component of hepatic membranes that is responsible for clearing asialoglycoproteins has been identified as a glycoprotein which contains subunits of molecular weights 4.0×10^4 and 4.8×10^4 in aggregates of molecular weight 5×10^5.[354] The carbohydrate structures (8) and (9) of the subunits were determined.[355] Evidence for this glycoprotein has also been provided by other workers, who noted that it does not occur in the livers of birds and reptiles (species possessing high levels of circulating asialoglycoproteins).[356]

Differences have been detected in the amounts of glycoproteins present in B and T lymphocyte membranes; in particular, there is a large concentration of a glycoprotein of molecular weight 3×10^4 in bursa-derived preparations.[357] Oxidation of human peripheral lymphocytes with D-galactose oxidase induced transformation, which was enhanced by prior treatment of the lymphocytes with

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α-NeuNAc-(2 → 2 or 4 or 6)-β-D-Gal*p*-(1 → 3 or 4)-β-D-Glc*NAcp*
1
↓
4 (or 2)
α-NeuNAc-(2 → 2 or 4 or 6)-β-D-Gal*p*-(1 → 2 or 4 or 6)-α-D-Man*p*-(1 → 3)-β-D-Man*p*-(1 → 4)-β-D-Glc*NAcp*-(1 → 3 or 4)-β-D-Glc*NAcp*-Asn
2 (or 4)
↑
1
α-NeuNAc-(2 → 3)-β-D-Gal*p*-(1 → 3 or 4)-β-D-Glc*NAcp*

(8)

(α-D-Man*p*)$_{6-n}$-β-D-Man*p*-(1 → 3 or 4)-β-D-Glc*NAcp*-(1 → 3 or 4)-β-D-Glc*NAcp*-Asn

(9)

neuraminidase.[358] Treatment of human lymphoblastoid cells with phytohaemagglutinin appeared to redistribute the surface array of sialoglycoproteins and hyaluronic acid.[359] Mild oxidation with periodate is thought to modify mainly the terminal sialic acid residue of the surface glycoprotein of calf submandibular lymph nodes.[360] Sensitization of murine spleen lymphocytes with periodate-treated autologous cells depends on the presence of aldehydic groups on the oxidized cells.[361]

Chromatography on Sepharose–concanavalin A has been used to separate the inside-out and non-inverted vesicles of porcine lymphocyte plasma membranes, by virtue of the carbohydrate residues exposed on the cell surface of the non-inverted vesicles.[362] These membranes contain 6.9% of carbohydrate consisting of L-fucose, D-ribose, D-mannose, D-glucose, D-galactose, and inositol in the molecular proportions 2 : 9 : 11 : 15 : 26 : 1, and of 2-amino-2-deoxy-D-glucose and 2-amino-2-deoxy-D-galactose in the molecular proportions 2 : 1.[363] Sialic acid is also present. Concanavalin A has also been used to study the distribution of lectin-receptor sites on the cell surface of rat thymocytes.[364] On exposure to mitogenic concentrations of concanavalin A, mouse lymphocytes exhibited a two-fold increase in the activity of the cell-surface sialyltransferase.[365] Differences have been found between the membrane glycoproteins of murine thymocytes and spleenocytes; the thymocytes contain mainly a glycoprotein of molecular weight $2.2—2.7 \times 10^4$, which is absent from spleenocytes, whereas the spleenocytes contain more glycoproteins of molecular weights $4.0—4.5 \times 10^4$ and 6.5×10^4.[366] The principal glycoprotein of the cell surface of rat thymocytes has been purified and found to consist of two species of similar, but not identical, molecular weight (*i.e.* 2.5×10^4 and 2.7×10^4).[367] A glycoprotein obtained from rat brain is probably identical to the form of lower molecular weight,[368] although detailed comparison suggests that there are differences in the carbohydrate moieties.[369] A receptor (mol. wt. 5.5×10^4) for concanavalin A on the thymocyte membranes of white New Zealand rabbits can be selectively cross-linked with glutaraldehyde.[370] The modified receptor exhibited high affinity for, and non-co-operative binding with, concanavalin A.

Alkaline phosphatase is one of the principal glycoproteins present in the microvillus plasma membrane of human placenta; sixteen protein and ten glycoprotein subunits of the enzyme were detected.[371] Purified brush-border peptidases (mol. wt. 2.8×10^5) from rat intestinal mucosa have been shown to

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contain similar amino-acid sequences, but they differ in the carbohydrate residues; they exist as dimers (subunit mol. wt. 1.4×10^5).[372, 373] Three glycopeptides (mol. wt. 2.5×10^3) located in the endoplasmic reticulum of rat intestinal mucosa have been characterized.[374] Cortisol could either stimulate or inhibit the synthesis of specific glycoproteins in the intestinal membranes of suckling rats.[375]

The rate of turnover of membrane glycoproteins in neuroblastoma cells during exponential growth is almost twice that of non-glycosylated proteins.[376] The alkaline phosphatase and arylamidase in the plasma membrane of bovine brain have been identified as glycoproteins.[377] D-Galactose oxidase has been used to study the distribution of D-galactosyl residues on the intact myelin sheath of rat spinal cord.[378] One of the principal glycoproteins is located either on the external surface of the sheath or is closely associated with the oligodendroglial plasma membrane. This glycoprotein was partially purified, and its carbohydrate composition during myelination was examined.[379] A molecular weight of 1.38×10^4 has been found for the glutamate-binding glycoprotein present in the synaptic membrane of rat brain.[380] Loss of some of the sialic acid residues from the surface of cloned lines of hamster astroblasts and neuroblasts led to a nine-fold increase in the inorganic pyrophosphatase activity.[381] The binding of concanavalin A by the myelin of rat sciatic nerve has provided information on the distribution of glycoproteins.[382] The phytohaemagglutinin of *Lens culinaris*, attached to an insoluble support, has been used in the fractionation of glycoproteins from rat cerebral cortex.[383]

Glycoproteins on the surface of Ehrlich ascites carcinoma cells have been purified by affinity chromatography on immobilized lectins.[384] Radioactive labelling has been used to identify receptors for IgE on the surface of leukaemic cells in rats.[385] The receptor is a glycoprotein (mol. wt. $5.0—7.0 \times 10^4$) containing residues of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and sialic acid. Further analysis revealed a possible homology with other immunoglobulin receptors. A series of heparan sulphates and a glycopeptide containing D-galactose, 2-amino-2-deoxy-D-galactose, 2-amino-2-deoxy-D-glucose, sialic acid, and traces of 2-amino-2-deoxy-D-mannose were isolated following digestion of the plasma membrane from an ascites hepatoma with pronase.[386] Despite treatment of leukaemic rat cells with neuraminidase, sialic acid was shown to reappear in the cells within 12 hours.[387] Removal of the sialic acid residues from

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the plasma membrane of a mouse-plasma cell tumour led to a loss of antigenicity; some of the sialic acid residues are located on the cell surface.[388] The isolation of these membrane sialoglycoproteins has confirmed that they are antigenic.[389] A major fibroblast that was morphologically changed after malignant transformation has been isolated and partially characterized.[390] Reconstruction experiments with one of the principal cell-surface glycoproteins indicated that it helps to maintain the shape, contact inhibition of movement, and, most important, the adhesion of the cells. The receptors for concanavalin A in transformed hamster fibroblasts are embedded in the lipid phase and differ from those for wheat-germ and soybean agglutinins.[391] Reduced aggregation has been observed in lines of BHK cells that are resistant to ricin, and two possible models were proposed to explain this behaviour.[392] An aggregation factor, which appears to be a glycoprotein, has been identified in, and extracted with acetone–lithium di-iodosalicylate–phenol from, rat fibroblasts.[393] The structure of a glycopeptide containing L-fucose that occurs on the surface of these cells has been elucidated.[394]

Studies of the glycoproteins of human and murine glomerular basement membranes revealed that D-glucose is present in some fractions.[395] The possibility that aldol-type linkages are formed between the disaccharide residues of collagen and the lysyl residues of structural glycoproteins was discussed. Two glycoproteins containing D-glucosyl-D-galactose residues have been isolated from bovine-liver basement membranes; the larger one also contains oligosaccharide side-chains composed of D-mannose, D-galactose, 2-amino-2-deoxyhexose, *N*-glycolylneuraminic acid, and L-fucose.[396] Another glycoprotein, having a different composition, is considered to be derived from the plasma membrane. Digestion of the tubular basement membrane from bovine kidneys with collagenase and pronase also afforded related peptide-bound carbohydrate moieties.[397] One of these glycoproteins (mol. wt. 7×10^4), which was purified to homogeneity, contains 8.6% of carbohydrate consisting of one heteropolysaccharide and four disaccharide units.[398] The amount of carbohydrate in the glomerular basement membrane of mouse kidneys has been shown to decrease with age, the greatest reduction (44%) being in the proportion of L-fucose, followed by sialic acid (34%), hexosamine (20%), and hexose (12%).[399]

Surface membranes of symbiote-containing and -free strains of *Crithidia oncopelti* (a parasitic trypanosomatid protozoan) possess different capacities to bind lectins, the symbiote-free strain being more readily agglutinated with concanavalin A.[400] Immunofluorescence studies, after *in situ* fixation, have shown

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that discoidin – a carbohydrate-binding protein present on the surface of aggregation-competent, but not vegetative, cells of *Dictyostelium discoideum* – is diffusely distributed on the surface of aggregating or streaming cells.[401] The cohesiveness of differentiated amoebae of *Polysphondylium pallidum* was inhibited by an antiserum raised against pallidin, the cell-surface lectin.[402] The surface carbohydrates of *Mycoplasma mycoides* var. *capri* may be located by treating the cells in turn with concanavalin A, horseradish peroxidase, and 1,4-diaminobiphenyl.[403]

Hormonal Glycoproteins

Hormone receptors[404] and their assay, structure, and function[405] have been reviewed. Chromatography on Sepharose–concanavalin A has been used to measure the concentration of glycoprotein hormones in human plasma.[406]

A simple radioactive assay has been developed to evaluate the combination of subunits from human chorionic gonadotrophin (hCG), thyrotrophin, follicle-stimulating hormone (FSH), and luteinizing hormone (LH).[407] The method is claimed to detect the formation of less than one picomole of the resulting hormone. An homology in the amino-acid sequences has been detected in the B-chain of cholera toxin and the β-subunits of thyrotrophin, LH, hCG, and FSH, and is thought to represent the site on these proteins that binds to the receptors on membranes.[408] Hybridization studies using the α- and β-subunits of thyrotrophin, lutrophin, and CG from different species (*e.g.* sheep, cattle, and humans) demonstrated increases in the α-helix and β-sheet contents of the proteins, comparable to those observed in the thyrotrophin subunit assembly.[409]

A highly purified preparation of LH from the snapping turtle (*Chelydra serpentina*) was shown to be a glycoprotein composed of L-fucose (0.7%), D-mannose (3.7%), D-galactose (0.6%), 2-amino-2-deoxy-D-glucose (1.2%), and 2-amino-2-deoxy-D-galactose (0.6%), but with no sialic acid, whereas FSH from the same source contains neutral sugars (4.6%), amino-sugars (6.6%), and sialic acid (1.7%).[410] Removal of the sialic acid residues from ovine FSH and LH had different effects on the biological activity of these hormones; FSH was inactivated, whereas the activity of LH was unimpaired.[411] Reduction of the five disulphide linkages in the α-subunits of LH and thyrotrophin is easily accomplished, in contrast to those in the β-subunits, which appear to be highly resistant to reduction.[412, 413] Re-oxidation of the reduced LH α-subunit afforded a glyco-

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protein that was indistinguishable from the original subunit, suggesting that the LH α-subunit is not synthesized in precursor form.[414] Synthetic gonadotrophin stimulated the incorporation of 2-amino-2-deoxy-D-glucose into LH.[415] The amino-acid sequences of the α- and β-subunits of porcine thyrotrophin have been determined; the amino-acid sequence of the α-subunit is identical to that of porcine LH, whereas the compositions of the oligosaccharide side-chains differ.[416] The primary structure of the β-subunit differs from that of bovine thyrotrophin by six amino-acid replacements and by the absence of a methionine residue at the carboxy-terminus. Following a report that the α- and β-subunits of thyrotrophin are present in the sera of patients with primary hypothyroidism, it has been shown that the sera of patients with pituitary tumours or 'empty sella' syndrome also contain elevated levels of the α-subunit.[417] β-Chains of thyrotrophin have been located in the rough endoplasmic reticulum by means of electron microscopy and immunochemical techniques.[418]

Porcine FSH has been found to contain 2.1% of sialic acid.[419]

The α- and β-subunits of human pituitary lutrophin have been separated, and the β-chain was shown to contain 115 amino-acid residues, with the only carbohydrate residue attached to asparagine-30.[420] Glycopeptides released from ovine lutrophin by digestion with trypsin contained similar proportions of D-mannose and 2-amino-2-deoxy-D-glucose, which are the principal components, and also L-fucose, D-galactose, 2-amino-2-deoxy-D-galactose, and sialic acid.[421] Prolactin granules isolated from the pituitary glands of female rats contain chondroitin 4- and 6-sulphates, heparin, and heparan sulphate.[422]

A purified, thyroxine-binding glycoprotein isolated from human plasma has been shown to have a molecular weight of 5.3×10^4 and to contain 7.5% of carbohydrate; it probably consists of four subunits (mol. wt. 1.35×10^4), each containing D-galactose (1.9), D-mannose (1.8), and 2-amino-2-deoxy-D-glucose (1.7 residues per molecule).[423] The interaction of retinol-binding protein with the glycoprotein has been examined, with the aid of fluorescent probes.[424]

Digestion of the gonadotrophins from intact and ovariectomized monkeys with neuraminidase appeared to abolish any differences in the molecular size.[425] The ability of hCG to bind to rat Leydig cells and to stimulate the production of testosterone and cAMP has been examined.[426] Sequential removal of the sugars led to a progressive loss of activity, which was attributed to the inability of the modified hCGs to stimulate adenyl cyclase, rather than to a failure to bind

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α-Neu*N*Ac/G-(2 → 6)-β-D-Gal*p*-(1 → 6)-β-D-Glc*N*Ac*p*-(1 → 2)-α-D-Man*p*-(1 → 6)-α-D-Man*p*-(1 → 6)-α-D-Man*p*-(1 → 6)-β-D-Glc*N*Ac*p*-Asn

2
↑
1

α-D-Man*p*-(1 → 6)-β-D-Glc*N*Ac*p*

(10)

α-Neu*N*Ac/G-(2 → 6)-β-D-Gal*p*-(1 → 2)-α-D-Man*p*-(1 → 6)-β-D-Glc*N*Ac*p*-(1 → 6)-α-D-Man*p*-(1 → 6)-β-D-Glc*N*Ac*p*-Asn

6 2
↑ ↖
X 1

β-D-Glc*N*Ac*p*

(11) G = glycolyl; X is an unknown substituent

to the cell. The synthesis of the α-subunit of hCG by cell-free extracts has been reported, and at least part of the carbohydrate moiety appears to be attached.[427] Sequences (10) and (11) of the carbohydrate moiety of the α-subunit of hCG have been determined, and it was noted that the sialic acid residues are either *N*-acetylated (93%) or *N*-glycolated (7%).[428]

A rapid, simple method for labelling erythropoietin with ^{125}I, with retention of biological activity, has been reported.[429]

Milk Glycoproteins

The structures of three new disialylfucosyl hexasaccharides,[430] two new nonasaccharides,[431] and a pentasaccharide [432] isolated from human milk have been elucidated. The structures indicate that, in contrast to those of the ABO and Lewis blood-groups, the H determinant is located only on the type-I carbohydrate chain of the branched moiety.[433] Extraction of bovine milk-fat globule membranes with phenol afforded three glycoproteins, which released the T antigen on treatment with alkaline borohydride and desialylation.[434]

Five closely related glycoproteins containing 50—80% of carbohydrate have been isolated from human colostrum whey.[435] They all promoted the growth of *Lactobacillus bifidus* var. *pennsylvanicus*, and this activity was not substantially altered by removal of the sialic acid residues. Human plasmin converted the high-molecular-weight form of UDP-D-galactose : D-glucose D-galactosyltransferase (mol. wt. 5.8×10^4) to a form of lower molecular weight (4.4×10^4), but proteolysis could be prevented by a plasmin-derived inhibitor.[436] Affinity chromatography of human-milk D-galactosyltransferase on immobilized α-lactalbumin revealed the presence of three forms (mol. wts. 3.8×10^4, 4.3×10^4, and 5.0×10^4) of the enzyme, which probably result from proteolysis.[437]

Serum Glycoproteins

The role of sialic acid residues in determining the life-time of circulating cells and glycoproteins and the importance of desialylation have been discussed.[438]

Chromatography on Blue Dextran 2000 coupled to agarose has been used in the rapid separation of factor X from citrated human plasma; a 2000-fold purification was achieved.[439] Inhibition by the antithrombin-heparin cofactor of the conversion of factor IX into its active form by factor IXa has been examined.[440] The process is time-dependent and requires a 1 : 1 combination

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of the cofactor and factor IXa. The carbohydrate moiety is considered to be of some importance to the function of factor VIII, since, in a study of three patients with von Willebrand's disease, the factor was found to be deficient in carbohydrate.[441] The partial chemical characterization of bovine factor VII has been reported; the amino-acid and carbohydrate compositions are closely similar to those of other vitamin-K-dependent factors.[442] Homologous sequences of amino-acids have been found at the NH_2-termini of factors II, IX, and X. The amino-acid sequences of purified human and bovine prothrombins show a high degree of homology.[443] About 70% of the carbohydrate residues of purified bovine prothrombin can be removed enzymically without affecting the coagulating activity.[444] Incomplete glycosylation cannot therefore account for the low coagulating activity of the prothrombin present on rough microsomes. The sialic acid residues of fibrinogen do not play any significant role in the conversion of fibrinogen into fibrin by thrombin, nor did their removal alter the clotting time, the solubility of clots, the extent of cross-linking in the fibrin polymer, or the firmness and elasticity of the clots.[445] The heterogeneity exhibited by a peptide chain of the D fragment isolated from a plasminolysate of human fibrinogen is attributed to glycosylation.[446] The γ-chain of human fibrin appears to contain 2-amino-2-deoxy-D-glucose,[447, 448] and the E fragment derived from fibrinogen is glycosylated on the γ-chain only.[449]

Comparisons of the properties of antithrombins III from human, bovine (mol. wt. 5.6×10^4), and equine (mol. wt. 5.2×10^4) sources showed that they contain 9, 12, and 16% of carbohydrate, respectively; each consists of a single polypeptide chain having a similar, but not identical, molecular weight.[450]

Rabbit plasminogen has been fractionated by affinity chromatography into two principal isoenzymes, one of which contains 2-acetamido-2-deoxy-D-glucose (4—5), 2-acetamido-2-deoxy-D-galactose (2), D-mannose (3), D-galactose (5), and sialic acid (3 residues per molecule).[451] The corresponding plasmin heavy-chain for this isoenzyme contains essentially all of the carbohydrate, and the plasmin light-chain appears to be devoid of carbohydrate. The second plasminogen isoenzyme contains 2-acetamido-2-deoxy-D-galactose (2), D-galactose (3), D-mannose (<1), sialic acid (2 residues per molecule), and trace amounts of 2-acetamido-2-deoxy-D-glucose, all of which appear on the heavy-chain of the plasmin.

The heterogeneity of the glycopeptides of human transferrin has been inferred from the complex catabolism of human transferrin and asialotransferrin in

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guinea-pigs. Removal of the sialic acid residues exposed structures with different affinities for the hepatic asialoglycoprotein receptor.[452] The metabolism of asialotransferrins in baboons and rhesus monkeys has also been examined.[453]

A procedure for the preparation of α_1-acid glycoprotein in a reasonably high degree of purity has been reported.[454] Procedures for desialylation and iodination were also described. The secondary structure of α_1-acid glycoprotein has been predicted, using the methods of Lim (*J. Mol. Biol.*, 1974, **88**, 873) and Chou and Fasman (*Biochemistry*, 1974, **13**, 211).[455] Four of the five sugar residues are considered to be attached either to a reverse β-turn or to regions containing many polar residues. Information on the tertiary structure of α_1-acid glycoprotein has been obtained by chemical modification of exposed residues,[456] and lysine, tyrosine, and tryptophan residues have been implicated in the binding of progesterone.[457] Hydrazinolysis of α_1-acid glycoprotein, followed by deamination with nitrous acid, afforded acidic and neutral mono- and oligo-saccharides (12)—(14) that contain 2,5-anhydro-D-mannose at the reducing end.[458] Asialo-α_1-acid [^{131}I]glycoprotein is rapidly cleared from the circulation after intravenous injection into rats.[459] The modified glycoprotein may provide additional information about the function of the liver.

$$\alpha\text{-D-Man}p\text{-}(1 \rightarrow 3)\text{-}[\alpha\text{-D-Man}p\text{-}(1 \rightarrow 6)]\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow 4)\text{-2,5-anhydro-D-Man}$$
(12)

$$\beta\text{-D-Gal}p\text{-}(1 \rightarrow 4)\text{-2,5-anhydro-D-Man}$$
(13)

$$\alpha\text{-Neu}N\text{Ac-}(2 \rightarrow ?)\text{-}[\beta\text{-D-Gal}p\text{-}(1 \rightarrow 4)\text{-2,5-anhydro-D-Man}]$$
(14)

The biology, biochemistry, metabolism, and oncogenesis of α-foetoprotein have been reviewed,[460] and a routine procedure for the isolation and purification of α-foetoprotein has been reported.[461] An alternative preparation of α-foetoprotein involved affinity chromatography on Sepharose–concanavalin A.[462] The question of whether the microheterogeneity of mouse α-foetoprotein during development results from different degrees of sialylation has been investigated, and large changes in the levels of sialyltransferase were found.[463] The accumulation of [^{125}I]asialofetuin in rat liver, and the subsequent clearance of the labelled glycoprotein (within 60 min), have been studied.[464] Each stage of heterophagy, including

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the uptake of proteins by the phagosomes, the transfer of the substrate to the lysosomes, peptide digestion, and the release of fragments from the lysosomes, was observed.

Structures have been proposed for the carbohydrate moieties of the low-density lipoprotein in human sera,[465] and the structures were compared with those in the sera of male patients with type II hyperlipoproteinemia.[466] Less D-mannose and sialic acid were found in the carbohydrate component of the lipoprotein from type II patients. Whereas concanavalin A and ricin both reacted with protein-deficient, low-density lipoprotein from human plasma, the native lipoprotein normally reacted with concanavalin A only.[467]

The structures of oligosaccharide components of the α_1-protease inhibitor in human plasma have been determined; two copies of two different chains are attached to each protein molecule.[468] The enzymic removal of sialic acid residues altered the electrophoretic mobility of serum α_1-antitrypsin; since neuraminidases are present in viruses and bacteria, the mobility of a contaminated sample of α_1-antitrypsin might be ascribed incorrectly to a slower-migrating variant.[469] The heterogeneity of the trypsin inhibitor in bovine colostrum has been shown to reside in the carbohydrate portion of the molecule.[470] The proportions of L-fucose, D-mannose, D-galactose, D-glucose, 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and sialic acid were determined for four fractions isolated.

The heterogeneity of carcinoembryonic antigen has been ascribed to variations in the extent of glycosylation.[471] A method capable of detecting less than 10 ng ml^{-1} of circulating carcinoembryonic antigen has been developed using counter-immunoelectrophoresis.[472] There appears to be some doubt concerning the cross-reactivity between carcinoembryonic antigen and the blood-group substances, since the technique using erythrocyte-eluted antibodies may be unreliable.[473]

The euglobin fraction of plasma appears to be a form of proteochondroitin 4-sulphate.[474]

α-L-Fucosidase of low activity has been detected in the plasma of some patients who did not exhibit the symptoms of fucosidosis.[475] The enzyme has a different pH profile from, and is less stable than, the normal enzyme. The L-fucose content of serum glycoproteins is considered to indicate better the disease-status of breast cancer than the corresponding levels of D-mannose, D-galactose, and sialic acid.[476]

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A glycoprotein (mol. wt. 3.1×10^4) that is present in human plasma, urine, and cerebrospinal fluid in concentrations of 100, 10, and 0.3 mg l^{-1}, respectively, contains 11.4% of sialic acid and 11% of neutral sugars and amino-sugars.[477] Glycoproteins in the blood of Pacific salmon have been separated by a combination of electrophoresis, precipitation, and chromatography.[478] Variations in the levels of α_1-antitrypsin, orosomucoid, transferrin, and α-foetoprotein in maternal and foetal sera and amniotic fluids during pregnancy have been monitored.[479] The levels of D-galactosyl- and sialyl-transferase activities in the sera of patients with renal failure are higher than normal; the findings were discussed in terms of the metabolism of glycoproteins.[480]

Immunoglobulins

Elucidation of the complete primary structure of human myeloma IgA1 immunoglobulin has revealed that all the carbohydrate at the carboxy-end of the α-1 chain occurs in the hinge region and Fc fragment.[481] Of the two types of oligosaccharide unit already reported for IgA1,[482] some are linked to either asparagine or serine in the hinge region. The oligosaccharide residues at the carboxy-end are attached to Asn-263 and -459, while another oligosaccharide residue is located at Asn-28. Statistical analyses of eleven IgA1 and six IgA2 myeloma proteins have also revealed differences in the L-fucose, D-mannose, and 2-amino-2-deoxy-D-glucose contents.[483] The sialic acid content of IgA1 was found to be inversely related to that of L-fucose, whereas a positive correlation was observed between the contents of D-galactose and sialic acid in IgA2. The heterogeneity of rabbit IgG immunoglobulin preparations has also been examined with regard to the variable amounts of attached carbohydrate residues.[484] Differences in the extents of glycosylation of the H and L chains derived from the IgM proteins of patients with Waldenström's disease have been shown to account for differences in the electrophoretic mobilities of the chains.[485] Two classes of oligosaccharide residues, one of which is located at Tyr-Asn-Val-Thr in the NH_2-terminal region of the Fc fragment, have been detected in human IgMDu myeloma protein.[486]

The amino-acid and carbohydrate compositions and parts of the sequences at the NH_2-termini have been reported for four HLA antigens, including the principal histocompatibility loci.[487] The HLA antigens have a common oligosaccharide chain composed of sialic acid (2), D-mannose (3), L-fucose (1), D-galactose (3 or 4), and 2-amino-2-deoxy-D-glucose (4 residues per mol. wt. 2.9×10^4). Human histocompatibility antigens have been purified using an

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immuno-adsorption column employing anti-β_2-microglobulin immunoglobulin.[488] Carbohydrate residues have been detected in a defective γ3 heavy-chain immunoglobulin which is thought to be derived from both variable and constant regions.[489] Fab′ fragments of cell-surface carbohydrates that bind immunoglobulin appear to be as mitogenic as the bivalent antibody, indicating that, at least for saccharide-specific ligands, multipoint attachment and receptor cross-linking on the cell may not be a stringent requirement for activation.[490] A striking homology is apparent between IgG1 and an Fc fragment of IgG3 derived from the 'heavy-chain-disease' protein ZUC.[491] Homologies also exist between IgG3 and IgG4 in the C_H2 and C_H3 domains. An internal molecular deletion of 81 residues occurring in a human λ-type immunoglobulin light-chain has been defined by sequence analysis; the carbohydrate residue is attached at position 25 of the first hypervariable region.[492] The production of a mutant murine myeloma protein lacking attached carbohydrate has indicated that the carbohydrate is not necessary for intracellular transport, assembly, or secretion of the immunoglobulin.[493] The interactions of homogeneous murine myeloma immunoglobulins with polysaccharide antigens, including (2 → 6)- and (2 → 1)-linked β-D-fructans, (1 → 6)-β-D-galactans, dextrans, and pneumococcal C polysaccharides, have been reviewed.[494]

2-Amino-2-deoxy-D-glucose, but not 2-amino-2-deoxy-D-galactose, is a constituent of murine J chain.[495]

Erythrocyte Glycoproteins

The role of erythrocyte-surface components, many of which are glycoproteins, as receptors has been reviewed.[496] The separation of these membrane constituents by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate, followed by chromatography in phenol–aqueous urea–acetic acid has been reported.[497] Twenty glycoproteins present in erythrocyte membranes have been resolved by electrophoresis on polyacrylamide slabs, following oxidation by D-galactose oxidase and reduction with sodium borotritide.[498] Two principal groups were observed; each of the glycoproteins in one group contains either D-galactose or 2-acetamido-2-deoxy-D-galactose linked to a sialic acid residue, which must be removed before labelling is possible, whereas those in the other group do not require such treatment.

Changes in the fluidity of erythrocyte membranes on binding influenza or Sendai virus have been ascribed to the interaction between the viral haem-agglutinin and a glycoprotein on the surface of the erythrocyte.[499]

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A quantitative yield of glycophorin (80% pure) was obtained following extraction of human-erythrocyte ghosts with sodium dodecyl sulphate and fractionation of the extracts on *N*-(3-carboxypropionyl)aminodecyl-Sepharose.[500] The method was less successful with spectrin. A one-step procedure for the isolation of glycophorin, involving extraction of erythrocyte ghosts with sodium dodecyl sulphate and chromatography on wheat-germ agglutinin attached to Sepharose, was also reported.[501] Another preparation of glycophorin involved extraction of erythrocytes with Tween 20, followed by chromatography on hydroxyapatite and pentyl-Sepharose.[502] Triton X-100 has been used to extract A, B, and O blood-group activities.[503] Fragmentation of purified glycophorin (MN glycoprotein) with cyanogen bromide afforded a peptide (C-2) from the CO_2H-terminus. When labelled with [^{35}S]methionylsulphone methyl phosphate and subjected to fingerprint analysis, C-2 gave rise to two labelled peptides, which were identical to two peptides derived from the region of glycophorin on the cytoplasmic side of the membrane. Hence, the CO_2H-terminus of glycophorin lies in the cytoplasm of red cells.[504] A sialoglycopeptide released by auto-digestion of human Rh(+) O-erythrocyte ghosts is probably derived from glycophorin.[505] An attempt to provide liposomes with cell-binding information has led to the incorporation of the major erythrocyte glycoprotein into their structures.[506] Sialoglycoproteins isolated from human-erythrocyte membranes did not act as substrates for cAMP-stimulated protein kinase.[507] Six sialoglycoproteins extracted from human-erythrocyte membranes have been examined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate.[508] A minor component (mol. wt. 4.1×10^4) was identified as a dimer of the Ss glycoprotein, thereby demonstrating appreciable aggregation in the dissociating medium. The study also indicated that some of the glycoproteins contain phosphate-binding sites, which account for the anomalies observed when phosphate buffers are used. Fragmentation of the transmembrane polypeptide (mol. wt. 9.5×10^4) in human red-cell membranes has revealed that the polypeptide has at least two sites of phosphorylation; the principal site of phosphorylation lies towards the NH_2-terminus and is, at least in part, within the cell.[509] Isolation and characterization of this glycoprotein revealed that the carbohydrate residues are heterogeneous.[510, 511] Circumstantial evidence has suggested that some of the glycoproteins spanning the cell membrane are involved in the transport of D-glucose across the membrane.[512] Proteins in erythrocyte membranes are produced asynchronously, and the synthesis of spectrin appears

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to diminish earlier during erythropoiesis than that of a protein of molecular weight 9×10^4.[513]

Another principal glycoprotein (mol. wt. 8.7×10^4) has been detected on the plasma membrane of platelets, where it appears to be located on the inner surface.[514] A glycoprotein of molecular weight 1.48×10^5, named glycocalicin, obtained on homogenization of platelets contains D-galactose, 2-acetamido-2-deoxy-D-galactose, 2-acetamido-2-deoxy-D-glucose, and sialic acid, in the proportions 2 : 1 : 1 : 2, and traces of D-glucose, D-mannose, and L-fucose; it afforded two glycoproteins (mol. wt. 1.18×10^5 and 4.5×10^4) on digestion with trypsin.[515] The aggregation of platelets obtained from patients with either Bernard–Soulier or von Willebrand's syndrome by ristocetin is brought about by glycoproteins on the membrane surface.[516]

An aldimine linkage between D-glucose and the α-amino-group of valine (the NH_2-terminal residue of the β-chain) appears to be responsible for the attachment of D-glucose to haemoglobin A_{1C}.[517] This linkage then rearranges, in a reversible manner, to form a keto-amine. The role of D-glucose 6-phosphate in the D-glucosylation of haemoglobin A_{1C} has been studied.[518]

Radiolabelling of surface proteins and chemical techniques have shown that human En(a−) erythrocytes (which lack the principal membrane sialoglycoprotein glycophorin) contain a decreased amount of the sialoglycoprotein PAS2.[519] A third membrane-penetrating glycoprotein (band 3) contains two oligosaccharide chains, and the more complex of the two oligosaccharides has a higher molecular weight in En(a−) cells than in normal cells. A fourth glycoprotein (PAS3) is present in normal amounts. Similar findings have been reported by other workers, and it is clear that a viable erythrocyte can exist despite the absence of one of the principal surface components.[520] A method that allows the characterization of the lectin-binding components of membrane glycoproteins involves the fixation of erythrocyte ghosts, after polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate, and incubation with ^{125}I-labelled lectins.[521] The principal membrane-penetrating glycoproteins (band 3) have been isolated from En(a−) and normal cells.[522] They differ only in the carbohydrate components, the glycoprotein component of En(a−) cells containing more D-galactose and 2-acetamido-2-deoxy-D-glucose.

The binding of concanavalin A to trypsinized and to non-trypsinized bovine erythrocytes has shown that the lectin-receptor and A antigen are independent cell-surface components.[523] This method should prove useful in defining the

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nature and heterogeneity of these components in cell membranes. The T-cell receptor on ovine erythrocytes has been identified as a glycoprotein of molecular weight 1×10^4 containing 2-acetamido-2-deoxy-D-glucose (3.6), 2-acetamido-2-deoxy-D-galactose (8.8), D-galactose (16.8), and sialic acid (9.7 residues per molecule).[524]

Spontaneous vesiculation of sheep-erythrocyte membranes is accompanied by an enrichment of specific membrane proteins in certain regions of the lipid bilayer.[525]

The receptor for infectious mononucleosis on goat erythrocytes has been identified as a glycoprotein (subunit mol. wt. 2.5×10^4) containing sialic acid, D-galactose, D-mannose, 2-amino-2-deoxy-D-galactose, and 2-amino-2-deoxy-D-glucose in the molar ratios of 3.1 : 2.1 : 0.1 : 1.6 : 1.0.[526] It interacted weakly with a number of lectins, although, in some cases, the interaction was improved after the removal of sialic acid residues.

Salivary and Mucous Glycoproteins

The possibility that disulphide bonds create a network in mucous gels has been examined, using the sputum of patients with chronic bronchitis or bronchorrhoea.[527] Fragments released by tryptic digestion of human neutral bronchial mucin contained the antigenic determinant, part of which is composed of carbohydrate residues.[528]

A new sialic acid, *N*-acetyl-9-*O*-(L-lactyl)-neuraminic acid, has been identified in glycoproteins isolated from bovine submandibular glands.[529] The acylated neuraminic acid fraction released by mild acidic hydrolysis contained 2% of this acid. A glycoprotein isolated from bovine cervical mucus has a molecular weight of 6×10^5 and contains 1200 amino-acid residues.[530] Analysis of the fragments released by cyanogen bromide suggested that the carbohydrate residues are distributed randomly on the core, and that the non-glycosylated regions are important in intermolecular binding.

Sodium thiocyanate has been used to disperse canine tracheal mucus, affording dissociation of up to 100%, with a high retention of elasticity and no demonstrable changes in the shapes or sizes of the component glycoproteins.[531]

Investigations into the response of murine sublingual and submandibular glands to stimuli have led to the characterization of the mucins in these glands.[532] Apparently homogeneous proteins were isolated, and the compositions of the carbohydrate residues were compared. α-L-Fucopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-D-galactitol and 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactitol were released from a water-soluble

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glycoprotein, isolated from the intestinal mucus of sterile rats, following removal of the sialic acid residues and cleavage with alkaline borohydride.[533]

Urinary Glycoproteins

The structures (15)—(17) of three glycosylated asparagines isolated from the urine of patients with aspartylglycosaminuria have been determined,[534, 535] and another glycosylated asparagine has been assigned the structure (18).[536] α-D-Mannopyranosyl-(1 → 3)-β-D-mannopyranosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose has been detected in human urine by g.l.c.–m.s., and the detection of this trisaccharide is recommended as a screening test for mannosidosis, since it could not be found in the urine of normal or heterozygote subjects.[537] Abnormally high amounts of low-molecular-weight carbohydrates rich in D-mannose have been found in the urine of an Angus calf with mannosidosis.[538] The most abundant oligosaccharide was shown to have the structure (19).

β-D-Glc*N*Ac*p*-Asn
(15)

β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-Asn
(16)

α-D-Man*p*-(1 → 6)-β-D-Man*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 4)-β-D-Glc*N*Ac*p*-Asn
(17)

α-Neu*N*Ac-β-D-Gal*p*-β-D-Glc*N*Ac*p*-β-D-Gal*p*-(1 → 4)-β-D-Glc*N*Ac*p*-Asn
(18)

α-D-Man*p*-(1 → 6)-β-D-Man*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 4)-β-D-Glc*N*Ac*p*-(1 → 4)-D-Glc*N*Ac*p*
(19)

Induced galactosuria is characterized by excretion in the urine of large amounts of oligosaccharides having structures that depend on the blood-group ABO, Lewis, and secretor phenotypes.[539] L-Fucose-rich oligosaccharides with blood-group activities have been found to be excreted in urine, and they appear to be formed by the glycosylation of free mono- and di-saccharides present in the organism; for example, D-glucose for normal and diabetic subjects, and lactose or D-galactose for lactosuric and galactosuric subjects, respectively.[540] Chromatographic methods have shown the existence of a complex system for transferring D-galactose from UDP-D-galactose to glycoproteins and for the hydrolysis of nucleotides in the urine of Balb/c mice.[541]

[533] J. K. Wold, B. Smestad, and T. Midtvedt, *Acta Chem. Scand.* (*B*), 1975, **29**, 703.
[534] A. Lundblad, P. K. Masson, N. E. Nordén, S. Svensson, P.-A. Öckerman, and J. Palo, *European J. Biochem.*, 1976, **67**, 209.
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[537] A. Lundblad, P. K. Masson, N. E. Nordén, S. Svensson, and P.-A. Öckerman, *Biomed. Mass Spectrometry*, 1975, **2**, 285.
[538] A. Lundblad, B. Nilsson, N. E. Nordén, S. Svensson, P.-A. Öckerman, and R. D. Jolly, *European J. Biochem.*, 1975, **59**, 601.
[539] G. Strecker, T. Riazi-Farzad, B. Fournet, S. Bouquelet, and J. Montreuil, *Biochimie*, 1976, **58**, 815.
[540] G. Strecker, C. Trentesaux-Chauvet, A. Poitau, and J. Montreuil, *Biochimie*, 1976, **58**, 805.
[541] N. Achy-Sachot and E. Morel, *Experientia*, 1976, **32**, 1126.

An acidic glycopeptide, possibly of diagnostic value, has been detected in the urine of patients with deficiencies in the metabolism of glycosphingolipids, or with multiple lysosomal lesions, or with defects in the organization of collagen fibrils.[542] The urine of pregnant women contains a glycoprotein of high molecular weight, composed of subunits of molecular weight *ca.* 1.3×10^4 and 1.6×10^4, having antisecretory activity.[543] The levels of neuraminyl-lactose and neuraminyl-D-galactosyl-(1 → 4)-2-acetamido-2-deoxy-D-glucose in urine have been shown to rise during normal pregnancy.[544] Measurements of the levels of acetamidodeoxyglucosidase and γ-glutamyltransferase in urine have been used to confirm the rejection of tissues after renal transplants.[545]

The isolation of kallikrein (mol. wt. 3.2×10^5), a protease that releases kinin from kininogen, from rat urine has been reported.[546, 547] Kallikrein contains five residues of 2-amino-2-deoxy-D-glucose per molecule. Excessive amounts of oligosaccharides rich in sialic acid have been detected in the urine of patients with I-cell disease or mucolipidosis, indicating that there is a deficiency of α-neuraminidase in these diseases.[548]

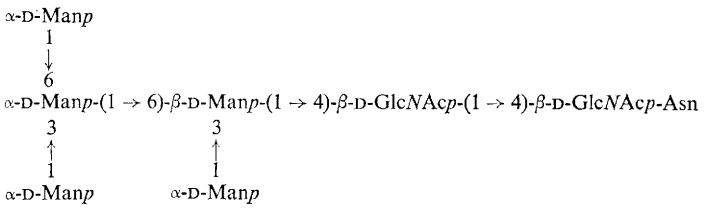
The structures of dermatan and heparan sulphates excreted in the urine of patients with Hurler and Hunter syndromes differ from those in normal urine; these differences are revealed by the resistance to periodate oxidation, the charge distribution (as judged by electrophoresis and ion-exchange chromatography), and the content of 2-sulphoamino-2-deoxy-D-glucose.[549]

Avian Glycoproteins

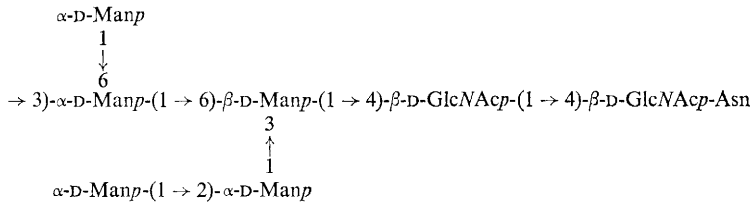
Procedures recommended for the isolation and purification of ovalbumin have been reported.[550] The structures (20) and (21) of two glycopeptides obtained from ovalbumin have been determined, and the specificity of an *endo*-β-acetamidodeoxyglucosidase from *Diplococcus pneumoniae* was defined by examining the action of the enzyme on the glycopeptides.[551]

The glycosylated asparagine residues of ovomucoid have been located at position 10, and between positions 49 and 60 and positions 69 and 75; each carbohydrate residue contains 2-amino-2-deoxy-D-glucose, D-mannose, and D-galactose in the ratios 10 : 6 : 1.[552] There is possibly a fifth site which is glycosylated. Two of the glycosylated asparagine residues occur in homologous sequences, and three of the regions of attachment occur in positions similar to

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[552] J. G. Beeley, *Biochem. J.*, 1976, **159**, 335.



(20)



(21)

those in quail ovomucoid. Using a predicted conformation, it is suggested that the carbohydrate is attached close to β-turns. A polypeptide containing 94 amino-acid residues and D-mannose (3) and 2-acetamido-2-deoxy-D-glucose (5 residues) has been isolated from hen ovotransferrin.[553] The oligosaccharide chain is attached at asparagine-13. A glycoprotein in the vitelline membrane of hen's egg has a molecular weight of 2.7×10^4 and contains L-fucose (3), D-mannose (5), D-galactose (5), D-glucose (1), and D-xylose (1 residue(s) per molecule).[554]

Miscellaneous Glycoproteins and Chitin

A review of complex carbohydrates, which includes a discussion of the structures, isolation, biosynthesis, and functions of glycoproteins, glycosaminoglycans, and blood-group substances, has appeared.[555] The structures of the carbohydrate moieties found in glycoproteins from such diverse sources as fungal and mammalian cells have been compared.[556]

The *in vitro* glycosylation of proteins continues to receive attention. The coupling of bovine pancreatic trypsin to dextran afforded a glycoprotein that was more resistant to inactivation by heat, autodigestion, and denaturing agents.[557] It was also less affected by trypsin inhibitor. Covalent binding of haemoglobin to dextran modified, but did not destroy, the oxygen-binding properties.[558] Experiments with rabbits showed that this complex is excreted by the kidneys, and that it is cleared more slowly than the native protein from the circulation.

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[554] S. Kido, M. Janado, and H. Nunoura, *J. Biochem.* (*Japan*), 1976, **79**, 1351.
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[557] J. J. Marshall and M. L. Rabinowitz, *J. Biol. Chem.*, 1976, **251**, 1081.
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The 'antifreeze' properties of the glycoprotein isolated from *Trematomus borchgrevinski* have been attributed to hydrogen-bonding between the carbohydrate residues and the surrounding water molecules.[559] This view is supported by the observation that the 'antifreeze' properties and the haemagglutinating activity were both lost on complexation of the glycoprotein with borate.[560]

Microtubular granules, but not microtubules, of the paragonial secretory cells of *Drosophila melanogaster* Meig. contain glycoproteins.[561] Some of the amino-acid sequences in glycoproteins derived from an 80-million-year-old mollusc shell exhibited homologies with those found in the glycoproteins of contemporary shells.[562] A glycoprotein (mol. wt. 3×10^4) found in the cytoplasm of unsporulated oocysts of *Eimeria tenella* (a parasitic protozoan) contains 40% of carbohydrate consisting of D-glucose, D-galactose, D-mannose, 2-amino-2-deoxy-D-glucose, and 2-amino-2-deoxy-D-galactose, but sialic acid is not found.[563] The abilities of the 1-thioglycosides of D-glucopyranose, D-galactopyranose, D-mannopyranose, and 2-acetamido-2-deoxy-D-glucopyranose attached to *Aspergillus oryzae* α-amylase, hen's egg lysozyme, and bovine serum albumin to bind to rabbit-liver membrane have been studied.[564] Only the 1-thio-D-galactopyranoside derivatives of the enzymes showed improved binding capacities. Using the procedure of Chou and Fasman (see page 319) to predict the secondary structure of the peptide segment around the carbohydrate–peptide linkage in glycoproteins, it has been found that serine and threonine residues involved in *O*-glycosidic linkages always occur at β-turns, whereas asparagine residues involved in *N*-glycosidic linkages are located at the surface of the protein, and often, but not always, belong to a β-turn.[565] Conformational studies of glycoproteins in the presence of sodium dodecyl sulphate have revealed that unfolding is a two-stage process.[566]

An examination of larval, white puparial, and fully sclerotized cuticles has shown that glycopeptides and peptidochitodextrins occur at specific times during the transformations.[567] An *in vitro* system for the synthesis of β-ecdysone-dependent chitin (which is found in cockroaches) has been developed using ^{14}C-labelling and inhibitors (TH6040 and polyoxin D) of chitin synthase.[568] The formation of 2-amino-2-deoxy-D-glucose 6-phosphate in the integument of *Locusta migratoria* before, and after, ecdysis has been studied.[569] The activity of the glutamine : D-fructose 6-phosphate aminotransferase during ecdysis correlates with the *in vivo* incorporation of D-glucose into chitin.

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[560] A. I. Ahmed, Y. Yeh, D. T. Osuga, and R. E. Feeney, *J. Biol. Chem.*, 1976, **251**, 3033.
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[562] S. Weiner, H. A. Lowestam, and L. Hood, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 2541.
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[566] B. Jirgensons, *Biochim. Biophys. Acta*, 1976, **434**, 58.
[567] S. Kimura, H. V. Strout, and H. Lipke, *Insect Biochem.*, 1976, **6**, 65.
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Analysis of Glycoproteins

The latest volume in the series *Methods in Carbohydrate Chemistry* reports a number of useful techniques, including the proteolytic digestion of membrane-derived glycoproteins, the acid-catalysed hydrolysis and methanolysis of glycoproteins, and the detection of residues linked *O*-glycosidically to serine and threonine, *etc.*[570] Among other methods described are the use of enzymes in the determination of structure, the removal and estimation of sialic acid residues, the determination of *O*-acetyl groups (by the Hestrin method) and of hexosamines and hexosaminitols (using an amino-acid analyser), and the identification of complex carbohydrates using immunofluorescence. The uses of g.l.c.–m.s. and ^{1}H n.m.r. spectroscopy in establishing the structures of TMS derivatives of methyl glycosides obtained on methanolysis of glycoproteins and glycopeptides have also been discussed.[571]

A cationic carbocyanine dye ('Stains-all') has been used to distinguish between sialoglycoproteins, proteins, and lipids following gel electrophoresis in the presence of sodium dodecyl sulphate.[572] Treatment in turn with periodate and dansylhydrazine is claimed to be a very sensitive method for detecting glycoproteins in polyacrylamide gels.[573]

Acetic anhydride in DMSO is an effective reagent for the selective location of polysaccharides containing pyranosyl residues in mammalian issue sections, since the oxidized residues can be visualized following treatment with Schiff's reagent or *o*-dianisidine.[574] Glycoproteins containing terminal or internal residues of D-mannose or terminal residues of 2-acetamido-2-deoxy-D-glucose can be detected using concanavalin A and horseradish peroxidase.[575]

The D-galactose oxidase from *Dactylium dendroides* oxidizes the primary hydroxy-group of non-reducing D-galactofuranosyl residues, a reaction that is useful in the quantitative determination of D-galactose and in determining the ring size of D-galactosyl residues in carbohydrate structures (*e.g.* a glycoprotein from the hyphal wall of the fungus *Pithomyces chartarum*).[576] The production of anti-D-galactose and anti-lactose antibodies has been described, and these antibodies are of potential use in structural work.[577] Anti-mannan antibodies have been used to investigate other structures containing D-mannosyl residues; for example, the glycopeptides of Taka-amylase A strongly inhibited the interaction between mannan and anti-mannan antibodies, implying that they contain structures similar to that of the mannan.[578] Reducing sugars (1—25 nmole) obtained on hydrolysis of glycoproteins have been determined by a spectrophotometric method that is based on their reaction with alkaline ferricyanide.[579]

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Biosynthesis of Glycoproteins

The biosynthesis and structure of polyprenol-linked sugars, and the role of such sugars, retinol phosphate, and vitamin K in the synthesis of glycoproteins, have been reviewed.[580] Information on proteins that serve as acceptors and the role of glycosyl-transfer reactions involving lipid-linked sugars are also discussed.

A serum transferase that catalyses the *in vitro* transfer of 2-acetamido-2-deoxy-D-glucose to the asparagine residue of the sequon Asn-Leu-Thr of bovine pancreatic ribonuclease has been reported.[581] Spermidine, Mn^{2+} ions, and CDP-choline are activators of this enzyme. Enzymes in preparations of yeast-cell membranes also accomplished this transglycosylation, which was inhibited by tunicamycin – an antibiotic that inhibits the synthesis of P^1-2-acetamido-2-deoxy-D-glucopyranosyl P^2-polyisoprenyl pyrophosphate.[582] Both 2-amino-2-deoxy-D-glucose and D-mannose were incorporated into nascent chains of ovalbumin by hen-oviduct fragments.[583] Although the asparagine residue to which the carbohydrate residue is attached is only 10 amino-acid residues from the NH_2-terminus, the majority, if not all, of the carbohydrate is attached to those peptide chains that are essentially complete.

The core of D-mannosyl residues in glycoproteins occurring in human diploid fibroblasts varies in size, depending on the rate of growth of the cells.[584] Oligosaccharyl-lipids isolated from calf-thyroid slices have been shown to contain 14 or 15 sugar residues, including D-glucose.[585] D-Mannose and D-glucose were similarly affected during pulse-chase experiments, suggesting that the intact oligosaccharide unit is transferred to the endogenous protein. Since neither of the two types of carbohydrate residue of calf thyroglobulin contains D-glucose, it must be assumed that this sugar is cleaved from the oligosaccharide after transfer. Some of the structural features of this lipid-linked oligosaccharide have been established[586] and compared with those of lipid-linked oligosaccharides isolated from other tissues.[587] The addition of D-mannose to glycoproteins in the thyroid rough endoplasmic reticulum is considered to involve dolichyl D-manno-pyranosyl phosphate and P^2-dolichyl P^1-D-manno-oligosaccharyl pyrophosphates.[588] Similar conclusions were drawn following an investigation of the synthesis of D-mannosylated glycoproteins in the white matter of calf brain.[589] The functions of lipid mono- and pyro-phosphates have also been studied using preparations from pig-liver microsomes.[590] A P^2-dolichyl P^1-oligosaccharyl pyrophosphate acts as the acceptor and GDP-D-mannose as the glycosyl donor, but there is also evidence that two pools of dolichyl D-mannopyranosyl phosphate exist, and that one of them is more closely involved with the transfer of D-mannose

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[587] M. J. Spiro, R. G. Spiro, and V. D. Bhoyroo, *J. Biol. Chem.*, 1976, **251**, 6420.
[588] C. Ronin and S. Bouchilloux, *Biochim. Biophys. Acta*, 1976, **428**, 445.
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to lipid-linked oligosaccharide residues. Related studies of the transfer of D-mannose to glycoproteins *via* lipid intermediates have been reported for porcine aorta,[591] chicken embryo,[592] and *Neurospora crassa*.[593] That oligosaccharyl-lipid intermediates are associated with plasma members, as well as with internal membranes, has been confirmed experimentally.[594] The synthesis of plant glycoproteins, particularly the transfer of D-mannose and of 2-acetamido-2-deoxy-D-glucose from nucleotide precursors in mung-bean seedlings, has been studied.[595] Particulate preparations obtained from maturing cotton fibres have been used to investigate the incorporation of D-mannose and 2-acetamido-2-deoxy-D-glucose into oligosaccharyl-lipids, the smallest carbohydrate moiety of which appears to be the trisaccharide β-D-Man*p* → β-D-Glc*NAc*p → D-Glc*NAc*p.[596] The transfer of 2-acetamido-2-deoxy-D-glucose from the UDP-nucleotide to form P^2-lipid P^1-di-*N*-acetylchitobiosyl pyrophosphate was also catalysed by this preparation, and the addition of GDP-D-mannose resulted in the appearance of the acetamido-sugar in the lipid-linked oligosaccharide. It is possible to distinguish between the D-mannosyltransferases which catalyse the formation of (1 → 2)-, (1 → 3)-, and (1 → 6)-linkages in microsomal preparations obtained from *Saccharomyces cerevisiae*.[597, 598] A cell-free preparation from *Mycobacterium smegmatis* was used to catalyse the synthesis of methyl 2-*O*-α-D-mannopyranosyl-α-D-mannopyranoside from methyl α-D-mannopyranoside and GDP-D-mannose.[599] Specific assays employing mannoprotein-derived oligosaccharides have been devised for several of the D-mannosyltransferases involved in the synthesis of yeast mannoproteins.[600]

The effects of potent antifertility agents (medroxyprogesterone acetate, ethynodiol, and chlormadinone acetate) on the synthesis of glycoproteins by human endometrium in culture have been examined with a view to elucidating further the mechanism of their action. When oestradiol or progesterone was added to the medium, the antifertility agents reduced the incorporation of 2-amino-2-deoxy-D-glucose into glycoproteins, and medroxyprogesterone acetate also reduced that of leucine into glycoproteins.[601]

A D-galactosyltransferase (pH optimum 6) requiring Mg^{2+} or Mn^{2+} ions has been found in alveolar lung cells; UDP is a non-competitive inhibitor of the enzyme.[602] High concentrations of cycloheximide competitively inhibited a D-galactosyltransferase present in isolated Golgi membranes from rat liver; the effect of the inhibitor appears to be primarily on the membrane, affecting the enzyme secondarily.[603] The observation that Zn^{2+}, Cd^{2+}, Fe^{2+}, Co^{2+}, and

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Pr^{3+} ions, as well as Mn^{2+} ions, stimulated the activity of UDP-D-galactose : 2-acetamido-2-deoxy-D-glucose β-4-D-galactosyltransferase (a component of lactose synthase) suggests that the enzyme might contain two cation-binding sites.[604] The interactions between this transferase and ovalbumin and α-lactalbumin have been compared.[605] α-Lactalbumin was shown to affect only the affinity of the transferase for monosaccharides.[606] Purification (19 600-fold) of the soluble transferase in foetal calf serum has indicated that it may be the same as that in cow's milk.[607] The high level of an abnormal D-galactosyltransferase in the ascitic fluid of Balb/c mice indicated that the enzyme may diffuse from the cancerous cells.[608] A D-galactosyltransferase present in rat-liver microsomes has a higher molecular weight than that of the corresponding serum enzyme, suggesting that proteolysis occurs after secretion of the enzyme from the liver.[609] The presence of a D-galactosyltransferase in spleen microsomes has been reported.[610] D-Galactosyltransferase activity in the Golgi membrane of rats is significantly enhanced on binding concanavalin A, owing to perturbation of the membrane on binding the lectin.[611] The levels of D-galactosyltransferase activity during the development of neural tissues in chicken embryos, although not showing a simple correlation, indicated that the enzyme is involved in the developmental processes.[612]

Kinetic parameters for the CMP-*N*-acetylneuraminic acid : glycoprotein sialyltransferase in human malignant melanoma have been reported.[613] An overall scheme for the metabolism of acylated neuraminic acids in animal tissues, based on a study of *O*-acylation of the sialic acid residues in bovine and equine submandibular glands, has been presented.[614]

Although different enzymes in liver Golgi membranes catalyse the transfer of D-mannose, D-glucose, and 2-acetamido-2-deoxy-D-glucose from the corresponding nucleotides, they use the same pool of dolichyl phosphate.[615] A study of the incorporation of sugars into ovalbumin revealed that either 1,4-dithiothreitol or glutathione inhibited the attachment of 2-amino-2-deoxy-D-glucose while stimulating that of D-mannose.[616] The incorporation of 2-deoxy-D-*arabino*-hexose into endogenous lipid and glycoprotein acceptors has been reported.[617] Fractions from the membrane of *Saccharomyces cerevisiae* used UDP-2-deoxy-D-*arabino*-hexose to synthesize glycolipids which displayed characteristics of sphingolipids or steroidal D-glycosides rather than of polyisoprenols. The synthesis of serum glycoproteins and proteins in liver microsomal fractions decreased in the early

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stages after partial hepatectomy.[618, 619] However, the effect is not lasting, since the synthesis of glycoproteins in the regenerating liver is restored to normal after several days. Colchicine inhibited the secretion of plasma glycoproteins by rat hepatocytes, causing them to accumulate in Golgi-derived secretory vesicles.[620] Inhibition occurs after D-galactose and sialic acid are added to the secretory proteins.

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6
Enzymes

BY J. F. KENNEDY

Introduction

General Aspects and Nomenclature.—A four-volume work entitled 'The Enzymes of Biological Membranes' deals with physical and chemical techniques used to identify the enzymes, the biosynthesis of cell components, membrane- and electron-transport systems, and receptors.[1]

The first supplement to 'Enzyme Nomenclature – Recommendations (1972) of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry' contains corrections and additions based on the literature up to 1975.[2] Various β-D-glucan hydrolases can be distinguished by determining whether or not they hydrolyse cellulose, reduced SIII pneumococcal polysaccharide, lichenin, and laminarin.[3]

Methods of Assay.—The second English edition of 'Methods of Enzymatic Analysis' has been published in four volumes.[4]

A method for locating the peaks of activity in eluates from chromatographic columns has been reported for polysaccharide hydrolases usually assayed by determining the reducing sugars released from substrates.[5] The enzymic activity of each fraction is tested, using appropriate substrates and conventional detection reagents on a droplet placed on a sheet of powdered paper.

Isolation.—Recent advances in affinity-chromatography techniques used in the purification and isolation of enzymes have been reviewed.[6]

A theory relating to the purification of enzymes by affinity chromatography takes account of steric blocking of ligand sites and interactions between the enzymes and the bound ligand, between the enzyme and the washing agent, and between two enzymes.[7] For small loadings, only the initial concentration of the ligand and the dissociation constant are important, but steric-exclusion effects assume importance as the loading becomes larger. Elution of the enzyme from the column depends on the ratio of the concentration of the washing agent to

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the dissociation constant of the enzyme-washing agent complex. High concentrations of the enzyme can be obtained when the washing agent is used to elute the enzyme after a step input. If two enzymes are absorbed, the results obtained with each enzyme apply only if small pulses are used, whereas they are not valid for step inputs and frontal development, since enzyme–enzyme interactions then become important. The concentration of the enzyme absorbed first on the column can be higher temporarily than that in its feed.

Properties.—The electron microscopy of enzymes has been reviewed.[8] A symposium on the physiological effects of carbohydrates in food includes articles on the associated mammalian glycoside and polysaccharide hydrolases.[9]

The kinetic properties and substrate specificities of known glycoside hydrolases have been tabulated and indexed.[10] The oligosaccharidases of small-intestinal brush borders have been reviewed.[11]

The properties of well-known and new starch-degrading enzymes have been discussed.[12] The relative rates of the reactions of various polysaccharide hydrolases (*e.g.* cellulase, xylanase, protease, amylase, and pectinase) can be readily determined using an oscillating capillary-tube recording viscometer; continuous readout allows the initial rate of reaction to be observed, and the complete curve for the enzymic reaction approximates to a rectangular hyperbola.[13]

New volumes in the third edition of 'The Enzymes' deal with dehydrogenases and electron-transfer,[14] oxidases, oxygenases and electron transfer,[15] and dehydrogenases, and oxidases and hydrogen-peroxide cleavage.[16]

Mechanisms of Action.—Reviews on enzyme mechanisms have been published,[17, 18] and one of them[18] includes diagrams of the chemistry that occurs at the active sites. A book on enzyme kinetics includes sections on one-substrate, linear, and branching mechanisms, inhibitors, activators, co-operative interactions, and isotopic exchange.[19]

The mechanisms of action of some glycoside hydrolases have been discussed,[20] and a review of the anomeric specificity of glycolytic enzymes outlines the techniques used and the implications of the enzyme's specificity in metabolic regulation.[21]

Studies on active-site-directed inhibitors of glycoside and polysaccharide hydrolases have been reviewed briefly.[22] It was stated that inhibitors used to

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[11] G. M. Gray, in ref. 9, p. 181.
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label functional groups at the active sites of these enzymes should have a structure similar to the substrate, in order to facilitate interaction with the substrate-binding site of the enzyme, and a reactive group (*e.g.* epoxide, α-haloacyl, diazoacyl, or azido) that is able to form a stable covalent bond with an amino-acid side-chain at the active site.

Structures.—A review of the occurrence of multiple forms of glycoside hydrolases in normal and pathological conditions has compared the relationships of the enzymes in storage disorders involving the metabolism of glycolipids, glycoproteins, and glycosaminoglycans.[23] A subunit hypothesis, which may be widely applicable, was invoked to explain the relationships among the multiple form

The collecting and processing of experimental data for mapping the subsites of enzymes (*e.g.* polysaccharide hydrolases) have been discussed,[24] and the problems involved in the synthesis of polymers that contain structures related to the active sites of enzymes have been reviewed.[25]

Functions.—The importance of the secretion of *exo*-enzymes through the apical cell walls of fungi has been questioned.[26] The roles of glycoside and polysaccharides hydrolases in the antifungal action of saponins have been investigated.[27]

A review of the chemistry and the biochemical control of hereditary glycolipid diseases refers to the deficiencies of glycoside hydrolases in these diseases.[28]

Applications.—The use and measurement of enzymes, including carbohydrases, in analytical chemistry have been reviewed.[29]

The uses of enzymic methods in the structural analysis of polysaccharides [30] and the carbohydrate chain(s) of glycoproteins [31] have been discussed.

Problems encountered in starch enzymology [32] and in the production of D-fructose-containing syrups from starch [33] have been described.

A section of the latest volume of 'Methods in Enzymology' deals with the enzymes involved in the synthesis of complex carbohydrates.[34] The methods and apparatus used to immobilize enzymes have been described, and in particular process development and design.[35]

D-2-Acetamido-2-deoxygalactosidases, D-2-Acetamido-2-deoxy-glucosidases, and β-D-2-Acetamido-2-deoxyhexosidases

A post-coupling method has been reported for the histochemical detection of β-D-2-acetamido-2-deoxyglucosidase in unfixed, frozen-tissue sections.[36]

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The levels of β-D-2-acetamido-2-deoxyglucosidase activity appear to be increased in diabetes mellitus.[37] Patients with symptomatic porphyria exhibit a higher activity of the lysosomal enzyme β-D-2-acetamido-2-deoxyglucosidase in their sera than did those of control groups.[38] The enzymic activity is higher during the active stage of the disease than during remission, which suggests that symptomatic porphyria is a lysosomal-enzyme disorder. A high correlation was found between the levels of the enzyme and those of serum porphyrins and urinary uroporphyrin.

The chemical and clinical diagnoses of Tay–Sachs disease, which involves a deficiency of β-D-2-acetamido-2-deoxyhexosidase A, have been reviewed.[39] An automated assay of β-D-2-acetamido-2-deoxyhexosidase has been modified by introducing a pH-inactivation stage in order to detect heterozygotes of Tay–Sachs disease.[40] The assignment of genes for the A and B forms of β-D-2-acetamido-2-deoxyhexosidase to individual chromosomes has been achieved in studies on the enzymology of Tay–Sachs and Sandhoff diseases.[41]

Interconversions based on a subunit model of the A, B, and S forms of isoenzymes of the β-D-2-acetamido-2-deoxyhexosidase in human tissues have been accomplished by means of preparative polyacrylamide gel electrophoresis.[42] It was concluded that the A, B, and S forms are composed of a heteropolymer containing α- and β-chains, a β-chain homopolymer, and an α-chain homopolymer, respectively. Separation of the isoenzymes of β-D-2-acetamido-2-deoxyhexosidase in human tissues by electrophoresis on cellulose acetate membranes has been used in the diagnosis of G_{M_2}-gangliosidosis.[43] The relative abundances and properties of the three isoenzymes were determined, and the relevance of the results to the disease was discussed.

After fractionation by isoelectric focusing, the activities of a crude, soluble preparation of human hepatic β-D-2-acetamido-2-deoxyhexosidase towards natural glycosphinolipids have been examined.[44] Profiles of the activities towards 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 4)-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 1)-ceramide (asialo-G_{M_2}-ganglioside) and 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1 → 4)-β-D-galactopyranosyl-(1 → 4)-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 1)-ceramide (globoside) were identical to those of non-specific β-D-2-acetamido-2-deoxyhexosidase, whereas hydrolytic activities towards 2-acetamido-2-deoxy-β-D-galactopyranosyl-(*N*-acetylneuraminyl)-D-galactopyranosyl-D-glucopyranosyl-(1 → 1)-ceramide (G_{M_2}-ganglioside) were associated only with the most acidic subfraction of the A form of the enzyme. On the basis of these findings, explanations were advanced to account for the accumulation of G_{M_2}-ganglioside in G_{M_2}-gangliosidosis (a partial deficiency of β-D-2-acetamido-2-deoxyhexosidase A) and the AB variant of the disease (normal activities of β-D-2-acetamido-2-deoxyhexosidases A and B).

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Human-liver β-D-2-acetamido-2-deoxyhexosidase A was converted into a form exhibiting identical electrophoretic and immunological properties and thermostability to those of β-D-2-acetamido-2-deoxyhexosidase B on incubation with commercial culture filtrates of *Vibrio cholerae*, whereas purified preparations of neuraminidase had no effect.[45] The factor responsible for the conversion was identified as merthiolate, a preservative added to commercial filtrates of *V. cholerae*. Thus, the conclusion that the A and B forms are glycoproteins containing different numbers of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid residues must be re-evaluated.

β-D-2-Acetamido-2-deoxyhexosidases A and B from human placenta have been purified by affinity chromatography on a matrix of concanavalin A and 2-acetamido-*N*-(6-aminocaproyl)-2-deoxy-β-D-glucopyranosylamine separately immobilized on agarose cyclic imidocarbonate.[46] The preparations were shown to be homogeneous by analytical ultracentrifugation and by electrophoresis on polyacrylamide gel. The A form contains 1.65 moles of sialic acid mol^{-1}, whereas the B form does not contain sialic acid. In the presence of guanidinium hydrochloride, the A and B forms (mol. wt. 1.00×10^5 and 1.08×10^5, respectively) yielded a species (mol. wt. 5.0×10^4) that could be further dissociated by reduction and alkylation. Identification of the subunits indicated that the A form is composed of two subunits (α_2 and β_2) linked by a disulphide bridge, and that the B form contains two subunits ($\beta_2\beta_2$). The models were discussed in the light of information that has accumulated on the relationships between the forms and metabolic disorders involving them.

Incubation of a purified β-D-2-acetamido-2-deoxyhexosidase A from human placenta with merthiolate or 4-hydroxymercuribenzoic acid or Ag^+ ions gave an enzyme having properties identical to those of β-D-2-acetamido-2-deoxyhexosidase B.[47] The findings can be reconciled with the view that the A enzyme consists of $(\alpha\beta)_3$ subunits and the B enzyme of $(\beta\beta)_3$ subunits. Thus, the reagents dissociate the α and β subunits of the A form, which reassemble as $(\beta\beta)_3$. It was suggested that the catalytic site is present only in the β subunit, whereas the α subunit controls the substrate specificity of the enzyme.

Three isoenzymes of β-D-2-acetamido-2-deoxyhexosidase, in addition to the isoenzymes A and B, have been separated from human body fluids by ion-exchange chromatography.[48] The chromatograms revealed a new isoenzyme, and the isoenzyme patterns of the fluids from normal and pregnant women, from carriers of Tay–Sachs disease and pregnant carriers, and from patients with the disease could be used to distinguish between normal and carrier pregnancies.

The β-D-2-acetamido-2-deoxyhexosidase activities in the sera of human divers before, during, and after simulated dives have been measured.[49] An automated assay for determining β-D-2-acetamido-2-deoxy-glucosidase in normal and pathological human urine has been described.[50]

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The α-D-2-acetamido-2-deoxyglucosidase activity in cultures of human skin fibroblasts has been studied.[51] Ion-exchange chromatography and gel filtration demonstrated the presence of different molecular forms of α- and β-D-2-acetamido-2-deoxyglucosidases in cultures of fibroblasts from human amniotic fluid; the isoenzyme patterns of the molecular forms are similar to those of enzymes in skin fibroblasts and liver tissues.[52] The intracellular exchange of β-D-2-acetamido-2-deoxyglucosidase between normal and enzyme-deficient human fibroblasts in confluent cultures has been examined.[53] The results were discussed in relation to Sandhoff's disease.

The β-D-2-acetamido-2-deoxy-galactosidase and -glucosidase activities of human epidermoid carcinoma KB cells have been measured.[54] Normal and malignant leucocytes were found to contain β-D-2-acetamido-2-deoxy-glucosidase activity, and the levels of activity were compared.[55]

The purification and properties of a 'B' form of β-D-2-acetamido-2-deoxy-hexosidase from monkey brain have been reported.[56]

β-D-2-Acetamido-2-deoxyhexosidases have been purified from bovine brain tissue by electrophoresis, ion-exchange chromatography, and gel filtration.[57] Two of the four fractions obtained exhibited both β-D-2-acetamido-2-deoxy-galactosidase and -glucosidase activities, whereas the others each contained only one of these activities.

Oligosaccharides derived from hyaluronic acid and chondroitin 4-sulphate have been used as substrates to evaluate the glycoside hydrolase and glycosyltransferase activities of the β-D-2-acetamido-2-deoxyglucosidases A and B from bovine spleen.[58] The glycoside hydrolase activity has a pH optimum of 4.5 and the glycosyltransferase has a pH optimum of 6.5. The B form exhibits equal hydrolase and transferase activities at pH 6.5, whereas the A form exhibits mainly hydrolase activity at this pH.

Inhibition by four isomeric 2-acetamido-2,3-dideoxy-D-hex-2-enonolactones (*viz.* 2-acetamido-2,3-dideoxy-D-*erythro*- and -D-*threo*-hex-2-enono-1,4-lactones and the corresponding 1,5-lactones) of the β-D-2-acetamido-2-deoxyglucosidase from bovine epididymus has been examined.[59] The D-*erythro*-lactones were shown to be weak competitive inhibitors, whereas the D-*threo*-lactones were inactive. The activity of 2-acetamido-2.3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone against this enzyme from a number of animal and plant sources was also tested, although 2-acetamido-1,5-anhydro-2-deoxy-D-*arabino*- and -L-*lyxo*-hex-1-enitol were much more effective inhibitors.

β-D-2-Acetamido-2-deoxyhexosidase from boar epididymis has been purified by affinity chromatography on 4-aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside coupled to an agarose matrix.[60] Although the enzyme

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preparation was heterogeneous, it did not contain other glycoside hydrolases. The interactions with glycosides and oligosaccharides of 2-acetamido-2-deoxy-β-D-glucopyranose have been used to study the absorption properties of the active site of the β-D-2-acetamido-2-deoxy-β-D-glucosidase from boar epididymus.[61]

The β-D-2-acetamido-2-deoxyglucosidase activity in embryonic fibroblasts of two hamster lines has been studied *in situ* in monolayers.[62]

The levels of non-specific α-D-2-acetamido-2-deoxygalactosidases in rat brains and kidneys have been compared with those of an endogenous *endo*-α-D-2-acetamido-2-deoxygalactosidase (*endo*-α-D-2-acetamido-2-deoxygalactanase) that is active towards a glycolipid (Forssman hapten).[63] Simultaneous adrenalectomy and thyroidectomy of rats decreased the rate of synthesis of β-D-2-acetamido-2-deoxygalactosidase in the small intestines.[64] The levels of β-D-2-acetamido-2-deoxyglucosidase activity in the kidneys and sera of streptozotocin diabetic rats have been measured, and the effects of insulin and glucagon on these levels have been investigated.[65]

The activity of β-D-2-acetamido-2-deoxyglucosidase in osteosarcoma homogenates of afflicted rats was found to be higher than the levels in the surrounding tissue and in the serum.[66] The levels of β-D-2-acetamido-2-deoxy-galactosidase and -glucosidase activities in Kupffer cells, hepatocytes, and peritoneal macrophages of rats have been compared.[67]

The effects of methyl D-glucopyranoside carbonates on the β-D-2-acetamido-2-deoxyglucosidase from hen egg-white have been studied.[68]

A fraction that cleaves 4-nitrophenyl 2-benzamido-2-deoxy-β-D-glucopyranoside has been isolated from the liver of the pecten *Mizuhopecten yessoensis*.[69] The enzyme, which is also active towards conventional substrates, contains both β-D-2-acetamido-2-deoxy-glucosidase and -galactosidase activities. The kinetic properties, *etc.*, of the partially purified enzyme were determined.

A β-D-2-acetamido-2-deoxyhexosidase possessing high activity at *ca.* pH 7 has been extracted from a Japan-sea animal, *Ophiura sarsi*.[70] After purification by gel filtration and ion-exchange chromatography, the specificity of the enzyme towards several D-glucosaminides modified at C-2 was examined. An enzyme from *Chaetopterus variopedatus* that hydrolyses 4-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside and -glucopyranoside and 4-nitrophenyl 2-deoxy-2-glycylamido-β-D-glucopyranoside has been reported.[71] Various properties of the β-D-2-acetamido-2-deoxyglucosidase found in the digestive juices of the giant snail *Achatina batteata* have been determined.[72]

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The properties of the β-D-2-acetamido-2-deoxyglucosidases in the blood and moulting fluid of the silkworm *Bombyx mori* have been examined.[73] The enzyme in the moulting fluid is active towards phenyl 2-acetamido-2-deoxy-β-D-gluco- and -galacto-pyranosides, whereas the enzyme in the blood is active only towards the D-glucoside. The enzymes have different molecular weights, pH optima, and K_m values, but they are both inhibited by mercuric chloride. However, the activity of the enzyme in the blood did not recover from inhibition with H_4edta and reagents active towards thiol groups.

The hydrolysis of 4-nitrophenyl 2-acylamido-2-deoxy-β-D-glucopyranosides showed that the β-D-2-acetamido-2-deoxyhexosidase from *Hohenbuehelia serotina* B has a broader specificity than that from other sources.[74] A β-D-2-acetamido-2-deoxyhexosidase that also hydrolyses 4-nitrophenyl 2-benzamido-2-deoxy-β-D-glucopyranoside has been extracted from the fruiting bodies of *H. serotina*.[75] The rate of hydrolysis was not significantly affected when the *para* or *meta* position of the aromatic ring of the aglycone was substituted, but it was reduced either appreciably or to zero on substitution of both *meta* positions or the *ortho* position, respectively.

A β-D-2-acetamido-2-deoxyglucosidase from *E. coli* has been purified to near homogeneity by electrophoresis on polyacrylamide gel in the presence of sodium dodecyl sulphate and urea.[76] Studies of the substrate specificity of the purified enzyme (pH optimum 7.7, K_m (for 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside 0.43 mmol l^{-1}) confirmed that it has an *exo*-action. The molecular weight (determined by gel filtration and by gel electrophoresis) of the enzyme in a dissociating medium is 2.6×10^4, showing that it does not contain subunits.

Three β-D-2-acetamido-2-deoxyhexosidases have been isolated from culture filtrates of a *Streptococcus* species by affinity chromatography on agarose derivatized with oxamic acid or 1-thio-β-D-galactopyranose.[77] The enzymes (pH optimum 5.0—5.5) hydrolysed synthetic substrates and glycopeptides, but not glycolipids.

A β-D-2-acetamido-2-deoxyhexosidase (mol. wt. 1.5×10^4, pH optimum 6.2) has been isolated from extracts of *Trichomonas foetus* by affinity chromatography on a matrix of 2-acetamido-*N*-(6-aminohexanoyl)-2-deoxy-β-D-glucopyranosylamine bound to macroporous agarose.[78] The enzyme hydrolysed 4-nitrophenyl 2-acetamido-2-deoxy-β-D-gluco- and -galacto-pyranosides, and the same active site appears to be responsible for both activities. The hydroxy-group at C-4 of the sugars plays no part in binding to the enzyme, but it does determine the rate of hydrolysis.

α-D- and -L-Arabino(furano)sidases

Competitive substrate inhibition of an enzyme isolated from human liver by ion-exchange chromatography showed that it hydrolysed α-L-arabinosides, β-D-galactosides, β-D-glucosides, β-D-fucosides, and β-D-xylosides.[79] Another

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α-L-arabinosidase activity, which was eluted with the principal β-D-galactosidase, exhibits a different thermal stability.

An α-1,3-D-arabinofuranosidase that degrades cell-wall material is synthesized by the potato dry-rot pathogen *Fusarium caeruleum*.[80]

β-D-Fructofuranosidases

The β-D-fructofuranosidase (see also sucrose α-D-glucohydrolases) released from the vesicles of human intestinal brush-border membranes by various enzymes, particularly pancreatic proteases, has been studied.[81] The results were discussed in terms of the location and chemical binding of the β-D-fructofuranosidase.

Although an 'alkaline' β-D-fructofuranosidase in carrot (*Daucus carota*) roots did not hydrolyse raffinose, an 'acid' β-D-fructofuranosidase in the same tissue did.[82] The activities of both enzymes were inhibited, but to different extents, by D-fructose. Inhibition of the 'acid' enzyme by D-fructose does not appear to have any physiological significance, whereas hexoses regulate the activity of the 'alkaline' enzyme. The involvement of 'alkaline' β-D-fructofuranosidase in regulating the sugar content of the roots was discussed.

Soluble and cell-wall fractions from radish (*Raphanus sativus*) seedlings exhibited β-D-fructofuranosidase activity.[83] Transfer of the enzyme from the cytosol to the cell wall in roots and hypocotyls is induced by light, which also increases the β-D-fructofuranosidase activity in hypocotyls. Cycloheximide prevented an increase in the enzymic activity, but did not affect the transfer of the enzyme to the cell wall.

A modified procedure for studying the secretion of β-D-fructofuranosidase in yeast sphaeroplasts, and the subcellular distribution of the enzyme have been reported.[84] The levels of β-D-fructofuranosidase in yeasts are regulated by binding the secreted enzyme to the cell surface.[85] The synthesis of β-D-fructofuranosidase by the cells and the protoplasts of the yeast *Pichia polymorpha* was affected by the presence of 2-deoxy-D-*arabino*-hexose in the culture medium.[86]

Immobilized forms of β-D-fructofuranosidase have been prepared by entrapment of the enzyme in poly(*N*-vinylpyrrolidone) gels[87] and in vanacryl and covanacryl (polyaldehyde) supports.[88]

α-D-, β-D-, and α-L-Fucosidases

α-L-Fucosidase contaminating preparations of glycoside hydrolases can be removed by affinity chromatography on immobilized *N*-(6-aminohexanoyl)-L-fucopyranosylamine.[89]

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Purification of an α-L-fucosidase from human liver also revealed the presence of an *endo*-β-D-2-acetamido-2-deoxyglucosidase activity.[90] Human-liver α-L-fucosidase has been purified to homogeneity, in 66% yield, by a two-step procedure involving affinity chromatography on agarose derivatized with *N*-(6-aminohexanoyl)-L-fucopyranosylamine.[91] Polyacrylamide gel electrophoresis of the purified enzyme, which contained traces of other glycoside hydrolases, demonstrated the presence of six isoenzymes. The enzyme [mol. wt. 1.75×10^5 (by gel filtration) and 2.30×10^5 (by sedimentation)] gave a single subunit (mol. wt. 5.01×10^4) on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate and has pH optima at 4.6 and 6.5.

An enzyme isolated from human liver by ion-exchange chromatography is able to hydrolyse α-L-arabinopyranosides, β-D-galactopyranosides, β-D-glucopyranosides, and β-D-xylopyranosides, as well as β-D-fucopyranosides.[79] The livers from patients with Gaucher's disease were shown to be deficient in β-D-fucosidase and β-D-glucosidase activities.

The kinetic properties and substrate specificity of an α-L-fucosidase from human placenta have been determined.[92] Ion-exchange chromatography separated the enzyme into two forms, which differ in molecular weight and thermal stability, and which are interconverted during storage and isoelectric focusing. Low levels of α-L-fucosidase activities have been found in normal human sera, and four main forms of α-L-fucosidase activity have been separated from normal sera by isoelectric focusing.[93] All the forms had less acid p*I* values after treatment with neuraminidase.[94] In cases of fucosidosis, only a single 'acid' peak, which is less affected by treatment with neuraminidase, was observed. The structure of the residual α-L-fucosidase found in patients with fucosidosis appears to be altered in such a way that the ability to form thermostable, high-molecular-weight aggregates is impaired.

The α-L-fucosidase activity in cultures of human-skin fibroblasts has been investigated.[51] Ion-exchange chromatography and gel filtration have revealed the presence of different molecular forms of α-L-fucosidase in cultured fibroblasts of human amniotic fluid.[52]

The molecular weights (2.0×10^5 and 1.4×10^5) of two forms of α-L-fucosidase isolated from porcine kidney have been established by gel filtration.[95] Only the former isoenzyme was able to bind to Sephadex by non-specific adsorption. Isoelectric focusing indicated that both forms of the enzyme are heterogeneous.

Affinity chromatography on a matrix containing residues of 2-amino-2-deoxy-L-fucose has been used to purify an α-L-fucosidase from rat epididymus, giving a preparation that is free from other glycoside hydrolases.[96] The enzyme preparation was used to investigate the structures of L-fucose-containing poly-

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saccharides. The levels of α-L-fucosidase and 'β-D-fucosidase' activities in Kupffer cells, hepatocytes, and peritoneal macrophages of rats have been compared.[67]

Both α-L- and β-D-fucosidase activities have been detected in the digestive juices of the giant snail *Achatina balteata*; the effects of temperature, pH, and trypsin on these activities were investigated.[72]

Measurements of the activities of glycoside hydrolases in *Bacillus* species have shown that the relative activity of α-L-fucosidase differs from one species to another.[97] The α-D-galactosidases in six strains of *Streptomyces* also displayed α-D-fucosidase activity, even after extensive purification, indicating that a single species is responsible for both activities.[98]

D-Galactosidases and D-Galactolipid-oriented Galactosidases

The mechanisms of hydrolysis by α- and β-D-galactosidases,[20] and the antibody-mediated activation of β-D-galactosidase mutants and complementing fragments [99] have been discussed.

Chemical and clinical diagnoses of Fabry's disease, which involves a deficiency of α-D-galactosidase, have been reviewed.[39] Variations have been found in assays of α-D-galactosidase activity using methylumbelliferyl α-D-galactopyranoside as a substrate; such variations can be misleading in the laboratory diagnosis of Fabry's disease.[100]

There is evidence to show that three distinct mammalian β-D-galactosidases are responsible for the hydrolysis of D-galactosylceramide, lactosylceramide, and G_{M_1}-ganglioside.[101] Comparisons of these activities in murine and human cells indicated that the β-D-galactosidases in humans can be detected in somatic cell hybrids by specifically inhibiting the murine activity. The low level of α-D-galactosidase activity in mammalian intestinal mucosa may be disadvantageous in the digestion of oligosaccharides present in legumes.[102]

Electrophoretic studies of the β-D-galactosidase activity in a patient with G_{M_1}-gangliosidosis revealed the presence of a variant form of the enzyme.[103] The level of lactosylceramide β-D-galactosidase was found to be extremely low in the tissues and in cultures of the fibroblasts from patients with Krabbe's disease; β-D-galactosidases active towards D-galactosylcerebroside, psychosine, and D-galactosyldiglyceride are also low.[104] These small enzymic defects are considered to explain chemical findings for the syndrome.

Because of conflicting reports, the levels of β-D-galactosylglucosylceramidase activities in the brains and livers of normal subjects and of patients with globoid cell leukodystrophy or G_{M_1}-gangliosidosis have been investigated in detail.[105] The level of activity measured was found to be affected by various components in the assay systems. The findings strongly suggest the presence of two genetically

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[105] H. Tanaka and K. Suzuki, *J. Biol. Chem.*, 1975, **250**, 2324.

distinct enzymes (lactosylceramidases I and II) that cleave lactosylceramide in these tissues. Lactosylceramidase I may be identical with D-galactosylceramide β-D-galactosidase, while lactosylceramidase II is closely related to the non-specific 4-methylumbelliferyl β-D-galactosidase. It is also clear that specific assays for lactosylceramide β-D-galactosidase must be used in the diagnosis of globoid cell leukodystrophy. The kinetic properties of the β-D-galactosidase from human-brain tissues have been studied.[106] The effect of bile salts on the hydrolysis of lactosylceramide by human β-D-galactosidases *in vitro* has been studied using liver and brain tissues and cultured skin fibroblasts.[107] Evidence that two distinct enzymes catalyse the hydrolysis of lactosylceramide was found when pure taurocholate, which stimulates lactosylceramidase I, was replaced with crude taurocholate or pure glycodeoxycholate, taurodeoxycholate, or taurochenodeoxycholate, which stimulate lactosylceramidase II. The use of these bile salts in the appropriate reaction showed that patients with Krabbe's disease exhibit almost normal lactosylceramide β-D-galactosidase activity, whereas patients with G_{M_1}-gangliosidosis exhibit little, if any, such activity. The implication of these findings to assays of β-D-galactosidases was discussed.

The levels of lysosomal β-D-galactosidase in subcellular fractions of eye tissues during treatment with corticosteroids have been measured.[108]

The release of β-D-galactosidase from the vesicles of human intestinal brush-border membranes by various enzymes, particularly pancreatic proteases, has been studied.[81] α-D-Galactosidases A and B have been extensively purified from human liver by classical procedures.[109] In contrast to previous reports, it was shown that the A form is not converted into the B form on treatment with neuraminidase, although an apparent transformation of the A form into the B form was revealed by Cellogel electrophoresis.

An enzyme isolated from human liver has been shown to hydrolyse α-L-arabinopyranosides, β-D-glucopyranosides, β-D-fucopyranosides, β-D-xylopyranosides, and β-D-galactopyranosides.[79]

Fractions obtained from homogenates of normal human liver by gel filtration and affinity chromatography on immobilized concanavalin A have been examined for β-D-galactosidase activity.[110] An 'acid' form of the enzyme has a high molecular weight and is converted into a form of lower molecular weight by treatment with sodium dodecyl sulphate. Electrophoresis and isoelectric focusing of the enzyme preparations in polyacrylamide gels revealed other minor components possessing enzymic activity. The enzymes are glycoproteins.

N-Bromoacetyl-β-D-galactopyranosylamine irreversibly inhibited the 'acid' and 'neutral' β-D-galactosidases present in human liver.[111] Inactivation of the 'acid' β-D-galactosidase appeared to involve a group of pK_a 4.5, whereas inhibition of the 'neutral' enzyme occurred above pH 8. The presence of substrates protected both enzymes against inhibition, indicating that the inhibitor reacts with the active sites of the enzymes.

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Fluorometric determination of the α-D-galactosidase activity in tears has been used to detect hemizygotes with Fabry's disease and heterozygous carriers.[112] Two components of the enzyme were distinguished by their thermal stabilities and behaviours on ion-exchange chromatography.

The α-D-galactosidase activity in cultures of human-skin fibroblasts has been studied.[51]

Ion-exchange chromatography and gel filtration have shown that multiple forms of α- and β-D-galactosidases are present in cultured fibroblasts of human amniotic fluid.[52] The intracellular exchange of β-D-galactosidase has been studied in confluent cultures of normal and enzyme-deficient human fibroblasts.[53]

The genetic heterogeneity of G_{M_1}-gangliosidosis has been studied by measuring the β-D-galactosidase activities in hybrid human cells.[113]

The α- and β-D-galactosidase activities in human epidermoid carcinoma KB cells have been measured.[54] Precipitation of the β-D-galactosidase in synchronized KB cells with the homologous antibody showed that changes in the activity of the enzyme during the cell's cycle result from fluctuations in the concentration of the enzyme. The levels of D-galactosyl-D-galactosyl-D-glucosylceramidase (ceramide trihexosidase) were also investigated at various stages of the cell's cycle, and two forms of the enzyme (pH optima 4.0 and 7.5) were identified.

The effect of 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone on the activity of the β-D-galactosidase from bovine epididymis has been investigated.[59]

Saline extracts of powdered sheep pancreas contained α- and β-D-galactosidases that hydrolyse D-galactosyl- and D-galactosyl-D-glucosyl-diglycerides.[114] The involvement of the enzymes in the system that completely degrades diglycerides was investigated.

One of the β-D-galactosidases from porcine small intestines that acts on glycoproteins was separated from other β-D-galactosidases ('lactase', *etc.*) by affinity chromatography on agarose-immobilized 4-chloromercuribenzoate.[115] The purified enzyme was unstable and hydrolysed the D-galactosidic linkages in the carbohydrate chains of human α_1-acid glycoprotein, ceruloplasmin, and desialylized glycoproteins and glycopeptides. The intracellular distributions of three β-D-galactosidases in mucosal cells from porcine small intestines have been studied: 'hetero' β-D-galactosidase was found in the cytosol, 'lactase' in the brush borders, and an 'acid' β-D-galactosidase in the lysosomes.[116]

A 'neutral' α-D-galactosidase from porcine serum has been purified by repeated ion-exchange chromatography and gel filtration.[117] The purified enzyme ($S_{20,w}$ 10.7 S, p*I* 4.0, mol. wt. 2.7×10^5 in the presence of sodium dodecyl sulphate) was most active against maltose and soluble starch in the pH range 6—7 and also possessed glucoamylase activity.

[112] D. L. Johnson, M. A. Delmonte, E. Cotlier, and R. J. Desnick, *Clinica Chim. Acta*, 1975, **63**, 81.
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The levels of α- and β-D-galactosidases in the livers of male and female mice of various ages have been compared.[118] No differences in the enzymic activities were found in young mice of either sex, but appreciable differences were found in older mice. The activities of β-D-galactosidases that hydrolyse cerebroside (*i.e.* D-galactosylceramide) and 4-nitrophenyl β-D-galactopyranoside are about one-third higher in the kidneys of male mice, but there is no difference in the α-D-galactosidase which is active against 4-nitrophenyl α-D-galactopyranoside. The variations of other D-galactose-metabolizing enzymes with sex were discussed.

β-D-Galactosidase from the livers of mice has been purified until it migrated as a single band on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate.[119] The molecular weight of the denatured, reduced enzyme is 6.3×10^4, and the parent enzyme appears to be a tetramer (mol. wt. 2.4×10^5) that reversibly dissociates to a dimer (mol. wt. 1.13×10^5) at an alkaline pH. Charged isomers were separated by electrophoresis and ion-exchange chromatography, and the electrophoretic mobilities were appreciably reduced after treatment with neuraminidase. Antisera to the enzyme cross-reacted with β-D-galactosidases from rats and hamsters, but not with those from humans, pigs, and cattle.

The relative activities of rat-brain β-D-galactosidase towards D-galactosylglycolipids and 4-methylumbelliferyl β-D-galactopyranoside did not change during purification of the enzyme by fractional precipitation, gel filtration, and ion-exchange chromatography.[120] Inhibition studies with monosaccharides, sphingolipids, and oligosaccharides derived from glycosphingolipids suggested that the enzyme, which cleaves lactosylceramide, differs from the enzyme(s) that is active towards other D-galactosylglycosphingolipids. Oligosaccharides were less effective inhibitors of the enzyme than intact glycosphingolipids.

A lower rate of synthesis of 'acid' β-D-galactosidase has been found in the small intestines of thyroidectomized and adrenalectomized rats.[64] The levels of α- and β-D-galactosidase activities in Kupffer cells, hepatocytes, and peritoneal macrophages of rats have been compared.[67]

Both α- and β-D-galactosidase activities have been detected in the digestive juices of the giant snail *Achatina balteata*, and the effects of temperature, pH, and trypsin on the enzymes were studied.[72]

Both α- and β-D-galactosidases have been detected in the mid-gut of several phytophagous lungs, including *Adelphocoris suturalis*, *Orthocephalus funestus*, and *Lygus saundersi*, with different feeding habits.[121] The salivary gland of *Coreus marginatus* contains α- and β-D-galactosidases, that of *Palomena angulosa* contains α-D-galactosidase, and that of *O. funestus* contains β-D-galactosidase.

The catalytic properties of the β-D-galactosidases from sweet almonds (*Amygdalus communis*)[122] and ripening apples[123] have been examined.

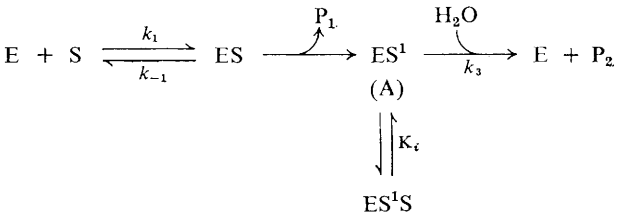
D-Xylose is a good competitive inhibitor of the α-D-galactosidases from *Arachis hypogaea*, *Cicer arietinum*, *Phaseolus mungo*, and *P. radiatus*.[124] The

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[124] T. N. Sharma and C. B. Sharma, *Phytochemistry*, 1976, **15**, 643.

apparent inhibition constant (K_i 0.4 × 10^{-2} mol l^{-1}) for the interaction and the number of inhibitor molecules (0.8) bound by each molecule of the enzyme from chick pea (*C. arietinum*) were determined using 4-nitrophenyl α-D-galactopyranoside as the substrate.

Depletion of the endogenous raffinose oligosaccharides and galactomannan in germinating seeds of carob, guar, lucerne, and soybean is accompanied by a rapid increase, followed by a decrease, in α-D-galactosidase activity.[125] Two, three, and four α-D-galactosidase activities are present in lucerne and guar, carob, and soybean, respectively, but only one of them is primarily involved in the hydrolysis of galactomannans. The α-D-galactosidases (mol. wt. 3.5 × 10^4 for guar, carob, and soybean, and 2.3 × 10^4 for leucerne) can be separated from the β-D-mannanase activities and appear to be located in the cotyledon embryo.

A purified α-D-galactosidase from *Coffea canephora* beans has been separated into two forms (mol. wts. 2.80 × 10^4 and 3.65 × 10^4).[126] The form of higher molecular weight hydrolysed aryl and alkyl α-D-galactopyranosides, but was inhibited by an excess of the substrate. The effects of *para* substituents, added alcohols, and non-competitive inhibition by methyl α-D-galactopyranoside on the rate of hydrolysis of substituted-phenyl α-D-galactopyranosides were studied. A two-step mechanism (Scheme 1) involving the formation of an enzyme-



(A) enzyme–D-galactosyl complex; p = product

Scheme 1

D-galactosyl complex (ES) was proposed for the hydrolysis, which was affected by pH in a complex way.

A β-D-galactosidase (p*I* 8.0, mol. wt. 7.5 × 10^4) isolated from Jack-bean meal has been purified to homogeneity by disc gel electrophoresis.[127] Examination of the specificity of the enzyme showed that the rates of hydrolysis of 2-acetamido-2-deoxy-3, 4, and 6-*O*-β-D-galactopyranosyl-D-glucose increased in the order (1 → 3) < (1 → 4) < (1 → 6). This property of the enzyme can be used to distinguish between the D-galactosidic linkages in the glycolipids (1) and (2). The

β-D-Gal*p*-(1 → 3)-β-D-Gal*p*NAc-(1 → 4)-β-D-Gal*p*-(1 → 4)-D-Glc-(1 → 1)-ceramide
(1)

β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc-(1 → 1)-ceramide
(2)

[125] B. V. McCleary and N. K. Matheson, *Phytochemistry*, 1974, **13**, 1747.
[126] H. Carchon and C. K. de Bruyne, *Carbohydrate Res.*, 1975, **41**, 175.
[127] S.-C. Li, M. Y. Mazzotta, S.-F. Chien, and Y.-T. Li, *J. Biol. Chem.*, 1975, **250**, 6786.

results suggest that not only does the type and linkage of the penultimate sugar residue affect the rate of hydrolysis, but also those of distal sugars. The pH optimum (2.8—4.0) of the enzyme depended on the buffer system and substrate used, and the K_m value also varied from one substrate to another. The enzyme is not inhibited by 4-aminophenyl 1-thio-β-D-galactopyranoside, unlike the β-D-galactosidase of *E. coli*.

The purification and properties of a β-D-galactosidase from *Aspergillus oryzae* have been reported.[128] Two soil organisms, a *Bacillus* species and a *Micrococcus* species, synthesize extracellular or only intracellular α-D-galactosidase, respectively.[129] The extracellular enzyme is constitutive, whereas the intracellular enzyme could be induced with D-galactose, melibiose, and raffinose. α-D-Galactosidases from alkaliphilic strains of *Bacillus* and *Micrococcus* species have been partly purified by ion-exchange chromatography and gel filtration.[130] The enzymes (pH optima 6.5—7.5) are stable over a relatively wide range of pH, but are thermolabile. Such metal ions as Ag^+ and Hg^{2+} completely abolished the enzymic activity, which was also inhibited by sulphydryl reagents. Among substrates tested with the enzymes, the rate of hydrolysis was 2-nitrophenyl α-D-galactopyranoside > melibiose > raffinose.

An intracellular, neutral α-D-galactosidase has been produced from *Bacillus stearothermophilus* in pilot-plant quantities.[131] The chromatographic procedures used in purifying the enzyme were described.

An α-D-galactosidase from a strain of *E. coli*, which was adapted to synthesize the enzyme, was stable in concentrated, but not in dilute, solution.[132] The enzyme (pH optimum 6.8, temperature optimum 37 °C, K_m for 4-nitrophenyl α-D-galactopyranoside, melibiose, and raffinose 1.07×10^{-4}, 2.33×10^{-3}, and 3.65×10^{-2} mol l^{-1}, respectively) also possesses weak glycosyltransferase activity.

1-Thio-β-D-galactopyranosides with either short aliphatic chains or short hydroxy-substituted chains on the sulphur atom are able to induce the synthesis of β-D-galactosidase in *E. coli*; cyanoalkyl, carbamoyl, methoxyalkyl, and epoxyalkyl 1-thio-β-D-galactopyranosides are also effective, whereas the sodium salts of thioalkyl 1-thio-β-D-galactopyranosides are able both to induce and to inhibit synthesis of the enzyme.[133] The mechanism of transport of β-D-galactosidase in membrane vesicles of *E. coli* has been discussed.[134]

The β-D-galactosidase in homogenates of *E. coli* has been purified by affinity chromatography on an iron oxide adsorbent containing attached residues of sebacic acid.[135] Related studies on the purification and specificity of *E. coli* β-D-galactosidase have been reported.[136]

A strain of *E. coli* with a deletion of the *lacZ* gene synthesized a new *ebg* β-D-galactosidase activity, which was shown to be a discrete protein immuno-

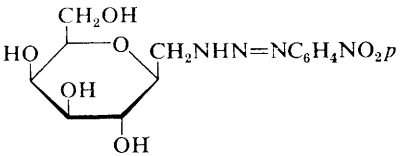
[128] Y. Tanaka, A. Kagamiishi, A. Kiuchi, and T. Horiuchi, *J. Biochem.* (*Japan*), 1975, **77**, 241.
[129] T. Akiba and K. Horikoshi, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 1845.
[130] T. Akiba and K. Horikoshi, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 1851.
[131] J. Delente, J. H. Johnson, M. J. Kuo, R. J. O'Connor, and L. E. Weeks, *Biotechnol. and Bioeng.*, 1974, **16**, 1227.
[132] S. Kawamura, T. Kasai, and T. Tanusi, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 641.
[133] V. Pačes, J. Frgala, and J. Šatava, *Coll. Czech. Chem. Comm.*, 1973, **38**, 2983.
[134] S. Schuldinger, G. Rudwick, R. Weil, and H. R. Kaback, *Trends in Biochemical Sciences*, 1976, **1**, 41.
[135] P. Dunnill and M. D. Lilly, *Biotechnol. and Bioeng.*, 1974, **16**, 987.
[136] S. Arakawa, T. Chiba, and S. Tejima, *Seikagaku*, 1974, **46**, 795.

logically unrelated to the *lacZ* β-D-galactosidase.[137] Kinetic analyses showed that the *ebg* β-D-galactosidase has a particularly high affinity for 2-amino-2-deoxy-D-galactose and D-galactono-1,5-lactone, binds D-galactose more tightly than lactose, and has a preference for monosaccharides rather than β-D-galactosides. It was concluded that the *ebg* β-D-galactosidase arises by modification of a gene involved in the metabolism of a monosaccharide, possibly a 2-amino-2-deoxy-sugar.

Examination of the hydrolase and D-galactosyltransferase activities of *E. coli* β-D-galactosidase with lactose as a substrate revealed that the rate of formation of D-galactose equalled that of D-glucose at low concentrations of lactose.[138] However, the relative rate of formation of D-galactose dropped appreciably at high concentrations of lactose and a number of tri- and tetra-saccharides were produced. The influence of the conditions, added reagents, and the anomeric configuration of lactose on the rates of formation of the oligosaccharides was studied.

The formation of the enzyme–substrate complex by *E. coli* β-D-galactosidase has been compared with the blocking of the active site of the enzyme with 2,6-anhydro-1-diazo-1-deoxy-D-*glycero*-L-*manno*-heptitol.[139]

A carbonium-ion precursor (3) deactivated Na^+- and Mg^{2+}-saturated *E. coli* β-D-galactosidase, although the enzyme could be protected against this irreversible inhibition by the competitive inhibitor methyl 1-thio-β-D-galacto-pyranoside.[140] The removal of Mg^{2+} ions increased the rate of deactivation of the enzyme by (3) and weakened the binding, and it was shown that inhibition



(3)

involves attachment of the β-D-galactopyranosylmethylene group derived from (3) to the protein.

Antibodies raised against certain mutant forms of *E. coli* β-D-galactosidase inactivated the normal enzyme, which was not inhibited by the homologous antibody.[141] The extent of inactivation was temperature-dependent and, in contrast to antibodies to the normal β-D-galactosidase, the inactivating antibodies altered the interaction of the enzyme with cations. A temperature-dependent equilibrium between two active forms of β-D-galactosidase has been suggested to explain the results, inactivation resulting from a conformational change induced by the binding of inactivating antibodies to one of the forms.

E. coli β-D-galactosidase has been used in determining [1-^{14}C]lactose[142] and human immunoglobulin IgG.[143]

[137] J. A. Arraj and J. H. Campbell, *J. Bacteriol.*, 1975, **124**, 849.
[138] R. E. Huber, G. Kurz, and K. Wallenfels, *Biochemistry*, 1976, **15**, 1994.
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[141] R. A. Roth and B. Rotman, *J. Biol. Chem.*, 1975, **250**, 7759.
[142] E. Davies, E. Bourke, and J. Costello, *Analyst*, 1975, **100**, 758.
[143] K. Kato, Y. Hamaguchi, H. Fukui, and E. Ishikawa, *J. Biochem.* (*Japan*), 1975, **78**, 423.

An *endo*-β-D-galactosidase of broad specificity, which is active towards glycoproteins and glycolipids, has been isolated from *E. coli*.[144]

Over two hundred leptospires belonging to known serogroups and water leptospires have been examined for β-D-galactosidase activity; since the enzyme was found in most species, it does not have any taxonomic value.[145]

The 'acid' β-D-galactosidase in culture filtrates of *Macrophomina phaseoli* has been purified to homogeneity by disc electrophoresis, ion-exchange chromatography, gel filtration, and isoelectric focusing (p*I* 3.6).[146] The enzyme (pH optimum 5.0, temperature optimum 60 °C, K_m for 2-nitrophenyl β-D-galactopyranoside and lactose 0.45 and 15 mmol l^{-1}, respectively) was stable at pH 4—8 and was completely inactivated by moderate concentrations of *N*-bromosuccinimide. The enzyme is a glycoprotein containing neutral sugars (12%), which are unchanged after isoelectric focusing.

Strains of *Neurospora sitophila* and *Rhizopus oligosporus* contain α-D-galactosidase activity.[147] Since these fungi are used in processing oilseeds, the action of the α-D-galactosidase on the carbohydrate components of oilseeds was examined. An α-D-galactosidase from *Pseudomonas atlantica* did not hydrolyse neoagaro-oligosaccharides.[148]

Two 'acid' β-D-galactosidases in extracts of *Sclerotium tuliparum* have been purified to homogeneity (disc electrophoresis) by ion-exchange chromatography, gel filtration, and isoelectric focusing (p*I* values 4.5 and 4.4).[149] Both enzymes (pH optimum 2.0, temperature optima 53 and 47 °C, K_m for 2-nitrophenyl β-D-galactopyranoside 1.4 and 1.2 mmol l^{-1}, and K_m for lactose 20 and 19 mmol l^{-1}, respectively) were stable at pH 3—6 and were completely inactivated by low concentrations of *N*-bromosuccinimide.

A β-D-galactosidase has been isolated from culture filtrates of a *Streptococcus* species by affinity chromatography on agarose derivatized with either oxamic acid or 1-thio-β-D-galactopyranose.[77] The substrate specificity of the enzyme is similar to that of the β-D-galactosidase from *E. coli*; the enzyme was inhibited by H_4edta and was activated by Mn^{2+}, Ca^{2+}, and Mg^{2+} ions.

Whereas the α-D-galactosidase from a *Streptomyces* species was active against macromolecular α-D-galactopyranosides and α-D-fucopyranosides, it hydrolysed neither β-L-arabinopyranosides nor macromolecular α-D-galactopyranosides (*e.g.* agarose and pectic acid).[150] The K_m and V values for α-D-fucopyranosides are much higher than those for the glycosides of D-galactopyranose, melibiose, and raffinose. Competitive inhibition was achieved with several sugars, a ratio of 10 : 1 being observed for the hydrolysis of α-D-galactopyranosides and α-D-fucopyranosides, respectively.

The properties of α-D-galactosidases in six *Streptomyces* strains have been examined.[98] α-D-Galactosidase activity was induced by D-galactose, but by

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[149] M. Sugiura, M. Suzuki, M. Sasaki, and T. Shimomura, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 788.
[150] K. Oishi and K. Aida, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 57.

neither D-fucose nor L-arabinose, and was always accompanied by an α-D-fucosidase activity that could not be removed by selective precipitation, ion-exchange chromatography, and gel filtration. Whereas both activities were inhibited by D-galactose and L-arabinose, only the α-D-fucosidase activity was inhibited by D-fucose.

β-D-Galactosidase in the form of a progesterone conjugate has been used in an immunoassay.[151]

α-D-Galactosidase has been immobilized, with retention of activity, by reaction with hydroxysuccinimide ester-activated nylon,[152] whereas immobilized forms of β-D-galactosidase have been prepared by covalent attachment to nylon tubing,[153] to a diazo derivative of glass,[154] and to hydroxysuccinimide ester-activated nylon,[152] by either glutaraldehyde-linking to, or carbodi-imide-assisted reaction with, an amino derivative of glass, by entrapment in cellulose fibres,[155] cellulose acetate,[156] and gels of poly(*N*-vinylpyrrolidone)[87] and poly(2-hydroxyethyl-methacrylate),[157] and by adsorption and cross-linking onto nylon.[156]

D-Glucosidases and D-Glucolipid-oriented Glucosidases

The mechanism of action of β-D-glucosidase,[20] inhibitors directed towards the active sites of α- and β-D-glucosidases,[22] and the solubilization of membrane-associated, tumour-specific antigens from rat tissues by β-D-glucosidase[158] have been discussed.

The lysosomal α-D-glucosidases present in mammalian tissues have been reviewed.[159] The levels of lysosomal β-D-glucosidase in subcellular fractions of eye tissues during treatment with corticosteroids have been measured.[108]

A series of studies on glycogen-storage diseases has revealed the existence of pathological variants of α-D-glucosidase, 'neutral' and 'acid' forms of the enzyme being detected by electrophoresis and immunochemical techniques.[160] The amylolytic activities of the enzymes are described as 'amylo-1,4-glucosidase' and 'amylo-1,6-glucosidase'. A new, sensitive assay for detecting individuals heterozygous and homozygous for Gaucher's disease uses 4-methylumbelliferyl β-D-glucopyranoside as a substrate under conditions optimal for determining β-D-glucocerebrosidase activity.[161] The use of this type of assay in the diagnosis of other diseases involving a deficiency of a glycoside hydrolase was discussed.

The release of α-D-glucosidase from the vesicles of human intestinal brush-border membranes by various enzymes, including pancreatic proteases, has been studied.[81]

[151] F. Dray, J.-M. Andrieu, and F. Renaud, *Biochim. Biophys. Acta*, 1975, **403**, 131.
[152] H. Faulstich, A. Schäfer, and M. Weckauf-Bloching, *F.E.B.S. Letters*, 1974, 48, 226.
[153] D. Narinesingh, T. T. Ngo, and K. J. Laidler, *Canad. J. Biochem.*, 1975, **53**, 1061.
[154] M. V. Wondolowski and J. H. Woychik, *Biotechnol. and Bioeng.*, 1974, **16**, 1633.
[155] D. M. Belen'kii, D. B. Tsukerman, and E. L. Rozenfel'd, *Biochemistry* (*U.S.S.R.*), 1975, **40**, 793.
[156] M. Charles, R. W. Coughlin, R. Tedman, and K. W. Beard, *Biotechnol. and Bioeng.*, 1974, **16**, 1549.
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[159] B. I. Brown, A. K. Murray, and D. H. Brown, in ref. 9, p. 223.
[160] J. C. Dreyfus, D. Proux, and Y. Alexandre, *Enzyme*, 1974, **18**, 60.
[161] S. P. Peters, R. E. Lee, and R. H. Glew, *Clinica Chim. Acta*, 1975, **60**, 391.

An 'acid' α-D-glucosidase in human liver has been purified by adsorption onto Sephadex, followed by desorption with methyl α-D-glucopyranoside, a competitive inhibitor.[155] The enzyme (p*I* 4.58) was shown to be homogeneous by ultracentrifugation and disc gel electrophoresis. Guanidinium hydrochloride, but not urea, dissociated the enzyme. The glucoamylase activity, but not the maltase activity, was inhibited by methyl α-D-glucopyranoside.

The β-D-glucosidase activities in fractions obtained from homogenates of normal human liver by gel filtration and selective adsorption onto, and elution from, immobilized concanavalin A have been measured.[110] The molecular forms of the enzyme were investigated by electrophoresis and isoelectric focusing in polyacrylamide gels. Inhibition of the 'neutral' β-D-glucosidase from human liver by *N*-bromoacetyl-β-D-galactopyranosylamine showed the same dependence on pH as that of the endogenous 'neutral' β-D-galactosidase, and both activities appear to reside in the same molecule.

An enzyme isolated from human liver by ion-exchange chromatography hydrolysed α-L-arabinopyranosides, β-D-galactopyranosides, β-D-fucopyranosides, β-D-xylopyranosides, and β-D-glucopyranosides.[79] Liver samples from patients with Gaucher's disease were shown to be deficient in β-D-glucosidase and β-D-fucosidase activities.

The α-D-glucosidase activity in cultures of human-skin fibroblasts has been measured,[51] and the intracellular exchange of 'acid' α-D-glucosidase in confluent cultures containing both normal and enzyme-deficient human fibroblasts has been investigated.[53]

Ion-exchange chromatography and gel filtration have revealed the presence of different molecular forms of α- and β-D-glucosidases in cultures of fibroblasts from human amniotic fluid; the patterns are similar to those in skin fibroblasts and liver tissues.[52]

The α- and β-D-glucosidase activities in human epidermoid carcinoma KB cells have been investigated.[54] The levels of D-glucosylceramidase (ceramide D-glucosidase) and ceramide trihexosidase were also investigated at various stages of the cell's cycle, and two forms of D-glucosylceramidase (pH optima 4.0 and 6.0) were detected.

Investigations of the substrate specificity of the 'neutral' α-D-glucosidase in porcine sera showed that the enzyme is particularly active towards malto-oligosaccharides, phenyl α-maltoside, and nigerose, whereas it is less active towards isomaltose and phenyl α-D-glucopyranoside; other isomalto-oligosaccharides are not attacked.[162] Amylopectin, β-limit dextrin, glycogen, and amylose are also substrates for the enzyme.

The isolation of a 'maltase–glucoamylase complex' from rabbit intestines has been described.[163]

Thyroidectomy and adrenalectomy of rats decreased the rate of synthesis of α-D-glucosidase.[64] The isolation and characterization of the β-D-glucosidase in the cytosol of rat-kidney cortex have been described,[164] and the levels of α- and β-D-glucosidase activities in Kupffer cells, hepatocytes, and peritoneal macrophages of rats have been compared.[67]

[162] S. Chiba, N. Hibi, and T. Shimomura, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1813.
[163] S. Sivakami and A. N. Radhakrishnan, *Indian J. Biochem. Biophys.*, 1973, **10**, 283.
[164] R. H. Glew, S. P. Peters, and A. R. Christopher, *Biochim. Biophys. Acta*, 1976, **422**, 179.

β-D-Glucosidases associated with the cellulase system of the octopus (*Octopus vulgaris*) are active against cellobiose and aryl β-D-glucopyranosides.[165] The effects of substrate concentration, pH, temperature, and added D-glucono-1,5-lactone and sodium and calcium chlorides on the enzymic activities were examined.

An α-D-glucosidase (mol. wt. 5.1—8.2 × 10^4, $S_{20,w}$ 4.0 S) isolated from the heads and abdomens of honey bees (*Apis mellifera*) was shown to be homogeneous by a number of criteria; it has a low substrate specificity and possesses D-glucosyltransferase activity.[166] The enzyme is a glycoprotein containing 2-amino-2-deoxy-D-glucose, -galactose, and -mannose (5.9, 8.2, and 100 μmol, respectively). Both α- and β-D-glucosidases have been found in the mid-gut of several phytophagous bugs, including *Lygus disponsi*, *Palomeno angulosa*, and *Eurydema rugosum*, with different feeding habits; the relations between the taxonomy or feeding habits of the bugs and the presence of carbohydrases in the alimentary system were discussed.[121]

The effects of methyl D-glucopyranoside carbonates on the activities of almond-emulsin β-D-glucosidase and yeast α-D-glucosidase have been examined.[68] Methyl 2- and 3-*O*-methoxycarbonyl-β-D-glucopyranosides caused only a small loss of activity of the β-D-glucosidase, whereas the corresponding α-analogues strongly inhibited the α-D-glucosidase. Loss of activity is caused by binding of the inhibitor at the active site, since the enzyme is protected by erythritol, a good competitive inhibitor of α-D-glucosidase. The effect of 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone on the activity of almond-emulsin β-D-glucosidase has been investigated.[59]

The β-D-glucosidase activities of several plants (*Arachis hypogaea*, *Cicer arietinum*, *Phaseolus mango*, and *P. radiatus*) were inhibited by D-xylose.[124]

Whereas a low level of α-D-glucosidase is found in ungerminated barley grain, *de novo* synthesis of the enzyme in grain with excised embryos can be induced by gibberellic acid, whereas it is suppressed by actinomycin D.[167] α-D-Glucosidases from barley and sorghum grains are insoluble in water, although the insoluble forms are active.[168] Although aqueous solutions of sodium chloride released the α-D-glucosidase from sorghum grain, it was not released to the same extent as with aqueous solutions of urea; the enzyme in barley grain was not solubilized by either solution. The stabilities and the modes of attachment to the insoluble grain matrix differ for these α-D-glucosidases.

Protein bodies and spherosomes in sorghum grain contain α-D-glucosidase activities and polysaccharide hydrolases.[169] The interaction of the α-D-glucosidase with α- and β-amylases was investigated.

The properties of β-D-glucosidases (cellobiases) used in the brewing industry have been investigated; all the enzymes exhibited maximum activity at 70 °C, and the stabilities of the enzymes to heat were compared with those of endogenous polysaccharide hydrolases.[170]

[165] K. Wakabayashi and W. Pigman, *Carbohydrate Res.*, 1974, **35**, 3.
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[167] D. G. Hardie, *Phytochemistry*, 1975, **14**, 1719.
[168] T. G. Watson and L. Novellie, *Phytochemistry*, 1974, **13**, 1037.
[169] C. A. Adams, T. G. Watson, and L. Novellie, *Phytochemistry*, 1975, **14**, 953.
[170] L. S. Salmanova and L. A. Zhdanova, *Priklad. Biokhim. i Mikrobiol.*, 1975, **11**, 43.

The effects of temperature, heat, pH, and trypsin on the β-D-glucosidase activity in the digestive juices of the giant snail *Achatina balteata* have been examined.[72]

A method for isolating β-D-glucosidase (pH optimum 6.4) from *Alcaligenes faecalis* has been described, and the kinetic properties of the enzyme were investigated.[171] The α-D-glucosidase activity in preparations of α-amylase from *Aspergillus aureus* has been measured using phenyl α-maltoside as a substrate.[172] The synthesis of an extracellular α-D-glucosidase by a thermophilic *Bacillus* species has been reported.[173]

A β-D-glucosidase that is active towards aryl β-D-glucopyranosides has been identified in the aquatic fungus *Lagenidium giganteum*, a parasite of mosquito larvae.[174]

An α-D-glucosidase in the mycelia of *Penicillium purpurogenum* has been isolated in an electrophoretically homogeneous form.[175] The enzyme (mol. wt. 1.2×10^5, p*I* 3.2, pH optimum 3.0—5.0, K_m for maltose 6.94×10^{-4} mol l^{-1}) hydrolysed maltose and also amylose, amylopectin, glycogen, and soluble starch to D-glucose only, and phenyl α-maltoside to phenyl α-D-glucopyranoside and D-glucose; it also exhibited D-glucosyltransferase activity.

The partial purification and characterization of an α-D-glucosidase from *Pseudomonas fluorescens* have been described.[176] The synthesis and role of β-D-glucosidase activity in a *Trichoderma* species in the saccharification of cellulose have been investigated.[177]

Active immobilized forms of β-D-glucosidase have been prepared by reaction of the enzyme with cellulose cyclic imidocarbonate,[171] glutaraldehyde-treated beads of polyacrylamide,[178] copolymers of acrylamide and 2-hydroxyethyl-methacrylate treated with cyanogen bromide, and copolymers of acrylamide and acrylic acid, and by entrapment of the enzyme in beads of polyacrylamide.

β-D-Glucuronidases

Although a reinterpretation of the course of hyperamylasemia during diabetic comas inferred a possible relationship between the levels of β-D-glucuronidase and amylase, the correlation actually obtained was poor.[179]

Incubation of normal human fibroblasts with chloroquine at physiological pH released β-D-glucuronidase activity into the medium, suggesting that chloroquine competes with the enzyme in binding to the cell membrane.[180]

Ion-exchange chromatography has shown that different molecular forms of β-D-glucuronidase are present in cultures of fibroblasts from human amniotic fluid; the pattern is similar to those in skin fibroblasts and liver tissues.[52]

[171] V. R. Srinivasan and M. W. Bumm, *Biotechnol. and Bioeng.*, 1974, **16**, 1413.
[172] S. Tanaka, H. Miyake, and H. Sekine, *J. Agric. Chem. Soc. Japan*, 1976, **50**, 409.
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[178] A. C. Johansson and K. Mosbach, *Biochim. Biophys. Acta*, 1973, **370**, 339.
[179] D. M. Goldberg, R. J. Spooner, and A. H. Knight, *Clinical Chem.*, 1974, **20**, 673.
[180] U. N. Wiesmann, S. DiDonato, and N. N. Herschkowitz, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 1338.

Purified bovine-liver β-D-glucuronidase (p*I* 5.1, mol. wt. 2.9×10^5, pH optimum 4.4) exhibited a broad pH-activity profile and contains 6.3% of carbohydrate consisting of D-galactose (8), D-glucose (7), D-mannose (45), 2-amino-2-deoxy-D-glucose (32), and 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (5 moles mol^{-1}).[181]

Gel electrophoresis showed that rabbit (*Oryctolegus cuniculus*) and, probably, rat (*Rattus norvegicus*) livers contain variants of β-D-glucuronidase corresponding to those in mouse (*Mus musculus*) tissues.[182] Microsomal and lysosomal fractions from rabbit liver differed only quantitatively in the β-D-glucuronidases present.

The lysosomal form of β-D-glucuronidase in mouse liver has been purified to homogeneity.[183] Electrophoresis on polyacrylamide gel showed that the enzyme is monomeric. However, microsomal forms of β-D-glucuronidase are spontaneously converted into the L form (mol. wt. $2.8—3.0 \times 10^5$), which is composed of four identical subunits (mol. wt. 7.5×10^4) and contains D-galactose (0.23%), D-glucose (0.44%), D-mannose (4.52%), and 2-amino-2-deoxy-D-glucose (2.10%), but neither L-fucose nor sialic acid.

The level of β-D-glucuronidase activity in proximal tubule cells of mouse kidneys increased rapidly following the administration of dihydrotestosterone, although the response depends on the particular strain of mouse used.[184] The rate of synthesis of β-D-glucuronidase was determined from the incorporation of [^{3}H]leucine into the antibody-purified enzyme. Synthesis of the enzyme is controlled by a structural gene residing on the same chromosome as an inducibility gene.

β-D-Glucuronidase (pH optimum 4.7, K_m for phenolphthaleinyl β-D-glucopyranosiduronic acid 1.18×10^4 mol l^{-1}) from the kidneys of mice treated with gonadotrophin has been purified by gel filtration, ion-exchange chromatography, and isoelectric focusing.[185] The preparation was shown to be homogeneous by electrophoresis and immunodiffusion, and was electrophoretically indistinguishable from the isoenzyme of the lysosomal form. The oligomeric parent enzyme (mol. wt. 3.0×10^5) contains subunits of molecular weight 7.4×10^4.

Thyroidectomy and adrenalectomy of rats produced a decrease in the rate of synthesis of β-D-glucuronidase activity.[64]

The isoenzymes of rat-liver microsomal β-D-glucuronidase showed different responses to such dissociating agents as urea, guanidinium hydrochloride, and sodium dodecyl sulphate.[186] Loss of catalytic activity in the denaturing media is accompanied by dissociation of the enzymes into subunits. Gel electrophoresis in the presence of sodium dodecyl sulphate revealed that the enzymes are tetramers consisting of different proportions of three types of glycoprotein subunit (mol. wts. 6.29×10^4, 6.02×10^4, and 5.87×10^4).

The rates of hydrolysis of 5-bromo-4-chloroindol-3-yl and 5-bromoindol-3-yl β-D-glucopyranosiduronic acids by rat-liver β-D-glucuronidase depended on the buffer, decreasing in the order acetate > citrate–phosphate > phosphate.[187]

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[182] R. T. Dean and M. Messer, *Comp. Biochem. Physiol.*, 1976, **54B**, 107.
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[184] R. T. Swank, K. Paigen, and R. E. Ganschow, *J. Mol. Biol.*, 1973, **81**, 225.
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[187] K. Yoshida, N. Iino, and I. Koga, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 1759.

The optimum pH of the enzyme is 4.75 in acetate buffer and 5.0 in citrate–phosphate and phosphate buffers.

The β-D-glucuronidase (a tetrameric glycoprotein) in the glands of female rats has been separated into several molecular forms by chromatography on hydroxyapatite.[188] Two of the three principal forms contain D-mannose (2.8%), 2-amino-2-deoxy-D-glucose (1.9%), L-fucose (0.2%), D-galactose (0.16%), and D-glucose (0.17%), whereas the other form is richer in L-fucose (0.6%), D-galactose (1.1%), and D-glucose (1.5%). G.l.c. was used to establish the presence of D-glucose, which does not appear to be a persistent contaminant. The glycopeptide linkages appear to involve 2-acetamido-(4-L-aspartoyl)-2-deoxy-β-D-glucopyranosylamine.

Large quantities of β-D-glucuronidase (p*I* 6.15) have been isolated from rat preputial gland.[189] Viscosimetry showed that the purified 12.5 S enzyme [mol. wt. 2.67×10^5 (by sedimentation-diffusion) and 2.83×10^5 (by sedimentation-equilibrium)] is a typical globular protein possessing little asymmetry. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate gave a single band (mol. wt. 7.2×10^4), indicating that the enzyme has a tetrameric structure. The c.d. spectrum showed that the enzyme contains *ca.* 14% of α-helix and more random-coil structure than β-structure.

The levels of the β-D-glucuronidase in homogenates of the osteosarcoma of afflicted rats were higher in the tumour than in the sera and surrounding tissues.[66] The β-D-glucuronidase activity in Kupffer cells of rats has been investigated.[67]

β-D-Glucuronidase activity has been detected in the digestive juices of the giant snail *Achatina balteata*, and the effects of temperature, pH, and trypsin on the enzyme were studied.[72] The β-D-glucuronidase activities in whole and in fractionated haemolymphs of the molluscs *Crassostrea virginica* and *Mercenaria mercenaria* have been measured, and possible functions of the enzymes in the sera of these species were discussed.[190] The effect of 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone on the activity of *Helix pomatia* β-D-glucuronidase has been investigated.[59]

β-D-2-Glycylamido-2-deoxyglucosidases

An enzyme (mol. wt. 1.25×10^5) that hydrolyses 4-nitrophenyl 2-deoxy-2-glycylamido-β-D-glucopyranoside has been obtained from an extract of *Chaetopterus variopedatus* by gel filtration and ion-exchange chromatography.[71] The K_m values obtained for 4-nitrophenyl 2-acetamido-2-deoxy-β-D-galacto- and -gluco-pyranosides and 4-nitrophenyl 2-deoxy-2-glycylamido-β-D-gluco-pyranoside are 0.5×10^{-3}, 0.13×10^{-3}, and 1.0×10^{-3} mmol l^{-1}, respectively, although high concentrations of 4-nitrophenyl 2-acetamido-2-deoxy-β-D-gluco-pyranoside inhibited the enzyme.

α-L-Iduronidases

Hurler's and Scheie's syndromes can be detected by assay of the levels of α-L-iduronidase in leucocytes.[191]

[188] D. R. P. Tulsiami, R. K. Keller, and O. Touster, *J. Biol. Chem.*, 1975, **250**, 4770.
[189] R. K. Keller and O. Touster, *J. Biol. Chem.*, 1975, **250**, 4765.
[190] T. C. Cheng and G. Rodrick, *Comp. Biochem. Physiol.*, 1975, **52B**, 443.
[191] K. O. Liem and G. J. M. Hodghwinkel, *Clinica Chim. Acta*, 1975, **60**, 259.

Not all preparations of α-L-iduronidase function as a corrective factor for Hurler's syndrome.[192] Corrective and non-corrective forms of the enzyme in urine have been separated by affinity chromatography on heparin immobilized on agarose; whereas they have similar catalytic properties, they differ in molecular weight (8.7×10^4, 6.7×10^4) and in binding to an immobilized castor-bean lectin. The corrective form was the only one efficiently taken up by Hurler's fibroblasts.

α-D-Mannosidases

The mechanism of action of α-D-mannosidase has been discussed.[20]

Oligosaccharides containing terminal, non-reducing α-(1 → 2)-, α-(1 → 3)-, and α-(1 → 6)-linked D-mannosyl residues, which were isolated from the urines of humans and cattle with mannosidosis, have been used to ascertain the substrate specificities of 'acid' α-D-mannosidases obtained from human and bovine livers.[193] Differences in the activities of A and B_2 forms of the enzyme were shown with all substrates. The C form (pH optimum *ca.* 7) did not hydrolyse any of the oligosaccharides at neutral pH, but it was active at a lower pH.

The 'acid' α-D-mannosidase in human liver has been purified by a relatively rapid procedure involving affinity chromatography on immobilized concanavalin A and immobilized *N*-(6-aminohexanoyl)-D-mannopyranosylamine.[89] The 'neutral' α-D-mannosidase did not bind to immobilized concanavalin A, so that the enzymes are separated early in the purification, and a contaminating α-L-fucosidase was later removed by affinity chromatography on immobilized *N*-(6-aminohexanoyl)-L-fucopyranosylamine. The p*I* of the purified 'acid' α-D-mannosidase was increased from 6.0 to 6.45 on treatment with neuraminidase. Although the p*I* and molecular weight (2.20×10^5) indicated that the purification procedure gave α-D-mannosidase A, ion-exchange chromatography suggested that the preparation contained mostly α-D-mannosidase B. This anomaly was discussed in terms of the multiple forms of human α-D-mannosidase. Antiserum raised against the purified B form of human-liver α-D-mannosidase precipitated both the A and B forms from solution, indicating that they are similar in structure.[194] 'Acid' α-D-mannosidases in human brain, kidneys, and leucocytes were also precipitated by the antiserum, but the 'neutral' α-D-mannosidases from human liver and plasma and the 'acid' α-D-mannosidase from cattle were not.

The isolation and properties of human-kidney α-D-mannosidase have been reported.[195] The levels of α-D-mannosidase activity in human body fluids, including sera and urine, have been monitored during ageing.[196]

The β-D-mannosidase activity in cultures of human-skin fibroblasts [51] and the α-D-mannosidase activities of human epidermoid carcinoma KB cells [54] have

[192] L. J. Shapiro, C. W. Hall, I. G. Leder, and E. F. Neufeld, *Arch. Biochem. Biophys.*, 1976, **172**, 156.
[193] B. Hultberg, A. Lundblad, P. K. Masson, and P. A. Öckerman, *Biochim. Biophys. Acta*, 1975, **410**, 156.
[194] N. Phillips, D. Robinson, and B. Winchester, *Biochem. J.*, 1975, **151**, 469.
[195] D. V. Marinkovic and J. N. Marinkovic, *Biochem. J.*, 1976, **155**, 217.
[196] J. M. Chéron, P. Rahimulla, and J. E. Courtois, *Compt. rend.*, 1975, **280**, *D*, 2393.

been investigated. Ion-exchange chromatography demonstrated that two molecular forms of α-D-mannosidase are present in cultures of fibroblasts from human amniotic fluid, and this pattern was compared with those in skin fibroblasts and liver tissues.[52]

The effects of 2-acetamido-2,3-dideoxy-D-*erythro*-hex-2-enono-1,4-lactone on the activities of α-D-mannosidases from bovine epididymis and almond emulsin have been investigated.[59]

The levels of α-D-mannosidase activities in Kupffer cells, hepatocytes, and peritoneal macrophages of rats have been compared.[67]

The effects of added nucleophiles, the concentration of water, and pH on the hydrolysis of substituted-phenyl α-D-mannopyranosides by leech (*Medicago sativa*) α-D-mannosidase have been investigated.[197] A two-step mechanism, involving the rate-limiting formation of a D-mannosyl-enzyme complex, was proposed. The activity of the enzyme is controlled by two dissociable groups (p*K ca.* 3 and 6), one of which functions as a proton-donor, while the other stabilizes the D-mannosyl–enzyme complex, either by forming ion-pairs or by covalent binding. The retention of configuration at C-1 of the D-mannopyranose ring during the enzyme-catalysed transmannosylation (from 4-nitrophenyl α-D-mannopyranoside) of methanol indicates that the reaction proceeds by a pathway involving the formation of a D-mannosyl–enzyme complex.[198]

The α-D-mannosidase (mol. wt. 2×10^5) from Jack-bean meal contains four subunits (mol. wts. 6.6×10^4 and 4.4×10^4).[199] The relative activities of α-D-mannosidases in *Bacillus* species vary from one species to another.[97] An active immobilized form of α-D-mannosidase supported on agarose has been prepared.[199]

Neuraminidases (Sialidases)

A new method for the assay of neuraminidase activity uses 5-acetamido-3,5-dideoxy-α-D-*glycero*-D-*galacto*-nonulosonyl-(2 → 3)-[^{3}H]lactitol as a substrate.[200] The unreacted substrate is removed by ion-exchange chromatography, whereafter the residual [^{3}H]lactitol is determined by scintillation counting.

The activities of membrane-bound neuraminidase in the brains of humans, pigs, cattle, rabbits, fish, and chickens towards exogenous and endogenous gangliosides and sialoglycoproteins have been compared.[201] Appreciable differences in the levels of activity and in the rates of hydrolysis of a particular substrate exist among the species. Guidelines for the accurate assay of membrane-bound neuraminidase in crude preparations were also suggested.

An assay using ^{3}H-labelled 5-acetamido-3,5-dideoxy-L-*arabino*-2-heptulosonic acid–fetuin as a substrate has shown that human-erythrocyte plasma membranes contain significant amounts of neuraminidase activity, which is active over a broad range of pH and which has a temperature optimum of 30—40 °C.[202]

[197] J. de Prijcker and C. K. de Bruyne, *Carbohydrate Res.*, 1975, **43**, 173.
[198] J. de Prijcker, C. K. de Bruyne, M. Claeyssens, and A. de Bruyne, *Carbohydrate Res.*, 1975, **43**, 380.
[199] V. Shepherd and R. Montgomery, *Biochim. Biophys. Acta*, 1976, **429**, 884.
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[201] G. Tettamanti, A. Preti, A. Lombardo, T. Suman, and V. Zambotti, *J. Neurochem.*, 1975, **25**, 451.
[202] H. B. Bosmann, *Vox Sanguinis*, 1974, **26**, 497.

No co-factors were necessary, and the enzymic activity was inhibited by Ca^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, and Hg^{2+} ions and 4-chloromercuribenzenesulphonic acid. The purified enzyme, like the neuraminidase from *Clostridium perfringens*, released terminal 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid residues from the external surface of intact human erythrocytes.

An extensive purification procedure, which included affinity chromatography on agarose derivatized with 4-nitrophenyloxamic acid, was necessary to isolate both forms of neuraminidase from porcine brain; the two forms were then separated by chromatography on a hydroxyapatite–cellulose gel.[203] The pH optima, maximum activities, and kinetic properties of the two forms differ and they are also activated by different cations.

Soluble and membrane-bound neuraminidase activities in regenerating rat liver have been investigated.[204]

A rapid procedure involving affinity chromatography on immobilized fetuin has been used to remove contaminating proteases from commercial preparations of neuraminidase.[205] The affinity-adsorbed enzyme was virtually free from protease activity, and the use of protease-free neuraminidase in studies of the metabolism of desialylized glycoproteins and of tumour cells was discussed.

The action of a neuraminidase (pH optimum 5.5—6.0, temperature optimum 38 °C) from a diphtheria *Bacillus* species on glycoproteins and glycopeptides has been examined.[206] Competitive inhibition was achieved with modified glycopeptides. The neuraminidase of *Clostridium perfringens* (*C. welchii*) has been purified by affinity chromatography on a 4-amino-oxanilic acid derivative of agarose, and the kinetics of the enzymic reactions with a neuraminyl-lactose and mixtures of gangliosides were determined.[207] The enzyme was reversibly transformed from a relatively inactive form into a highly active form in strong electrolytes, which shielded the active functional group from unfavourable interactions with polyanionic ganglioside micelles. This effect explains the low pH optima and skewed pH-profiles previously reported for the enzyme. Neuraminidase has been purified on a new affinity-chromatography matrix composed of α_1-acid glycoprotein immobilized on agarose, which did not display non-specific adsorption characteristic of other matrices.[208] The immobilized glycoprotein was used to isolate *C. perfringens* neuraminidase in an electrophoretically homogeneous form. Another report has described the purification and characterization of *C. perfringens* neuraminidase.[209]

The properties of neuraminidases from *C. perfringens* and *Diplococcus pneumoniae* have been compared,[210] and an attempt has been made to uncover

[203] B. Venerando, G. Tettamanti, B. Cestaro, and V. Zambotti, *Biochim. Biophys. Acta*, 1975, **403**, 461.
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[205] J. L. Winkelhake and G. L. Nicolson, *Analyt. Biochem.*, 1976, **71**, 281.
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[208] M. J. Geisow, *Biochem. J.*, 1975, **151**, 181.
[209] S. Nees, R. W. Veh, and R. Schauer, *Z. physiol. Chem.*, 1975, **356**, 1027.
[210] N. Houdret, A. Scharfman, G. Martin, and P. Roussel, *Ann. Microbiol.*, 1975, **126B**, 175.

the biological relation between the neuraminidase and the haemagglutinin in *C. perfringens*.[211]

The purification and characterization of siastatin, a sialidase inhibitor produced by a *Streptomyces* species, have been described.[212]

Vibrio comma neuraminidase was inactivated by Ca^{2+}-binding anions (*e.g.* citrate, H_4edta, oxalate, phosphate, and tartrate), whereas the neuraminidases from *Erysipelothrix insidiosa* and *Streptococcus viridans* were not.[213] Pyruvate and, to a lesser extent, citrate inactivated all the neuraminidases, but they did not interfere with the sites binding Ca^{2+} ions.

Vibrio cholerae neuraminidase was activated by divalent cations, and the effects of these ions were thoroughly investigated.[214] The effect of *V. cholerae* neuraminidase on the uptake of 5-hydroxytryptamine by human platelets has been studied.[215]

β-D-Xylosidases

The mechanism of action of β-D-xylosidase has been discussed.[20]

An enzyme isolated from human liver by ion-exchange chromatography hydrolysed β-D-xylopyranosides, β-D-glucopyranosides, β-D-galactopyranosides, β-D-fucopyranosides, and α-L-arabinopyranosides.[79]

The levels of β-D-xylosidase activity in Kupffer cells, hepatocytes, and peritoneal macrophages of rats have been compared.[67]

The effects of temperature, pH, and trypsin on the β-D-xylosidase activity in the digestive juices of the giant snail *Achatina balteata* have been examined.[72]

Measurements of the activities of the glycoside hydrolases in many *Bacillus* species have shown that the relative activity of β-D-xylosidase varies from one species to another.[97] The properties of a β-D-xylosidase from *Bacillus pumilus* have been investigated.[216]

β-D-Xylosidase activity was detected in cell-free extracts and culture fluids of *Cryptococcus albidus* growing on D-glucose as the only source of carbon.[217] Ion-exchange chromatography of the enzyme, which appears to be constitutive, showed that there are two forms in the cell-free extracts and one form in the culture fluid.

endo-D-2-Acetamido-2-deoxygalactanases

An enzyme that cleaved the terminal, non-reducing 2-acetamido-2-deoxy-α-D-galactopyranosyl residue from 'Forssman hapten' (4) has been detected in

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-(1 → 1)-ceramide

(4)

[211] J. I. Rood and R. G. Wilkinson, *J. Bacteriol.*, 1976, **126**, 831.
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[216] M. Claeyssens, E. Saman, H. Kersters-Hilderson, and C. K. de Bruyne, *Biochim. Biophys. Acta*, 1975, **405**, 475.
[217] V. Notario, T. G. Villa, and J. R. Villaneuva, *Canad. J. Microbiol.*, 1976, **22**, 312.

soluble fractions isolated from the brains and kidneys of adult rats.[63] The enzyme, which was identified as an *endo*-α-D-2-acetamido-2-deoxygalactanase (pH optimum 4.4), requires taurocholate as an activator. The level of the enzyme decreases with age.

Commercial preparations of *Clostridium perfringens* (*C. welchii*) neuraminidase and culture filtrates of *Diplococcus pneumoniae* contained an *endo*-glycosidase that hydrolysed the *O*-glycosidic linkages between 2-acetamido-2-deoxy-D-galactosyl and serine or threonine residues, but it did not hydrolyse phenyl 2-acetamido-2-deoxy-α-D-galactopyranoside.[218] The enzyme is classified as an *endo*-α-D-2-acetamido-2-deoxygalactanase on the basis of its substrate specificity. Cultures of *D. pneumoniae* also contained an enzyme that cleaved 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-D-galactose from a desialylized glycoprotein from human-erythrocyte membranes.[219] This disaccharide was also released from glycopeptides and glycoproteins in which it is linked to serine or threonine residues. Purification of the enzyme (pH optimum 6.0), which can be classified as an *endo*-β-D-2-acetamido-2-deoxygalactanase, was achieved by gel filtration and ion-exchange chromatography.

endo-β-D-2-Acetamido-2-deoxyglucanases

An *endo*-β-D-2-acetamido-2-deoxyglucosidase from human liver has been shown to hydrolyse the proline-rich glycoproteins in saliva.[90]

The substrate specificities of the *endo*-β-D-2-acetamido-2-deoxyglucosidases from *D. pneumoniae* and *Streptomyces griseus* have been compared.[220] Because of their different substrate requirements, they have been named as D and H forms, respectively.[221]

endo-α-D-2-Amino-2-deoxygalactanases

An *endo*-D-2-amino-2-deoxygalactosidase isolated from culture filtrates of *S. griseus* cleaved 2-amino-2-deoxy-D-galactose linked to 2-amino-2-deoxy-D-galactose in an oligosaccharide and in high-molecular-weight material from a *Neurospora* mutant.[222] Since some or all of these residues are α-(1 → 4)-linked, and the enzyme is inactive against chitosan and the *N*-acetylated *Neurospora* oligosaccharide, it appears to be an *endo*-α-D-2-amino-2-deoxygalactanase.

Agarases

Characterization of the agarase synthesized by *Cytophaga flevensis* has been reported.[223]

An agarase has been isolated from the mollusc *Littorina mandshurica* by affinity chromatography on agarose.[224] The preparation was free from endogenous cellulase and xylanase activities.

[218] V. P. Bhavanandan, J. Umemoto, and E. A. Davidson, *Biochem. Biophys. Res. Comm.*, 1976, **70**, 738.
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[222] J. L. Reissig, W.-H. Lai, and J. E. Glasgow, *Canad. J. Biochem.*, 1975, **53**, 1237.
[223] H. J. van der Meulen and W. Harder, *J. Microbiol. Serol.*, 1975, **41**, 431.
[224] A. I. Usov and L. I. Miroshnikova, *Carbohydrate Res.*, 1975, **43**, 204.

Alginases and Alginate Lyases

A viscometric assay for the alginolytic activities in bacteria has been reported.[225]

α-Amylases

The proceedings of an international symposium on enzymes and proteins from thermophilic micro-organisms includes papers on amylases.[226]

Methods for the assay of α-amylases and the uses of α-amylases in clinical chemistry have been reviewed.[29] Problems associated with the standardization and calibration of assays for amylase that use Phadebas Blue Starch have been noted.[227, 228]

A method using a chromogenic substrate has demonstrated the presence of multiple forms of amylases following electrophoresis on a slab-type polyacrylamide gel.[229] Slabs of the gel are layered onto thin-film plates of agar impregnated with Remazobrilliant Blue Starch, where the amylases appear as clear zones against a blue background.

Several groups have examined the action patterns of depolymerizing enzymes by studying the distribution of products during the early stages of hydrolysis, and mathematical models have been devised to account for the products formed.[230] Published data for several α-amylases have been re-examined in terms of the mathematical models, and it was concluded that, after the initial random attack on a long polymer chain, the enzyme often releases only one of the resulting fragments of the bound substrate. The retained fragment may be repeatedly hydrolysed near one end, with the release of a series of oligosaccharides, before the complex of the enzyme and the fragmented substrate finally dissociates. At near-optimum conditions, the α-amylase from *Bacillus subtilis* is unique insofar as it does not repeatedly attack fragments of the substrate.

Several models have been proposed to account for the non-random distribution of oligosaccharides formed when polysaccharides are hydrolysed by α-amylase.[231] The preferred-attack model assumes that the probability of bond-cleavage depends on the position of the bond in the chain; the repetitive (or multiple-attack) model assumes that α-amylase can form a cage-like complex with the substrate and attack it several times during a single encounter; the multiple-enzyme (or dual-site) model assumes that the substrate is hydrolysed by the combined actions of *exo*- and *endo*-enzymes. The effects of pH, inhibitors, and the chain length of the substrates have been studied in an attempt to decide which of the three models best fits the action of α-amylase. The effects of these variables on either the distribution of products or the action pattern of the enzyme were incorporated into the models, which were then used to interpret experimental data obtained with porcine pancreatic α-amylase.

[225] R. A. Stevens and R. E. Levin, *Appl. Environ. Microbiol.*, 1976, **31**, 896.
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[229] A. M. Spiekerman, P. Perry, N. C. Hightower, and F. F. Hall, *Clinical Chem.*, 1974, **20**, 324.
[230] J. A. Thoma, *Biopolymers*, 1976, **15**, 729.
[231] J. A. Thoma, *Carbohydrate Res.*, 1976, **48**, 85.

Substances from plants that inhibit α-amylase activity have been reviewed.[232] Wheat flour contains proteinaceous inhibitors of α-amylase, and the interactions of immobilized forms of the inhibitor with α-amylases were investigated.[233]

An oligosaccharide from *Streptomyces diastaticus* has been shown to inhibit α-amylase.[234] The oligosaccharide, which consists mainly of D-glucose, inhibited microbial and mammalian α-amylases and glucoamylase, but not β-amylase, α-D-glucosidase, dextranase, cellulase, or β-D-glucosidase.

The isoenzymes of amylase can be separated in an agarose gel containing a linear polyacrylamide polymer, which is introduced to reduce the endo-osmotic flow.[235] Characteristic changes in the isoenzyme patterns were noted in such diseases as cystic fibrosis and pancreatitis, *etc.* The occurrence of multiple forms of α-amylase in humans has been suggested to result from *in vivo* digestion of the enzyme by either glycoside hydrolases or proteases.[236] It was pointed out that autodigestion and *in vivo* changes of α-amylase should be carefully considered in diagnosing hyperamylasemia.

Cyclohepta-amylose inhibited an undefined pancreatic α-amylase by binding to the active site.[237] Preparative ultracentrifugation showed that the enzyme binds three molecules of cyclohepta-amylose, although the binding is characterized by a single dissociation constant; only one molecule of cyclohepta-amylose appears to interact with a tryptophanyl residue of the enzyme. The purification of α-amylases on immobilized inhibitors of the enzyme from wheat has been reported.[233] Purification of the α-amylase from human saliva by this method gave a preparation having activities and physicochemical characteristics similar to those obtained by other methods. Electrophoretically homogeneous forms of the α-amylases from chicken pancreas and yellow mealworm (*Tenebrio molitor*) larvae were eluted from the matrix with high concentrations of maltose.

Isoenzymes of the amylases in human saliva, sera, and urine have been characterized by isoelectric focusing, and the relationships among the isoenzymes were discussed.[238] Proline-rich glycoproteins that are isolated during the purification of human parotid α-amylase may modify or protect the enzyme.[239]

The levels of amylase in normal urine and sera have been measured using Phadebas Starch as a substrate.[240] The sera of patients undergoing haemodialysis or treatment with heparin appear to show a rise in the levels of amylase when the amylase is measured by a starch–iodine procedure.[241] A corresponding increase was not observed in *in vitro* experiments, and it was concluded that interactions between lipoproteins and starch *in vivo* prevent the starch–iodide complexes from forming, thereby giving a false measurement.

[232] J. J. Marshall, in ref. 9, p. 244.
[233] V. Buonocore and E. Poerio, *J. Chromatog.*, 1975, **114**, 109.
[234] S. Murao and K. Ohyama, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2271.
[235] G. Skude, *Scand. J. Clin. Lab. Invest.*, 1975, **35**, 41.
[236] T. Takeuchi, T. Matsushima, T. Sugimura, T. Kozo, T. Takeuchi, and T. Takemoto, *Clinica Chim. Acta*, 1975, **60**, 205.
[237] S. Móra, I. Simon, and P. Elödi, *Mol. Cell. Biochem.*, 1974, **4**, 205.
[238] S. Scharpé, A. Lauwers, and W. Cooreman, *Biochimie*, 1973, **55**, 1003.
[239] P. Degand, J. P. Aubert, A. Boersma, C. Richet, M. H. Loucheux-Lefebvre, and G. Biserte, *F.E.B.S. Letters*, 1976, **63**, 137.
[240] K. Ojala and A. Hamoinen, *Scand. J. Clin. Lab. Invest.*, 1975, **35**, 163.
[241] A. Pasternack and U.-H. Stenman, *Clinica Chim. Acta*, 1975, **65**, 213.

A rapid method for determining the amylase isoenzymes in human sera and urine is based on separation of the isoenzymes by electrophoresis on a cellulose acetate membrane and visualization using a Blue Starch–agar plate.[242] The two isoenzymes which are separated possess the same characteristics as salivary and pancreatic amylases. The relative proportions of the isoenzymes are useful in distinguishing hyperamylasemias arising from disorders of the pancreas and parotid gland, *etc.*

α-Amylases from human sera and urine have been separated by isoelectric focusing into two principal and one minor isoenzymes (p*I* values of 6.4—6.5, 6.9—7.0, and 5.9, respectively); salivary α-amylase gave one principal and two minor isoenzymes (p*I* values of 6.5, 6.0, and 6.9, respectively), and pancreatic α-amylase also gave one principal and two minor isoenzymes (p*I* values of 7.0, 6.0, and 6.5, respectively).[243] The antigenicities of the isoenzymes were also studied. Chromatographic studies of the amylase isoenzymes in human sera, urine, and milk have been reported.[244] An unidentified isoenzyme was found in urine, as well as enzymes of the salivary and pancreatic types. Salivary and unidentified isoenzymes are present in milk.

Amylases with unusually fast electrophoretic mobilities have been found in the sera of certain patients.[245] Since digestion of the amylases with neuraminidase markedly modified the electrophoretic mobility, it was concluded that neuraminic acid residues are incorporated into the enzyme, possibly by attachment to the glycoprotein.

Isoenzymes of amylases released by acid from partially purified human macroamylase complexes are indistinguishable from the pancreatic and salivary α-amylases found in human urine and sera.[246] The proportions of the two isoenzymes released from macroamylase complexes showed greater variation than those found in normal sera.

The affinity characteristics of amylase-binding substance(s) prepared from macroamylase complexes have been studied to see if they resemble those of pancreatic and salivary amylases, and if the aetiology of macroamylasemia results from altered affinity characteristics.[247] A reinterpretation of the chemical data relating to hyperamylasemia in diabetic comas has considered the possibility that the release of 'serum' amylase occurs from tissues other than the hepatocytes.[179]

Ovine pancreatic α-amylase has been purified by fractional precipitation, ion-exchange chromatography, and specific adsorption onto Sephadex.[248] The enzyme was obtained as a homogeneous glycoprotein, which was shown, by various physicochemical criteria, to exist as a single species. The properties and amino-acid composition of the enzyme (mol. wt. 5.6—5.8 × 10^4, p*I* 3.2) were compared with those of porcine pancreatic α-amylase.

[242] T. Takeuchi, T. Matsushima, T. Sugimura, T. Kozu, T. Takeuchi, and T. Takemoto, *Clinica Chim. Acta*, 1974, **54**, 137.
[243] T. Takeuchi, T. Matsushima, and T. Sugimura, *Clinica Chim. Acta*, 1975, **60**, 207.
[244] L. Fridhandler, J. E. Berk, K. Montgomery, and D. Wong, *Clinical Chem.*, 1974, **20**, 547.
[245] K. Sudo and T. Kanno, *Clinica Chim. Acta*, 1975, **64**, 303.
[246] L. Fridhandler, J. E. Berk, and K. Montgomery, *Clinical Chem.*, 1974, **20**, 26.
[247] L. Fridhandler, J. E. Berk, and D. Wong, *Clinical Chem.*, 1974, **20**, 22.
[248] M. Ettalibi, A. B. Abdeljlil, and G. Marchis-Mouren, *Biochimie*, 1975, **57**, 995.

A solid-state radioimmunoassay has been developed for the detection and measurement of porcine pancreatic α-amylase.[249] A double-antibody radioimmunoassay that is able to determine and detect small amounts of pancreatic amylase, even in the presence of amylase isoenzymes, has been reported.[250] The assay was used to ascertain the effects of pancreatectomy on the levels of amylase in porcine tissues, and it was concluded that the pancreas contributes significantly to the levels of circulating amylase.

When the Cl^- effector binds to porcine pancreatic α-amylase, k_{cat} of the amylolytic reaction is increased 30-fold, whereas the affinity of the enzyme for substrates is unchanged.[251] Derivatization of the enzyme and other studies indicated that the effector-binding site is a single ε-amino-group of lysine close to the active site.

Preparative ultracentrifugation has shown that three molecules of cyclohepta-amylose bind specifically to porcine pancreatic α-amylase, although the binding can be characterized by a single dissociation constant.[252] Only one of the bound cyclohepta-amylose molecules interacts with a tryptophanyl side-chain of α-amylase. The radius of gyration of the enzyme (measured by *X*-ray diffraction) decreased on binding cyclohepta-amylose.[253] On partial saturation of the α-amylase with cyclohepta-amylose, the solution contains only saturated α-amylase–cycloamylose complexes and free α-amylase, consistent with all-or-none binding. The bound molecules are accommodated in a trough of the α-amylase molecule, with their planes perpendicular to the longitudinal axis of the trough.

Two masked sulphydryl groups, which are activated by the removal of Cu^{2+} ions, have been found in porcine pancreatic α-amylase.[254] The number of reactive sulphydryl groups in active α-amylase depends on the method of preparation, which may cause —SH → —S—S— interconversion.

The relations between the α-amylases in the parotid gland, liver, pancreas, and serum of rats have been investigated by immunodiffusion, immunoelectrophoresis, and immunoinhibition.[255] The enzymes in the serum and liver are identical and are very similar to that of the parotid gland; however, pancreatic α-amylase is quite distinct, possessing only some of the determinants present on parotid-gland α-amylase. These observations reinforce the suggestion that the liver is the main source of serum α-amylase. Sex-linked dependences have been observed in the levels of α-amylase activity in the salivary glands and saliva of rats.[256]

The relation between the secretion of α-amylase by rat pancreas and the endogenous accumulation of cAMP has been studied *in vitro*.[257] Secretin stimulated the secretion, and pancreozymin the release, of the α-amylase. The

[249] H. J. Wedner, L. N. Parker, and M. G. Rosenfeld, *Analyt. Biochem.*, 1975, **65**, 175.
[250] J. P. Ryan, H. E. Appert, J. Carballo, and R. H. Davis, *Proc. Soc. Exp. Biol. Med.*, 1975, **148**, 194.
[251] R. Lifshitz and A. Levitski, *Biochemistry*, 1976, **15**, 1987.
[252] S. Móra, I. Simon, and P. Elödi, *Mol. and Cell. Biochem.*, 1974, **4**, 205.
[253] I. Simon, S. Móra, and P. Elödi, *Mol. and Cell. Biochem.*, 1974, **4**, 211.
[254] M. Telegdi, F. Fabian, S. M. El-Sewedy, and B. F. Straub, *Biochim. Biophys. Acta*, 1976, **429**, 860.
[255] M. Messer and R. T. Dean, *Biochem. J.*, 1975, **151**, 17.
[256] A. P. Levitsky, R. D. Barabash, and V. M. Konovetz, *Zhur. obshchei Biol.*, 1974, **35**, 149.
[257] S. Heisler, G. Grondin, and G. Forget, *Life Sci., Part II*, 1974, **14**, 631.

effects of detergents and trypsin in stimulating the secretion of α-amylase by either pancreozymin or sodium fluoride in perfused rat pancreas have been investigated.[258]

The origin and excretion of serum α-amylase in mice have been studied by cellulose acetate electrophoresis of partially purified preparations of α-amylase from various tissues; the liver and serum were shown to contain mainly an α-amylase variant that is electrophoretically indistinguishable from parotid α-amylase, whereas urine contains only pancreatic α-amylase.[259] Murine pancreatic α-amylase was rapidly cleared from the serum when injected intravenously, appearing in the urine; parotid α-amylase was also cleared from the serum, but little appeared in the urine. It was concluded that murine liver secretes both pancreatic and parotid α-amylases into the blood, but that only the pancreatic α-amylase is excreted.

A method for the rapid determination of the α-amylase 'diastase' in honey has been reported.[260]

α-Amylase from yellow mealworm (*Tenebrio molitor*) larvae consists of a single polypeptide chain (mol. wt. 6.8×10^4, p*I* 4.0), which has a very low proportion of sulphur-containing amino-acids.[261] The enzyme (pH optimum 5.8, temperature optimum 37 °C) is Ca^{2+}-dependent, and the removal of Ca^{2+} ions by exhaustive dialysis caused irreversible inactivation. The enzyme is activated by Cl^- and other inorganic anions, but is inhibited by F^- anions, and it exhibits identical kinetic behaviour towards starch, amylose, and amylopectin, but has a higher affinity constant for glycogen.

The amylase activities in whole and in fractionated haemolymphs of the molluscs *Crassostrea virginica* and *Mercenaria mercenaria* have been measured.[190] Amylase occurs only in whole haemolymphs and in the sera of *C. virginica*, originating from the crystalline style. Possible roles for the serum enzyme were discussed.

The distribution of α-amylase activity during the development of barley grain has been reported.[262] Gibberellic acid induced α-amylase activity in barley (*Hordeum vulgare*) grains with excised embryos.[167] This increase was ascribed to *de novo* protein synthesis, which was suppressed by prior incubation of the grains with actinomycin D.

Both gel filtration and centrifugation have been used to examine the distribution of α-amylase activity in homogenates of barley aleurone layers following incubation with gibberellic acid.[263] The results support the view that α-amylase is secreted by membrane-bound vesicles. The amount of α-amylase retained by the cell wall is influenced by the buffer in which the layers are incubated and by the presence of actinomycin D.

Kidney beans (*Phaseolus vulgaris*) contain a proteinaceous inhibitor of α-amylase named 'phaseolamin'.[264] After purification by heat treatment, ion-

[258] P. Robberecht, M. Deschodt-Lanckman, J. Camus, and J. Christophe, *Biochem. Pharmacol.*, 1975, **22**, 1623.
[259] P. I. Mackenzie and M. Messer, *Comp. Biochem. Physiol.*, 1976, **54B**, 103.
[260] R. A. Edwards, R. Faraji-Heremi, and M. Wooton, *J. Apicultural Res.*, 1975, **14**, 47.
[261] V. Buonocore, E. Poerio, V. Silano, and M. Tomasi, *Biochem. J.*, 1976, **153**, 621.
[262] M. J. Allison, R. P. Ellis, and J. S. Swanston, *J. Inst. Brewing*, 1974, **80**, 488.
[263] R. D. Firn, *Planta*, 1975, **125**, 227.
[264] J. J. Marshall and C. M. Lauda, *J. Biol. Chem.*, 1975, **250**, 8030.

exchange chromatography, and gel filtration, the inhibitor was shown to be specific for animal α-amylases, possessing no activity towards plant, bacterial, and fungal α-amylases. Gel filtration demonstrated that a 1 : 1-complex is formed between animal α-amylases and the inhibitor. The enzyme–inhibitor complex could be dissociated, with degradation of the enzyme, only at low pH.

Attempts to fractionate the amylases of lentil (*Lens culinaris*) roots by gel filtration gave only one active peak, although three amylase components were detected by polyacrylamide gel electrophoresis; two of the components are α-amylases, whereas the other is a β-amylase.[265] An assay for the α-amylase inhibitor in legumes has been reported.[266]

The development of amylase activity in extracts of embryo-free and of GA_3-treated, embryo-free maize kernels has been determined.[267] The increase in amylase activity was accompanied by the appearance of several starch-degrading enzymes. Actinomycin D and cycloheximide prevented the amylolytic activity from developing. Other results indicated that the development of α-amylase activity in embryo-free maize kernels does not depend on gibberellic acid, but involves the *de novo* synthesis of protein.

Amylase isoenzymes in six varieties of oats (*Avena sativa*) have been examined by agar gel electrophoresis.[268] Three distinct zymograms were detected in aqueous extracts of the seedling endosperms, but no differences were detected among the varieties. The results indicate that amylase zymograms could be used to identify varieties of oats.

An α-amylase in the spherosomes of sorghum has been shown to act in conjunction with an endogenous α-D-glucosidase activity.[169] Both activities are manifest by a single enzyme complex.

The purity of four isoenzymes of an α-amylase isolated from red spring wheat has been established by chromatography, electrophoresis, and sedimentation studies.[269] The isoenzymes have identical diffusion coefficients, molecular weights [4.51×10^4 (by sedimentation) and 4.20×10^4 (by electrophoresis)], p*I* values (6.05—6.20), and pH optima (5.6—5.5). The proteins contain relatively large proportions of glutamic and aspartic acids, but no sulphydryl groups. The inhibition of wheat α-amylase by L-ascorbic acid and derivatives of isoalloxazine has been studied.[270]

A method for determining bacterial amylases in the presence of fungal α-amylases is based on differences in the thermal stabilities of the enzymes.[271]

The role of α-amylase in the metabolism of exogenous and endogenous carbohydrates by *Aerobacter aerogenes*, *E. coli*, and *Streptococcus mutans* has been studied.[272]

Abscisic acid inhibited the α-amylase activities in an *Aspergillus* species and *Bacillus subtilis*.[273]

[265] P. A. Simonin and P.-E. Pilet, *Experientia*, 1974, **30**, 23.
[266] J. J. Marshall and C. M. Lauda, *Stärke*, 1975, **27**, 274.
[267] L. D. Goldstein and P. H. Jennings, *Plant Physiol.*, 1975, **55**, 893.
[268] J. B. Smith and M. D. Bennett, *J. Sci. Food Agric.*, 1974, **25**, 67.
[269] R. Tkachuk and J. E. Kruger, *Cereal Chem.*, 1974, **51**, 508.
[270] J. C. Palla and J. Verrier, *Ann. Technol. Agric.*, 1974, **23**, 151.
[271] M. Berger and P. Grandvoinnet, *Ann. Technol. Agric.*, 1974, **23**, 215.
[272] T. N. Palmer, G. Wöber, and W. J. Whelan, *European J. Biochem.*, 1973, **39**, 601.
[273] T. Hemberg, *Physiol. Plantarum*, 1975, **35**, 11.

An acid-stable α-amylase (mol. wt. 6×10^4, p*I* 3.55) has been isolated from an aqueous extract of wheat-bean cultures of *Aspergillus aureus* by fractional precipitation, ion-exchange chromatography, and gel filtration.[172] The preparation is more stable than Taka-amylase A (one of the α-amylases of *Aspergillus oryzae*) and did not separate into subunits on polyacrylamide gel electrophoresis at pH 2.3—9.4. The addition of Ca^{2+} ions stabilized the α-amylase and markedly influenced the pH-activity profile and dependence on temperature. Metal ions such as Al^{3+}, Fe^{3+}, and Pb^{2+} inhibited the enzymic activity, whereas metal-chelating agents and sulphydryl reagents were without effect. In the hydrolysis of soluble starch to D-glucose, maltose, and maltotriose, the extent of hydrolysis at the achromatic point in the starch–iodine reaction was 16% and the limit of hydrolysis was 43%.

The interactions of cycloamyloses with fluorescent probes [*e.g.* 6-(4-toluidyl)-naphthalene 2-sulphonate] have been used to monitor the rate of hydrolysis of cyclohepta-amylose by Taka-amylase A and inhibition of the hydrolysis by maltose and cyclohexa-amylose.[274] Various kinetic parameters of the hydrolyses of cyclohexa-, cyclohepta-, and cyclo-octa-amyloses by Taka-amylase A at pH 5.3 and 20 °C have been determined.[275] Glucoamylase was used in an analytical procedure that distinguished between cleavage of the dextrin ring and subsequent hydrolysis of the linear products. Whereas the molecular activity (k_0) increased with increasing size of the dextrin ring, the K_m values did not show much variation. The extent of multiple attack was estimated to be 1.5, regardless of the size of the dextrin ring.

Two groups have independently developed theoretical and experimental approaches that enable the binding energies of monomeric substrates to depolymerases to be determined.[24] However, these approaches initially gave different results when applied to the same enzyme–substrate system (*e.g.* α-amylase–malto-oligosaccharide), although proper data-management resolved the differences. It is clear that the complexities of the subsite model demand extensive data-gathering and -processing and verification that the computed parameters for the model faithfully reproduce the experimental data.

The immunological properties of α-amylases from transformable, transformant, and DNA-donor strains of *Bacillus amyloliquefaciens*, *B. natto*, and *B. subtilis* have been compared.[276] Most of the enzymes cross-reacted with each other's antibodies and contain at least twelve common peptide sequences. However, distinct structural differences were noted; *e.g.* only two of the enzymes contain carbohydrate, which consists of neutral sugars and 2-amino-2-deoxy-D-glucose (9—11% total).

The molecular size of the extracellular amylase produced by intact cells and protoplasts of *B. caldolyticus* has been shown to vary.[277] Factors that possibly regulate the synthesis of α-amylase by wild-type *B. licheniformis* and by mutants synthesizing high levels of α-amylase have been discussed.[278] The α-amylases synthesized by transformable transformant and DNA-donor strains of *B. natto*

[274] H. Kondo, H. Nakatani, and K. Hiromi, *J. Biochem.* (*Japan*), 1976, **79**, 393.
[275] N. Suetsugu, S. Koyama, K. Takeo, and T. Kuge, *J. Biochem.* (*Japan*), 1974, **76**, 57.
[276] H. Matsuzaki, K. Yamane, and B. Maruo, *Biochim. Biophys. Acta*, 1974, **365**, 248.
[277] M. Kessel and J. N. Eloff, *Arch. Mikrobiol.*, 1975, **106**. 201.
[278] N. Saito and K. Yamamoto, *J. Bacteriol.*, 1975, **121**, 848.

and *B. subtilis* have molecular weights in the range 3.4—5.5 × 10^3, but possess different catalytic properties.[279] It appears that the α-amylases synthesized by the transformants are hybrids of the two α-amylases in the parent strains.

B. subtilis α-amylase was absorbed onto a benzylated agarose derivative by hydrophobic interactions, and the enzyme could be eluted from the gel without denaturation.[280]

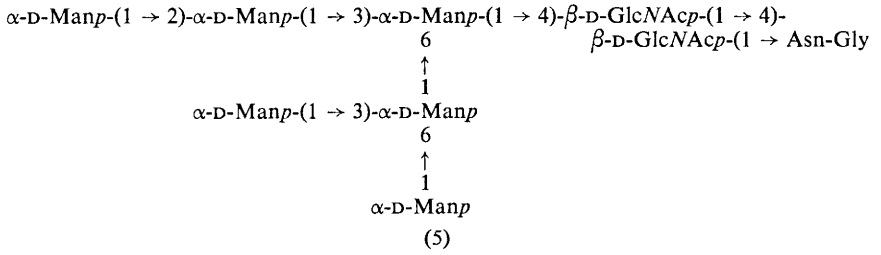
The rates of hydrolysis of various maltodextrins (d.p. 3—117) by *B. subtilis* α-amylase have been determined; the K_m values decreased with increasing d.p.[281] The dependence of K_m and k_0 on the d.p. suggested that four subsites comprise the active site of the enzyme.

Inhibition by substrate analogues of the hydrolysis of phenyl α-maltoside to phenol and maltose by *B. subtilis* α-amylase was largely competitive, except that β- and αβ-maltose showed non-competitive or mixed-type inhibition, respectively.[282] α-Maltose is a competitive inhibitor. The results were compared with those for Taka-amylase A and glucoamylase.

A tyrosine residue, which is responsible for the productive binding of substrates, appears to be located at a subsite near the catalytic site of *B. subtilis* α-amylase.[283] Changes in the u.v. spectrum of *B. subtilis* α-amylase on the addition of maltotriose, a substrate that is hydrolysed slowly, were used to investigate enzyme–substrate binding.[284]

Membrane-bound amylase is synthesized adaptively by a dextranase-producing strain of *Cytophaga johnsonii*.[285]

The structure of one of the carbohydrate moieties of an α-amylase from a *Rhizopus* species has been determined.[286] Six D-mannosyl residues and a 2-acetamido-2-deoxy-D-glucosyl residue were released on digestion of a glycopeptide in turn with α-D-mannosidase and β-D-2-acetamido-2-deoxyglucosidase. The structure (5) was proposed on the basis of the results of enzymic hydrolysis, methylation, partial acetolysis, and Smith degradation.

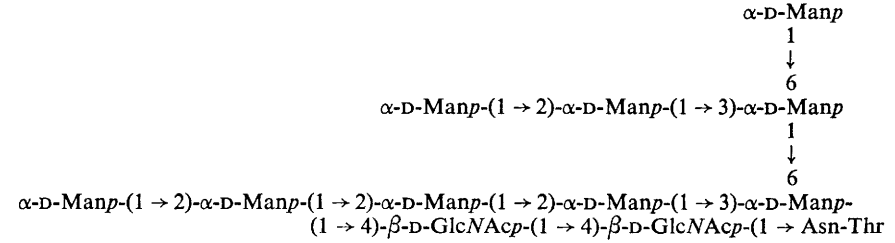


(5)

The structure of the carbohydrate residue of one of three glycopeptides released by pronase from *Rhizopus javanicus* α-amylase has also been investigated

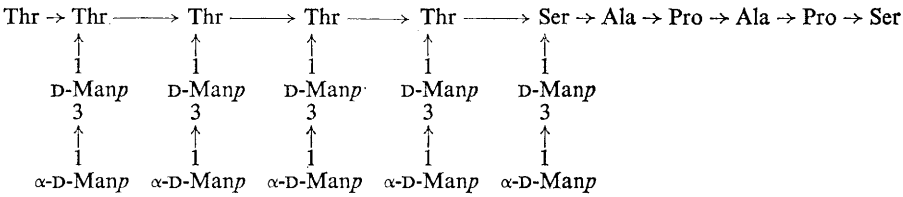
[279] H. Matsuzaki, K. Yamane, K. Yamaguchi, Y. Nagata, and B. Maruo, *Biochim. Biophys. Acta*, 1974, **365**, 235.
[280] T. Låås, *J. Chromatog.*, 1975, **111**, 373.
[281] T. Shibaoka, K. Miyano, and K. Takahashi, *J. Biochem. (Japan)*, 1974, **76**, 475.
[282] T. Shibaoka, K. Ishikura, T. Inatani, H. Fukube, K. Hirome, and T. Watanabe, *J. Biochem. (Japan)*, 1974, **76**, 909.
[283] M. Ohnishi, T. Suganuma, and K. Hiromi, *J. Biochem. (Japan)*, 1974, **76**, 7.
[284] M. Ohnishi, H. Kegai, and K. Hiromi, *J. Biochem. (Japan)*, 1975, **78**, 247.
[285] J. C. Janson, *J. Gen. Microbiol.*, 1975, **88**, 205.
[286] K. Watanabe and T. Fukimbara, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 1973.

by enzymic studies, methylation, acetolysis, and Smith degradation.[287] The results of this investigation, in conjunction with previous information, indicated the structure (6) for the glycopeptide. Alkaline degradation and methylation



(6)

analysis of another glycopeptide isolated from a pronasic digest of *R. javanicus* α-amylase showed that the carbohydrate residue contains only 3-*O*-α-D-mannopyranosyl-D-mannopyranose units.[288] Both serine and threonine are involved in the carbohydrate–peptide linkages, and the structure (7) was proposed for the glycopeptide.



(7)

The affinity of an extracellular α-amylase from *Streptomyces aureofaciens* for cross-linked starches is affected by the presence of soluble substrates, the pH of the medium, and the presence of proteins.[289]

The properties of an amylase present in the thermophilic actinomycete *Thermomonospora vulgaris* have been reported.[290]

α-Amylase has been immobilized, with retention of enzymic activity, by adsorption onto a phenol–formaldehyde resin,[291] by covalent attachment to activated, cross-linked poly(4-methacryloxybenzoic acid),[292] and by cross-linking with glutaraldehyde.[249]

β-Amylases

The β-amylases from barley, soybean, *Bacillus megaterium*, and *B. polymyx* can be isolated by adsorption onto starch without loss of enzymic activity.[293]

[287] K. Watanabe and T. Fukimbara, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 1711.
[288] K. Watanabe, *J. Biochem.* (*Japan*), 1976, **80**, 379.
[289] E. Hostinová and J. Zelinka, *Stärke*, 1975, **27**, 343.
[290] A. M. Allam, A. M. Husein, and A. M. Ragab, *Z. allgem. Mikrobiol.*, 1975, **15**, 393.
[291] J. A. Boundy, K. L. Smiley, C. L. Swanson, and B. T. Hofreiter, *Carbohydrate Res.*, 1976, **48**, 239.
[292] G. J. Bartling, S. K. Chattopadhyay, H. D. Brown, C. W. Barker, and J. K. Vincent, *Biotechnol. and Bioeng.*, 1974, **16**, 1425.
[293] M. Hoshino, Y. Hirose, K. Sano, and K. Mitsugi, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 2415.

Whereas a β-amylase isolated from the roots of the lentil *Lens culinaris* could not be distinguished from the endogenous α-amylases by gel filtration, the enzymes were separated by polyacrylamide gel electrophoresis.[265]

One of the isoenzymes of the amylase in oat (*Avena sativa*) seedlings is labile to heat and does not attack β-limit dextrin; the enzymic activity was inhibited by heavy-metal ions and appears to be a β-amylase.[268]

Sweet-potato β-amylase has been purified by affinity chromatography on cyclohexa-amylose bound covalently to agarose.[294] Inhibition studies indicated that the size of the active site of the enzyme is complementary to cyclohexa-amylose.

Polyacrylamide gel electrophoresis has been used to obtain zymograms for the β-amylase from rice (*Oryza sativa*) seeds, and the relationships among the multiple forms of this β-amylase were discussed.[295]

The β-amylase in the protein bodies of sorghum has been shown to act in conjunction with an endogenous α-D-glucosidase activity; both activities are manifest by a single species.[169]

The role of β-amylase in the metabolism of exogenous and endogenous carbohydrates in *Aerobacter aerogenes*, *E. coli*, and *Streptococcus mutans* has been discussed.[272]

A strain of *Bacillus cereus* contains a β-amylase that, together with an endogenous pullulanase, converted starch into maltose (95%) at pH 6.0—6.5 and 50 °C.[296] This β-amylase was purified to homogeneity by disc gel electrophoresis, fractional precipitation, adsorption onto starch and Celite, and gel filtration.[297] The enzyme (optimum pH 7, mol. wt. *ca.* 3.5×10^4), which released only β-maltose from starches and glycogen, was inhibited by mercuric chloride and 4-chloromercuribenzoate; in the latter case, cysteine restored the enzymic activity.

β-Amylase has been immobilized, with retention of activity, by attachment to a copolymer of acrylic acid and acrylamide.[298]

Amylo-1,6-D-glucosidases

The role of amylo-1,6-D-glucosidase in the metabolism of exogenous and endogenous carbohydrates in *Aerobacter aerogenes*, *E. coli*, and *Streptococcus mutans* has been discussed.[272]

Arabinanases

Studies on the hydrolytic and macerating activities of the enzyme preparation 'Pectawamorin P10X' showed that arabinose is released from the polysaccharides in the skin and juice of grapes.[299]

Strains of *Aspergillus niger*, *Rhodotorula flava*, a *Streptomyces* species, and a mould contain arabinanase activities having pH optima of 3.4—3.9, 2.5—3.0,

[294] P. Vretblad, *F.E.B.S. Letters*, 1974, **47**, 86.
[295] H. Matsui, S. Chiba, T. Shimomura, and N. Takahashi, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 2239.
[296] Y. Takasaki, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 1515.
[297] Y. Takasaki, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 1523.
[298] K. Mårtensson, *Biotechnol. and Bioeng.*, 1974, **16**, 1567.
[299] E. N. Datunashvili and V. N. Ezhov, *Prinklad. Biokhim. i Mikrobiol.*, 1974, **10**, 117.

4.8—5.2, and 3.5—4.0, respectively, and temperature optima of 65, 65, 55, and 50—55 °C, respectively.[300] The enzymes hydrolysed an arabinan to different extents (100, 84, 59, and 100%, respectively) to give mainly arabinose. A xylanase from *Aspergillus niger* cleaved the α-(1 → 3)-linked D-arabinofuranosyl residues of arabinoxylo-oligosaccharides.[301]

An arabinanase has been isolated from culture fluids of *B. subtilis* by fractional precipitation, repeated chromatography on hydroxyapatite, and gel filtration.[302] The purified *endo*-enzyme hydrolysed arabinans to arabinose and arabinobiose, but was inactive towards phenyl α-L-arabinofuranoside, 4-nitrophenyl β-D-galactopyranoside, arabinoxylan, and gum arabic.

Cellulases

Papers presented at a symposium on 'Cellulose as a Chemical and Energy Resource' have dealt with cellulases from the viewpoints of the enzyme system, the substrates, the processes, and the products.[303] Active-site-directed inhibitors of cellulases have been discussed briefly.[22]

The enzymic activity in the digestive glands of the octopus (*Octopus vulgaris*) that is responsible for the cleavage of some water-soluble derivatives of cellulose has been investigated.[304] An *endo*-β-D-1,4-glucanase and β-D-glucosidase activities were also detected. The effects of the substrate concentration, pH, and temperature, and of added D-glucono-1,5-lactone, sodium chloride, and calcium chloride on the cellulase activity were determined. The absence of the so-called C_1 enzyme, which allows cellulase to attack insoluble cellulose, indicated that octopuses are unable to digest cellulosic foods.

A study of the cellulase activity and aetiology of freshwater gastropods was designed to see if the dietary preferences allow the species to coexist.[305] The cellulase activities in various species of Prosobranchia and Pulmonata, including *Lymnaea stagnalis*, were measured. Investigations of the hydrolysis of cellulose *in vitro* by a cellulase isolated from termites (*Microtermes tragardhi*) have indicated that Zn^{2+} ions activate the enzyme.[306] Cellulolytic activity has been detected in the mid-gut of the phytophagous bugs *Palomena angulosa*, *Eurydema rugosum*, and *Coreus marginatus*.[121]

Extracts from citrus orange (*Citrus sinensis*) pedicles contained cellulase activity.[307] The effects of pH, ionic strength, and ethylene on the extraction of cellulase from the abscission zones of citrus-leaf (*Citrus sinensis*) explants have been studied.[308] Prior treatment of the explants with ethylene increased the solubility of the enzyme in phosphate buffers, regardless of pH.

The synthesis of cellulolytic enzymes by *Alternalia* species during the pathogenesis of tomatoes has been studied.[309] Cellulolytic enzymes from

[300] I. Kusakabe, T. Yasui, and T. Kobayashi, *J. Agric. Chem. Soc. Japan*, 1975, **49**, 295.
[301] S. Takenishi and Y. Tsujisaka, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2315.
[302] A. Kaji and T. Saheki, *Biochim. Biophys. Acta*, 1975, **410**, 354.
[303] 'Cellulose as a Chemical and Energy Resource', ed. C. R. Wilke, *Biotechnol. and Bioeng.*, Symposium No. 5, 1975.
[304] M. Furia, L. Gianfreda, and V. Scardi, *Comp. Biochem. Physiol.*, 1975, **52B**, 529.
[305] P. Calow and L. J. Calow, *Nature*, 1975, **255**, 478.
[306] F. T. Abushama and M. A. Kambal, *Experientia*, 1976, **32**, 19.
[307] F. M. Basiouny and R. H. Biggs, *Planta*, 1976, **128**, 271.
[308] M. Huberman, R. Goren, and Y. Birk, *Plant Physiol.*, 1975, **55**, 941.
[309] P. Mehta, K. M. Vyas, and S. B. Saksena, *Hindustan Antibiotic Bull.*, 1974, **16**, 210.

Aspergillus niger and a *Streptomyces* species were unable to hydrolyse reduced SIII pneumococcal polysaccharide, allowing them to be distinguished from other β-D-glucan hydrolases.[3]

The cellulase activities in *Cellvibrio fulvus*,[310] *Chaetomium thermophile*, *Sporotrichum thermophile*, and *Thermoascus aurantiacus*[311] have been examined. The development of cellulolytic enzymes in cultures of *Chrysosporium lignorum* during growth has been monitored.[312]

Two cellulase-active fractions have been obtained from the phytopathogenic fungus *Fusarium moniliforme* by fractional precipitation and ion-exchange chromatography.[313] One of the cellulases (mol. wt. 2.5×10^4, pH optimum 4.5, temperature optimum 60 °C), which was homogeneous on examination by disc gel electrophoresis and ultracentrifugation, is particularly stable to heat; it was inhibited by Hg^{2+} ions, *N*-bromosuccinimide, and sodium picryl sulphate, and was activated by Co^{2+} and Zn^{2+} ions, hydroquinone, and L-ascorbic acid. The enzyme contains up to 26% of neutral carbohydrate, is not thiol-dependent, and hydrolysed insoluble celluloses but not cellobiose and monosaccharide glycosides (*i.e.* the preparation was free from β-D-glucosidase activity).

In order to establish the relations between the cellulase activities in various strains of *Pyricularia oryzae* and the optimum pH and between the intra- and extra-cellulases, the gel-filtration profiles of the enzymes have been compared.[314] It appears that some of the molecular properties of one of the cellulase components change on excretion into the culture medium. A number of cellulases from *P. oryzae* are active at pH > 7.

The isolation, morphology, and nutritional characteristics of a cellulolytic pythiaceous fungus (*Pythium* sp.) have been described.[315] The function of cellulolytic enzymes in the invasion of soybeans by *Rhizobium japonicum* has been investigated.[316] Optimal conditions for the synthesis of cellulase by *Rhizoctania solani* have been sought to determine at what stage the enzyme appears.[317]

The synthesis of cellulase C_x when *Sphaeropsis malorum* grows on apples has been investigated.[318] The degradation of natural insoluble cellulose by *Sporocytophaga myxococcoides* afforded cellobiose, D-glucose, and cellodextrins; the anomeric configuration of the terminal, reducing residue of these products on release from cellulose was determined.[319]

Factors affecting the synthesis of cellulases by a thermophilic *Sporotrichum* species have been investigated.[320] An enzyme that oxidizes cellulose has been found in culture filtrates of *Sporotrichum pulverulentum*.[321] The presence of this

[310] B. Berg, *Canad. J. Microbiol.*, 1975, **21**, 51.
[311] R. A. Romanelli, C. W. Houston, and S. M. Barnett, *Appl. Microbiol.*, 1975, **30**, 276.
[312] U. Westermark and K.-E. Eriksson, *Acta Chem. Scand.* (*B*), 1974, **28**, 209.
[313] K. Matsumoto, Y. Endo, N. Tamiya, M. Kano, K. Miyauchi, and J. Abe, *J. Biochem.* (*Japan*), 1974, **76**, 563.
[314] T. Sudo, H. Nagayama, and K. Tamari, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 1509.
[315] D. Park, *Trans. Brit. Mycol. Soc.*, 1975, **65**, 249.
[316] W. J. Hunter and G. H. Elkan, *Canad. J. Microbiol.*, 1975, **21**, 1254.
[317] N. Lisker, J. Katan, and Y. Henis, *Canad. J. Microbiol.*, 1975, **21**, 1298.
[318] G. Bompeix and B. Poiret, *Compt. rend.*, 1974, **279**, *D*, 275.
[319] M. Charpentier and D. Robic, *Compt. rend.*, 1974, **279**, *D*, 863.
[320] A. D. Coutts and R. E. Smith, *Appl. Environ. Microbiol.*, 1976, **31**, 819.
[321] K.-E. Eriksson, B. Pettersson, and U. Westermark, *F.E.B.S. Letters*, 1974, **49**, 282.

enzyme produced at least twice the amount of degradation of cellulose than that of a mixture of *endo-* and *exo*-D-glucanases.

The role of a β-D-glucosidase in a *Trichoderma* species in the saccharification of cellulose has been investigated.[177] Waste cellulose (*e.g.* newspaper) is a suitable source of carbon for the synthesis of cellulase by *Trichoderma viride*.[322] The economics of using this enzyme in the disposal of cellulosic wastes were discussed. Several cellulases are present in *T. viride*, and the purification and properties of two of these enzymes have been reported.[323] 'Avicelase', a cellulase component of the cellulose-degrading enzyme system of *T. viride*, has been further purified and its action pattern was shown to be less random than that of other cellulases.[324] Two cellulases (A and B) isolated from *T. viride* hydrolysed cellopentaose at either pH 3.5 or pH 5 with retention of configuration at the anomeric carbon atoms.[325] The K_m values for the hydrolysis of cellotetraose by the enzymes differ, but the V_{max} values are similar; the K_m and V_{max} values for cello-oligosaccharides increased with increasing length of the oligosaccharide chain. Both cellulases yielded mainly cellobiose and D-glucose from cellulosic substrates and higher cello-oligosaccharides. Cellulase A preferentially attacked the intersaccharide linkage of 4-nitrophenyl β-D-cellobioside, whereas cellulase B mainly attacked the aglyconic linkage. Both cellulases catalysed the synthesis of cellotriose from 4-nitrophenyl β-D-cellobioside, possibly by transfer of a D-glucosyl residue to cellobiose, and of cellotetraose from cellobiose, with the formation of cellotriose and D-glucose by secondary, random hydrolysis of cellotetraose.

Chitinases

The effects of a chitinase complex on the antigenicity of yeast-form cell walls of *Blastomyces dermatidis* and *Histoplasma capsulatum* have been studied.[326] The properties of a chitinase from *Phycomyces blakesleeanus* have been investigated.[327]

Chitosanases

A *Bacillus* species synthesizes two activities that degrade the cell walls of *Rhizopus* species; one of them, a chitosanase, is active towards glycolchitosan and chitosan.[328] The activity of the enzyme (mol. wt. 3.1×10^4, p*I* 8.30, pH optimum 5.6, temperature optimum 40 °C) was destroyed by sulphydryl reagents, but it was restored by either reduced glutathione or cysteine. The enzyme appears to act by an *endo*-mechanism.

The synthesis of an extracellular chitosanase by a *Streptomyces* species could be induced by 2-amino-2-deoxy-D-glucose.[329] The enzyme, which was purified to homogeneity by gel filtration and ion-exchange chromatography, acted on

[322] M. Mandels, L. Hontz, and J. Nystrom, *Biotechnol. and Bioeng.*, 1974, **16**, 1471.
[323] G. Okada, *J. Biochem.* (*Japan*), 1975, **77**, 33.
[324] Y. Tomita, H. Suzuki, and K. Nisizawa, *J. Ferment. Technol.*, 1974, **52**, 233.
[325] G. Okada and K. Nisizawa, *J. Biochem.* (*Japan*), 1975, **78**, 297.
[326] F. C. Odds, L. Kaufman, D. McLaughlin, C. Callaway, and S. O. Blumer, *Sabouraudia*, 1974, **12**, 138.
[327] R. J. Cohen, *Life Sci., Part II*, 1974, **15**, 289.
[328] Y. Tominaga and Y. Tsujisaka, *Biochim. Biophys. Acta*, 1975, **410**, 145.
[329] J. S. Price and R. Storck, *J. Bacteriol.*, 1975, **124**, 1574.

chitosan to give the corresponding di- and tri-chitosaccharides, but was inactive towards chitin. The chitosanase (pH optimum 4.5—6.5, temperature optimum 60 °C, mol. wt. 2.6—2.9 × 10^4) contains no carbohydrate residues.

Dermatan Sulphate Lyases

An enzyme that hydrolyses dermatan sulphate, but neither chondroitin 4-sulphate nor chondroitin 6-sulphate, has been isolated from *Flavobacterium heparinum* grown in the presence of glycosaminoglycans.[330] Chondroitin and dermatan sulphates and disaccharides obtained from them induced the synthesis of this enzyme, which was separated from a chondroitin sulphate lyase AC. The enzyme acted on dermatan sulphate to give an unsaturated, sulphated disaccharide and higher oligosaccharides.

Dextranases

Dextranase NG is absorbed, without denaturation, onto a benzylated agarose derivative by hydrophobic interactions.[280]

The properties of a dextranase synthesized by an oral strain of *Actinomyces israelii* have been reported.[331] Chemical analysis of the *exo*-dextranase ($S_{20,w}$ 4.35, p*I* 4.17, mol. wt. 5.5 × 10^4) from *Brevibacterium fuscum* showed that it is a glycoprotein containing 429 amino-acid residues, neutral sugars (11 residues), and 2-amino-2-deoxyhexoses (3 residues), whereas the *endo*-dextranase ($S_{20,w}$ 4.37, p*I* 4.9, mol. wt. 4.4 × 10^4) from *Penicillium funiculasum* contains 349 amino-acid residues (including cystine), neutral sugars (10 residues), and 2-amino-2-deoxyhexose (1 residue).[332, 333]

Dextran induced the synthesis of a dextranase, which is attached to the outer membrane of the envelope, by an aerobic, Gram-negative bacterium closely related to *Cytophaga johnsonii*.[285] The use of proteolytic enzymes, snake venoms, and detergents in solubilizing a dextranase from *C. johnsonii* has been examined.[334] Chymotrypsin was the only protease that efficiently solubilized the enzyme without destroying the enzymic activity.

An extracellular *endo*-dextranase (mol. wt. 3.9 × 10^4, temperature optimum 55 °C, pH optimum 5.5, K_m for dextran 1.1 × 10^{-4} mol l^{-1}) from *Fusarium moniliforme* hydrolysed dextran to isomaltose.[335] This enzyme was not inhibited by either iodoacetate or H_4edta and could be distinguished from commercial dextranases by electrophoresis.

The properties of a dextranase from *Oershovia xanthineolytica* have been reported.[336]

The effects of methyl D-glucopyranosides on a dextranase from a *Penicillium* species have been investigated.[68] Dextranase activities have been detected in *P. lilacinum*[337] and *P. purpurogenum*.[338]

[330] Y. M. Michelacci and C. P. Dietrich, *Biochem. J.*, 1975, **151**, 121.
[331] R. H. Staat and C. F. Schachtele, *Infection and Immunity*, 1975, **12**, 556.
[332] M. Sugiura and A. Ito, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1304.
[333] M. Sugiura and A. Ito, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1532.
[334] J. C. Janson, *J. Gen. Microbiol.*, 1975, **88**, 209.
[335] L. G. Simonson, A. E. Liberta, and A. Richardson, *Appl. Microbiol.*, 1975, **30**, 855.
[336] A. C. Hayward and L. I. Sly, *J. Appl. Bacteriol.*, 1976, **40**, 355.
[337] S. Murao, R. Yamamoto, and M. Arai, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 23.
[338] M. E. Preobrazhenskaya and A. L. Minakova, *Doklady Akad. Nauk S.S.S.R.*, 1975, **224**, 482.

A Blue Dextran–agar complex has been used to detect dextranase activity in strains of *Streptococcus mutans*.[339] The hydrolysis of [^{3}H]dextran to tritiated oligosaccharides by one of the strains was investigated. Possible roles for dextranases in the metabolism of dextrans by *S. mutans* were discussed. The isolation and properties of a dextranase from *S. mutans* have been described.[340]

Active immobilized forms of dextranases have been prepared by reaction of the enzymes with agarose cyclic imidocarbonate.[341]

β-1,4-*N*,6-*O*-Diacetylmuramidases

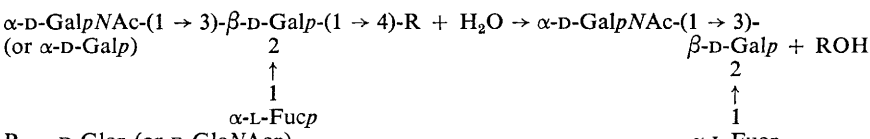
Features of the primary structure [342] and the complete amino-acid sequence [343] of β-1,4-*N*,6-*O*-diacetylmuramidase from a *Chalaropsis* species have been elucidated.

β-D-Galactanases

An enzyme that acts on the β-D-galactosidic linkages of glycoproteins has been separated by affinity chromatography, *etc.*, from other β-D-galactosidases in porcine small intestines.[115]

endo-β-D-Galactanases

An *endo*-β-D-galactanase that acts on blood-group A and B substances has been found in culture fluids of *Diplococcus pneumoniae*.[344] The enzyme, which was free from *exo*-glycosidases, *endo*-β-D-2-acetamido-2-deoxyglucosidase, and proteases, liberated trisaccharides from the blood-group substances (Scheme 2).



Scheme 2

endo-α-1,3-D-Glucanases

An *endo*-enzyme that hydrolyses the sticky α-(1 → 3)-linked D-glucan from *Streptococcus mutans* has been recovered from cultures of a *Flavobacterium* species by ion-exchange chromatography.[345] This basic enzyme (mol. wt. 6.5×10^4, p*I* 8.5, pH optimum 6.3, temperature optimum 42 °C) released isomaltose, nigerose, and nigerotriose from the *S. mutans* D-glucan, but was inactive towards D-glucans containing α-(1 → 6)-, α-(1 → 4)-, β-(1 → 3)-, β-(1 → 4)-, and β-(1 → 6)-linkages.

[339] R. H. Staat and C. F. Schachtele, *Infection and Immunity*, 1974, **9**, 467.
[340] B. Guggenheim and J. J. Burckhardt, *Helv. Odontol. Acta*, 1974, **18**, 101.
[341] M. Sugiura and A. Ito, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 3223.
[342] N. Wahba, J. W. Felch, J. W.-K. Shih, and J. H. Hash, *J. Biol. Chem.*, 1975, **250**, 3709.
[343] J. W. Felch, T. Inagami, and J. H. Hash, *J. Biol. Chem.*, 1975, **250**, 3713.
[344] S. Takasaki and A. Kobata, *J. Biol. Chem.*, 1976, **251**, 3603.
[345] S. Ebisu, K. Kato, S. Kotani, and A. Misaki, *J. Bacteriol.*, 1975, **124**, 1489.

endo-β-1,3-D-Glucanases

An *endo*-β-1,3-D-glucanase that acts on β-(1 → 3)-linked D-glucans has been extracted from malted barley and purified by ion-exchange chromatography.[346] Inhibition of the enzyme by group-specific reagents was investigated.

Adsorption chromatography was used to isolate a β-1,3-D-glucanase possessing high lytic activity from culture fluids of an *Arthrobacter* species, whereas a β-1,3-D-glucanase possessing much less lytic activity was not adsorbed.[347]

An *endo*-β-1,3-D-glucanase from *Nicotiana glutinosa* is inactive against reduced SIII pneumococcal polysaccharide, which is also hydrolysed by other β-D-glucan hydrolases.[3]

Plasmodia of the acellular slime mould *Physarum polycephalum* synthesize an *endo*-β-1,3-D-glucanase (mol. wt. 1.8×10^4, pH optimum 5.0, temperature optimum 40 °C).[348] Procedures for isolating this enzyme were reported.

An *endo*-β-1,3-D-glucanase isolated from culture filtrates of *Rhizopus chinensis* has been obtained in crystalline form.[349] Although the enzyme (mol. wt. 2.3×10^4, K_m for laminarin 3.4 mol l^{-1}) did not degrade the cell walls of living yeast, it degraded the yeast D-glucan.

exo-β-1,3-D-Glucanases

The synthesis and excretion into the culture medium of large amounts of an *exo*-β-1,3-D-glucanase by a *Basidiomycetes* species were triggered off by a critically low concentration of the source of carbon.[350] The addition of D-glucose or other sources of carbon to the culture, after the original source of carbon had been consumed, resulted in inactivation of the extracellular *exo*-β-1,3-D-glucanase by a system that could be separated from the cells. Although the enzyme is particularly stable, its electrophoretic mobility and immunological properties indicated that significant changes occur in the protein during inactivation.

An *exo*-β-D-1,3-glucanase prepared on a large scale from culture filtrates of a *Basidiomycetes* species was homogeneous on examination by disc gel electrophoresis (under non-dissociative conditions), ultracentrifugation, and isoelectric focusing.[351] Leucine and serine are present at the NH_2-terminus and alanine, serine, and glycine at the carboxy-terminus of this glucanase. The pH-profile showed that there are no titratable groups directly involved in substrate-binding at pH 2.5—8.0 and that a single group (pK_a 6.5) is involved in catalysis. Photo-oxidation studies, in conjunction with chemical modification of the enzyme, suggested that a histidine residue is located at the active site, and that a tryptophan residue, although not essential, may participate in catalysis.

Plasmodia of the acellular slime mould *Physarum polycephalum* produce an *exo*-β-D-1,3-glucanase (mol. wt. 1.4×10^4, pH optimum 5.0, temperature optimum 48 °C).[348] Procedures for isolating the enzyme were reported.

exo-β-1,3-D-Glucanases (mol. wt. 4.3×10^4) have been isolated from cell extracts and culture fluids of *Schizosaccharomyces japonicus* by gel filtration and

[346] D. J. Manners and G. Wilson, *Carbohydrate Res.*, 1976, **48**, 255.
[347] K. Doi, A. Doi, and S. Nakamura, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 1669.
[348] D. R. Farr. A. Schuler, and M. Horisberger, *Experientia*, 1975, **31**, 719.
[349] S. Yamamoto and S. Nagasaki, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2163.
[350] B. Friebe and A. W. Holldorf, *J. Bacteriol.*, 1975, **122**, 818.
[351] D. R. Peterson and S. Kirkwood, *Carbohydrate Res.*, 1975, **41**, 273.

ion-exchange chromatography.[352] The purified enzymes hydrolysed β-(1 → 6)- as well as β-(1 → 3)-D-glucosidic linkages, both activities being manifest by a single species.

An assay for yeast *exo*-β-D-1,3-glucanase activity in column eluates is based on treatment of an aliquot with laminarin on a plate of powdered paper, followed by reaction of the products of hydrolysis with silver nitrate, *etc.*[5]

endo-α-1,4-D-Glucanases

An enzyme isolated from *Pseudomonas statzeri* hydrolyses α-(1 → 4)-D-glucosidic linkages in amylose-like polysaccharides by the successive removal of maltotetraose units from the non-reducing end of the chain.[353] The enzyme was bound by α-(1 → 6)-linked dextrans, so that it could be easily separated from other proteins by adsorption onto Sephadex and elution with either a salt gradient or a buffer containing starch. The unexpected adsorption of this *exo*-amylase on Sephadex was compared with that of other amylases.

endo-β-1,4-D-Glucanases

The section on cellulases should also be consulted.

The degradation of barley D-glucan by a purified *endo*-β-1,4-D-glucanase isolated from a snail (*Helix pomatia*) has been reported.[354] The principal product of hydrolysis is β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-D-glucopyranose, which arises from regions of the D-glucan where two cellotetraose units are joined by a β-(1 → 3)-linkage.

A strong synergistic response was found when an *exo*- and five *endo*-β-1,4-D-glucanases [obtained from cultures of the rot fungus *Sporotrichum pulverulentum* (*Chrysosporium lignorum*)] acted on wax-free cotton and Avicel, whereas none was found with acid-swollen cellulose.[355] The *endo*-1,4-β-D-glucanases are considered to act randomly on the cellulose chain, thereby facilitating the action of the *exo*-1,4-β-D-glucanase.

exo-β-D-1,4-Glucanases

The action of an *exo*-1,4-β-D-glucanase from *Sporotrichum pulverulentum* (*Chrysosporium lignorum*) on celluloses showed a synergistic effect in the presence of *endo*-1,4-β-D-glucanases.[355] Products released by the *exo*-1,4-β-D-glucanase have the α-configuration.

An *exo*-D-glucanase, which accompanies a cellulase activity in *Sporocytophaga myxococcoides*, appears to release products of inverted anomeric configuration from the substrate.[319]

endo-β-1,6-Glucanases

An *endo*-β-1,6-glucanase isolated from culture filtrates of *Mucor hiemalis* has been purified by fractional precipitation and gel filtration.[356] The homogeneous

[352] G. H. Fleet and H. J. Paff, *Biochim. Biophys. Acta*, 1975, **410**, 318.
[353] H. Dellweg, M. John, and J. Schmidt, *European J. Appl. Microbiol.*, 1975, **1**, 191.
[354] J. J. Marshall, *Carbohydrate Res.*, 1975, **42**, 203.
[355] M. Streamer, K.-E. Eriksson, and B. Pettersson, *European J. Biochem.*, 1975, **59**, 607.
[356] T. Miyazaki and N. Oikawa, *Carbohydrate Res.*, 1976, **48**, 209.

enzyme is stable over wide ranges of temperature and pH, and exhibits a high substrate specificity.

exo-β-1,6-D-Glucanases

Both *exo*-β-1,6- and *exo*-β-1,3-D-glucanase activities present in cell extracts and culture fluids of *Schizosaccharomyces japonicus* appear to be manifest by the same species.[352]

D-Glucanases (Miscellaneous)

The properties of new starch-degrading enzymes have been reviewed.[12] Studies on glycogen-storage diseases, concerned mainly with α-D-glucosidases, have also included discussions on 'amylo-1,4-glucosidase' and 'amylo-1,6-glucosidase' activities.[160]

The properties of an amylase preparation (free from α-amylase activity) from human liver have been investigated.[357] This enzyme hydrolysed maltose, malto-oligosaccharides, starch, glycogen, and β-limit dextrin, liberating D-glucose from the non-reducing terminus, although it is unable to hydrolyse isomaltose. The enzyme is distinguishable from glucoamylase, since it has a much higher activity towards maltose than towards glycogen and starch; it appears to be similar to amylases present in urine.

A 'neutral' α-D-glucosidase from porcine serum preferentially hydrolysed malto-oligosaccharides.[162] The substrate specificity and kinetic properties, *etc.*, of a β-1,4-D-glucanase (mol. wt. 5.1×10^4, pH optimum 5.5—6.0, temperature optimum 45 °C) isolated from the intestinal juices of a snail (*Helix pomatia*) have been determined.[358] The purified enzyme, which degrades poly- and oligo-saccharides (d.p. >3) containing β-(1 → 4)- and β-(1 → 3)-linked D-glucosyl residues, was inhibited by H_4edta and appeared to be activated by Ca^{2+} ions.

A purified *endo*-β-D-glucanase from barley was found to be highly specific for the endogenous β-D-glucan (either in free or dyed form).[346] The enzyme had no action on β-(1 → 3)- or β-(1 → 4)-linked D-glucans and was inhibited by group-specific reagents.

D-Glucose was released from polysaccharides in grape skin and juice by at least one of the polysaccharide hydrolases present in 'Pectawamorin P10X'.[299]

The *endo*-β-D-glucanase and β-D-glucanase activities in preparations used in the brewing industry are highest at 50 and 40 °C, respectively, and, in some instances, the stabilities of the enzymes to heat were increased by the presence of substrates.[170]

An *exo*-enzyme (mol. wt. 5.4×10^4) isolated from *Aerobacter aerogenes* has been purified to homogeneity by disc electrophoresis, ion-exchange chromatography, and gel filtration.[359] The enzyme is stable over a wide range of pH, and at its optimum pH (6.80) and temperature (50 °C) it hydrolysed starch, amylose, and amylopectin to maltohexaose, but it did not hydrolyse cyclohexa- or

[357] K. Tsujino, M. Yoshimura, K. Umeki, N. Minamiura, and T. Yamamoto, *J. Biochem. (Japan)*, 1974, **76**, 1235.
[358] J. J. Marshall and R. J. A. Grand, *Comp. Biochem. Physiol.*, 1976, **53B**, 231.
[359] K. Kainuma, K. Wako, S. Kobayashi, A. Nogami, and S. Suzuki, *Biochim. Biophys. Acta*, 1975, **410**, 333.

cyclohepta-amyloses, pullulan, or maltohexaitol. The β-limit dextrins of amylopectin and glycogen were hydrolysed to branched oligosaccharides.

The actions of an 'isomalto-dextranase' from *Arthrobacter globiformis* on D-gluco-oligosaccharides and dextran have been reported.[360] The enzyme released isomaltose from some oligosaccharides by cleaving not only α-(1 → 6)-linkages, but also α-(1 → 2)-, α-(1 → 3)-, and α-(1 → 4)-linkages. The enzyme acts by an *exo*-mechanism – which was confirmed by analysis of the products released from isomaltohexaitol – and also cleaved α-D-glucopyranosyl-(1 → 2 and 3)-α-D-glucopyranosyl-(1 → 6)-D-glucose from a tetrasaccharide and D-glucans. A mechanism was suggested for the action of the enzyme on dextrans.

Gel filtration showed that the hemicellulase from *Aspergillus awamori* is a mixture of *exo*-xylanase and *endo*-glucanase activities.[361] Purification of the *endo*-glucanases was reported.

β-D-Glucanases have been detected in cell-free extracts of the yeast *Cryptococcus albidus* growing on D-glucose as the only source of carbon.[362] The synthesis of β-1,3- and β-1,6-D-glucanases by the cells is highest during the log and stationary phases of growth. On gel filtration of the enzymes, the β-D-glucanase activities showed profiles that depended on the period of growth, but all the fractions exhibited both β-1,3- and β-1,6-activities. A low-molecular-weight form of β-D-glucanase has a molecular weight of 2.1×10^3.

The synthesis of β-D-glucanase (*cf.* β-D-fructofuranosidase, p. 343) by either cells or protoplasts of the yeast *Pichia polymorpha* occurred in the presence of 2-deoxy-D-*arabino*-hexose.[86] The properties of some of the β-D-glucanase activities in *P. polymorpha* have been studied.[363]

Cultures of *Streptomyces chartreusis* and *S. werraensis* contain enzymes that readily solubilized the D-glucans elaborated by cariogenic Streptococci.[364] These enzymes may be useful in preventing and controlling the formation of dental plaque.

Glucoamylases

An 'acid' α-D-glucosidase activity isolated from human liver exhibits glucoamylase activity, which was competitively inhibited by methyl α-D-glucopyranoside, and 'maltase' activity.[155]

Porcine-serum α-D-glucosidase exhibits glucoamylase activity.[117] A simple procedure for isolating the 'glucoamylase–maltase' complex from rabbit intestines by affinity chromatography on Sephadex G-200 has been reported.[163] Other Sephadex gels did not bind the enzyme to any significant extent.

A strain of *Aspergillus awamori* selectively synthesized one of three types of glucoamylase, depending on the conditions of growth.[365] The glucoamylases

[360] M. Torii, K. Sakakibara, A. Misaki, and T. Sawai, *Biochem. Biophys. Res. Comm.*, 1976, **70**, 459.
[361] V. I. Rodzevich, M. V. Dalin, N. G. Portnova, and N. S. Mazur, *Priklad. Biokhim. i Mikrobiol.*, 1974, **10**, 861.
[362] V. Notario, T. G. Villa, T. Benttez, and J. R. Villanueva, *Canad. J. Microbiol.*, 1976, **22**, 261.
[363] T. G. Villa, V. Notario, and J. R. Villanueva, *Arch. Mikrobiol.*, 1975, **104**, 201.
[364] M. Inoue, T. Egami, K. Yokogawa, H. Kotani, and T. Morioka, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1391.
[365] S. Hayashida, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2093.

could be distinguished from one another by their actions on various starches. Modification of *A. awamori* glucoamylase with subtilisin gave a species which, although still appreciably active, exhibited starch-degrading properties that differ from those of the parent enzyme.[366]

A partially purified glucoamylase from *A. niger* has been characterized by biochemical, physicochemical, and optical methods.[367] Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate indicated that the enzyme consists of two principal components (mol. wts. 6.30×10^4 and 5.75×10^4). Small proportions of dissociated and aggregated species are also present, but the size of the monomer (3.0×10^4) was deduced from sedimentation studies, *etc.* Chemical modification of the enzyme indicated that tyrosyl residues are located at the active site. The tertiary structure of the molecule contains 15—25% of α-helix, as well as β-structure and disordered segments.

Glucoamylases from black *Aspergillus* species (*A. niger*, *A. cinnamomeus*, and *A. awamori*) were freed from all traces of α-amylase by gel filtration.[368] Glucoamylase I retained its ability to hydrolyse rabbit-liver glycogen rapidly, whereas glucoamylase II hydrolysed glycogen slowly. The addition of α-amylase to the digests did not enhance the activity of glucoamylase II towards glycogen, demonstrating that α-amylase is not involved in the hydrolysis of glycogen by this glucoamylase.

A β-amylase isolated from culture filtrates of *Bacillus polymyxa* released maltose from amylose-like substrates, but it had only limited or no action on amylopectin, periodate-oxidized amylose, and cycloamyloses.[369]

Two glucoamylases (I and II) have been obtained from a crude glucoamylase preparation from a *Rhizopus* species; glucoamylase II was less active than glucoamylase I towards raw starch.[370] The digestion of waxy corn starch by glucoamylase I – but not by glucoamylase II – was accelerated by intra- and extra-cellular pullulanases, and it seems that the presence of amylose in raw, non-waxy corn starch hinders the action of glucoamylase. The results of inhibition studies with the glucoamylase from *R. delemar* have been compared with those for α-amylases.[282]

The kinetics of the transient phase of the hydrolysis of maltodextrin (average d.p. 11) by *R. niveus* glucoamylase have been studied using a fluorescent stopped-flow method.[371] The fluorescence decreased rapidly on mixing solutions of the enzyme and the substrate, but slowly reappeared as the reaction proceeded; the two phases are considered to represent the formation of an enzyme–substrate complex and the release of the free enzyme on breakdown of the complex into products. The importance of tryptophanyl residues at the subsite of *R. niveus* glucoamylase has been studied by modifying them with *N*-bromosuccinimide.[372]

[366] S. Hayashida, T. Nomura, E. Yoshino, and M. Hongo, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 141.
[367] I. M. Freedberg, Y. Levin, C. M. Kay, W. D. McCubbin, and E. Katchalski-Katzir, *Biochim. Biophys. Acta*, 1975, **391**, 361.
[368] H. J. Gasdorf, P. Atthasampunna, V. Dan, D. E. Hensley, and K. L. Smiley, *Carbohydrate Res.*, 1975, **42**, 147.
[369] J. J. Marshall, *F.E.B.S. Letters*, 1974, **46**, 1.
[370] S. Ueda and R. Ohba, *Stärke*, 1976, **28**, 20.
[371] K. Hiromi, M. Ohnishi, and T. Yamashita, *J. Biochem.* (*Japan*), 1974, **76**, 1365.
[372] M. Ohnishi and K. Hiromi, *J. Biochem.* (*Japan*), 1976, **79**, 11.

An oligosaccharide (see p. 365) has been found to inhibit *Streptomyces diastaticus* α-amylase and *R. niveus* glucoamylase.[234]

Active immobilized forms of glucoamylase have been prepared by reaction of the enzyme with dialysis tubing that had been treated with cyanogen bromide [373] and with derivatives of carboxymethylcellulose, DEAE–cellulose, and agarose,[374] by cross-linking with glutaraldehyde to gelatin and other proteins using bentonite fillers,[375] by covalent attachment to porous silica particles,[376] by absorption onto DEAE–cellulose [377] and paper made from it,[373] and by entrapment in gels of poly(*N*-vinylpyrrolidone).[87]

An active, water-soluble, immobilized form of glucoamylase was obtained when the enzyme reacted with derivatives of testosterone.[378] Highly-charged, water-soluble conjugates of glucoamylase and a copolymer of either ethylene and maleic acid or styrene and maleic acid are absorbed onto particles of DEAE–cellulose to give immobilized derivatives of the enzyme.[379]

Glycanases (Miscellaneous)

A glycanase activity associated with *Klebsiella* bacteriophage No. 11 catalysed the depolymerization of the alkali-treated capsular polysaccharide of *Klebsiella* serotype 11 to oligosaccharides consisting of one or two repeating units.[380] The glycanase will probably also depolymerize all polysaccharides containing the unsubstituted chain-trisaccharide repeating unit present in the natural substrate.

A fungus, *Penicillium lilacineum*, isolated from soil synthesizes an enzyme that degraded the cell walls of red yeast.[337]

Enzymes induced by two *Rhizobium trifolii* bacteriophages depolymerized the *exo*-polysaccharides of most strains of *R. trifolii* and *R. leguminosarum*, but they did not attack the *exo*-polysaccharides of *R. meliloti* or slow-growing Rhizobia or *Agarobacterium* species.[381] Ca^{2+} or Mg^{2+} ions or both are required to activate the enzyme.

Heparin Hydrolases

An enzyme that degrades heparan sulphate is present in human platelets.[382] The presence of this enzyme in platelets, which do not contain endogenous heparan sulphate, suggests that platelets are able to degrade heparan sulphate in other cells (*e.g.* endothelial cells) in the vascular space.

A heparin-degrading enzyme isolated from a transplantable murine mastocytoma hydrolysed some of the bonds in macromolecular mastocytomal [^{35}S]heparin, yielding products similar in size to commercial heparins.[383]

[373] A. Emery, J. Sorenson, M. Kolarik, S. Swanson, and H. Lim, *Biotechnol. and Bioeng.*, 1974, **16**, 1359.
[374] J. Kučera and J. Hanus, *Coll. Czech. Chem. Comm.*, 1975, **40**, 2536.
[375] B. Solomon and Y. Levin, *Biotechnol. and Bioeng.*, 1974, **16**, 1393.
[376] D. D. Lee, Y. Y. Lee, and G. T. Tsao, *Stärke*, 1975, **27**, 384.
[377] E. N. Oreshkin, L. A. Nakhapetyan, and L. M. Vainer, *Priklad. Biokhim. i Mikrobiol.*, 1974, **10**, 856.
[378] K. Tateishi, H. Yamamoto, T. Ogiwara, C. Hayashi, and M. Kitagawa, *J. Biochem. (Japan)*, 1976, **80**, 191.
[379] B. Solomon and Y. Levin, *Biotechnol. and Bioeng.*, 1974, **16**, 1161.
[380] H. Thurow, H. Niemann, and S. Stirm, *Carbohydrate Res.*, 1975, **41**, 257.
[381] Y. M. Barnet and B. Humphrey, *Canad. J. Microbiol.*, 1975, **21**, 1647.
[382] A. Wasteson, M. Höök, and B. Westermark, *F.E.B.S. Letters*, 1976, **64**, 218.
[383] S. Ögren and U. Lindahl, *J. Biol. Chem.*, 1975, **250**, 2690.

Although the linkages cleaved in macromolecular heparin were only tentatively identified, the enzyme appears to act on regions of the chain containing 2-acetamido-2-deoxy-D-glucosyl residues. D-Glucopyranosyluronic acid residues are exposed by the action of this enzyme, which is a heparin hydrolase (an *endo*-D-glucuronidase).

Heparin Lyases and Heparan Sulphate Lyases

The isolation and partial characterization of a heparin lyase and two heparan sulphate lyases from *Flavobacterium heparinum* have been reported.[384]

The structure required for a polysaccharide to induce the synthesis of a heparin-degrading system by *F. heparinum* has been studied.[385] It was concluded that the polysaccharide must contain a uronic acid residue (with a free carboxy-group) attached to either a 2-acetamido-2-deoxy- or 2-deoxy-2-sulphamido-hexose residue by an α-(1 → 4)-linkage. Neither *O*- nor *N*-sulphated residues are essential for inducing the synthesis of this enzyme system. The reason why some heparan sulphates are more effective than heparin in inducing enzymic activity was discussed.

Hyaluronate Lyases

A hyaluronate lyase from group A Streptococci has been purified (1000-fold) by ion-exchange chromatography and gel filtration; two fractions with identical amino-acid compositions were obtained.[386] The enzyme was inhibited by *N*-(toluene-*p*-sulphonyl)phenylalanine chloromethyl ketone and *N*-(toluene-*p*-sulphonyl)lysine, and did not depolymerize hyaluronic acid methyl ester. Photo-oxidation in the presence of Methylene Blue inactivated the enzyme, whose action probably involves the transfer of a proton between a histidyl residue of the enzyme and a carboxy-group of the substrate.

Investigations of a streptococcal, bacteriophage-borne enzyme that depolymerizes hyaluronic acid have shown that it is a hyaluronate lyase.[387]

Hyaluronidases

A new semimicro assay for hyaluronidase has used [^{3}H]hyaluronic acid as a substrate.[388] After enzymic hydrolysis of the substrate, cetylpyridinium chloride is added to the hydrolysate to precipitate any unreacted substrate, and the soluble [^{3}H]oligosaccharides are then determined by scintillation counting.

The levels of lysosomal hyaluronidase in subcellular fractions of eye tissues during treatment with corticosteroids have been measured.[108]

The properties of the hyaluronidase in human synovial fluid have been compared with those of hyaluronidases from human sera and bovine testicles.[389] The pH optimum, molecular size, action pattern, substrate specificity, *etc.*, of the

[384] M. E. Silva and C. P. Dietrich, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 965.
[385] M. E. Silva and C. P. Dietrich, *Biochimie*, 1973, **55**, 1101.
[386] H. Greiling, H. W. Stuhlsatz, T. Eberhard, and A. Eberhard, *Connective Tissue Res.*, 1975, **3**, 135.
[387] H. Niemann, A. Birch-Andersen, E. Kjems, B. Mansa, and S. Stirm, *Acta Pathol. Microbiol. Scand.*, 1976, **84B**, 145.
[388] C. J. Coulson and R. Girkin, *Analyt. Biochem.*, 1975, **65**, 427.
[389] R. W. Stephens, J. Sutherland, and T. K. F. Taylor, *Proc. Austral. Biochem. Soc.*, 1975, **8**, 42.

hyaluronidases in human sera and synovial fluids differ from those of testicular hyaluronidase.[390]

The chondroitin sulphate in rat liver has been shown to be degraded by the combined actions of *endo*- and *exo*-glycosidases, whereas that in human fibroblasts – in which hyaluronidase activity is not detected – is degraded by *exo*-glycanases.[391]

The properties of a purified, homogeneous, bovine testicular hyaluronidase (pH optimum 5.0—5.5) have been studied using radiolabelled oligomers of *N*-acetylhyalobiouronic acid as substrates and acceptors.[392] Transfer of a glycosyl residue to an acceptor occurred with retention of configuration. On the basis of the cleavage and transglycosylation reactions, it was proposed that the active site of the enzyme consists of five subsites that bind disaccharide units.

The level of hyaluronidase activity in the seminal plasma has been used to assess the integrity of bulls' sperm.[393] A hyaluronidase isolated from bulls' sperm by fractional precipitation and ion-exchange chromatography was homogeneous on examination by disc gel electrophoresis.[394] The enzyme (mol. wt. 6.2×10^4, pH optimum 3.8, K_m for hyaluronic acid 3.7 mmol l^{-1}) has an absolute requirement for cations: K^+ and Na^+ ions are more effective than Ca^{2+}, Mg^{2+}, and Mn^{2+} ions, whereas Co^{2+}, Cu^{2+}, and Zn^{2+} ions are ineffective.

Greatly improved yields of the hyaluronidase in bovine seminal plasma have been obtained by fractional precipitation, ion-exchange chromatography, gel filtration (to remove β-D-glucuronidase and β-D-2-acetamido-2-deoxyglucosidase), and affinity chromatography on immobilized concanavalin A (indicating that the enzyme is a glycoprotein).[395] One main protein band was detected by electrophoresis on polyacrylamide gel.

The purification and properties of a hyaluronidase from ram-sperm acrosomes have been reported.[396]

A substrate-film technique has been used to examine the enzyme system that hydrolyses hyaluronic acid (and chondroitin sulphate) in isolated macrophages and in developing and healing tuberculosis lesions in rabbits.[397] The pH optimum of the enzyme shifted towards neutrality in 'intact' cells and tissues. The level of hyaluronidase in the kidneys of rats suffering from dehydration has been investigated.[398]

The levels of hyaluronidase in osteosarcoma homogenates from afflicted rats are higher in the tumour than in the sera and surrounding tissues.[66]

Hyaluronidase activity has been detected in the mucus accessory glands of drone bees (*Apis mellifera*); the pH and temperature optima of the enzyme are

[390] R. W. Stephens, J. Sutherland, P. Ghosh, and T. K. F. Taylor, *Biochem. Pharmacol.*, 1976, **25**, 1507.
[391] B. Arbogast, J. J. Hopwood, and A. Dorfman, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 376.
[392] S. Highsmith, J. H. Garvin, jun., and D. M. Chipman, *J. Biol. Chem.*, 1975, **250**, 7473.
[393] J. A. Foulkes and P. A. Watson, *J. Reprod. Fertility*, 1975, **43**, 349.
[394] C.-H. Yang and P. N. Srivastava, *J. Biol. Chem.*, 1975, **250**, 79.
[395] C.-H. Yang and P. N. Srivastava, *Biochim. Biophys. Acta*, 1975, **391**, 382.
[396] C.-H. Yang and P. N. Srivastava, *J. Reprod. Fertility*, 1974, **37**, 17.
[397] T. Tsuda, A. M. Dannenberg, M. Ando, O. Rojas-Espinosa, and K. Shima, *J. Reticuloentothelial Soc.*, 1974, **16**, 220.
[398] L. N. Ivanova, R. E. Goriunova, and V. P. Klimova, *Doklady Akad. Nauk S.S.S.R.*, 1975, **224**, 1209.

4.5 and 53 °C, respectively.[399] The enzyme also hydrolyses chondroitin 4- and 6-sulphates.

Isoamylases

A study of the metabolism of exogenous and endogenous carbohydrates by *E. coli* indicated that isoamylase, rather than pullulanase, degrades the glycogen in the cell, liberating maltodextrins.[272]

Keratan Sulphate Hydrolases

An enzyme that degrades keratan sulphate, porcine colonic mucin, and milk oligosaccharides has been isolated from *E. coli*.[144] Desialylized, porcine colonic mucin was degraded by this enzyme to 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose, 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl 6-sulphate)-D-galactose, and an enzyme-resistant polymer. Lacto-*N*-tetraose (8)

β-D-Gal*p*-(1 → 3)-β-D-Glc*p*NAc-(1 → 3)-β-D-Gal*p*-(1 → 4)-D-Glc

(8)

and lacto-*N*-tetraitol released D-glucose and D-glucitol, respectively, demonstrating that the enzyme is an *endo*-β-D-galactosidase (or *endo*-β-D-galactanase) possessing a broad substrate specificity.

Laminarinases

Laminarinase activity has been detected in culture filtrates of *Penicillium lilacinum*.[337]

The ability of *Rhizopus arrhizus* laminarinase to hydrolyse reduced SIII pneumococcal polysaccharide distinguishes it from other β-D-glucan hydrolases.[3] Laminaribiose is the principal product, although the corresponding tetra-, hexa-, and octa-saccharides, each having a 3-substituted D-glucosyl residue at the reducing end, are formed transiently.

Lichenanases

An enzyme (mol. wt. 2.7×10^4) that hydrolyses β-D-glucans and lichenan, but neither laminarin nor carboxymethylcellulose, has been obtained in partially purified form from a strain of *Bacillus pumius*; it cleaves a β-(1 → 4)-linkage next to a β-(1 → 3)-linkage in these polymers.[400] It was suggested that enzymes of this type should be named 'lichenanases' to distinguish them from cellulases and laminarinases.

The ability of *Bacillus subtilis* lichenanase to hydrolyse reduced SIII pneumococcal and other polysaccharides distinguishes it from other β-D-glucan hydrolases.[3] The enzyme behaved as a laminarinase (see above) towards the reduced SIII polysaccharide.

Limit Dextrinases

Gibberellic acid induced the *de novo* synthesis of limit dextrinase in barley grains with excised embryos.[167]

[399] D. Allalouf, A. Ber, and J. Ishay, *Experientia*, 1974, **30**, 853.
[400] H. Suzuki and T. Kaneko, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 577.

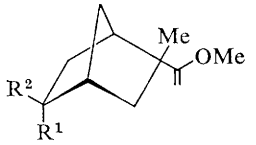
A limit dextrinase isolated from broad-bean (*Vicia faba*) flour readily hydrolysed branched α-dextrins containing maltosyl or maltotriosyl side-chains, pullulan, and amylopectin β-limit dextrin, whereas it hydrolysed glycogen β-limit dextrin and amylopectin slowly and glycogens not at all.[401]

The substrate specificities of purified limit dextrinases from ungerminated oats (*Avena sativa*) and rice (*Oryza sativa*) have been compared with that of a bacterial isoamylase.[402] The cereal enzymes are able to hydrolyse α-(1 → 6)-D-glucosidic linkages in oligosaccharides, α-dextrins, pullulan, amylopectin, and the β-limit dextrins of amylopectin and glycogen, but are unable to hydrolyse glycogens.

Lysozymes

A sensitive fluorometric assay for lysozyme uses as the substrate *Bacillus subtilis* cell walls that have been labelled with fluorescamine on the free amino-groups of the diaminopimelic acid residues.[403] The method is particularly suitable for measuring the competition between cell-wall preparations for the same enzyme.

Syntheses of the bicyclo[2,2,1]heptyl carboxylic acids (9) and (10) have been described, and it was argued that the electrostatic effect of the ionized *endo*-carboxylic acid group on the hydrolysis of (9) should be a reasonable model for



(9) $R^1 = CO_2H$; $R^2 = Me$
(10) $R^1 = Me$; $R^2 = CO_2H$

that of aspartic acid-52 in lysozyme on the hydrolysis of oligosaccharides.[404] The modest acceleration in the rate indicated that the developing carbonium ion is not significantly stabilized by the ionized carboxy-group in the hydrolysis of (9) and, by implication, in that of glycosidic linkages by lysozyme.[405]

Inhibitors directed towards the active site of lysozyme [22] and the effects of lysozymes on the ultrastructure of the cell walls of *Caryophanon lactum*[406] have been discussed.

Several bacteriolytic enzymes have been purified from crude animal and microbial extracts by chromatography on a lysate from the cell walls of *Micrococcus lysodeikticus* coupled to agarose cyclic imidocarbonate.[407] The enzymic and bacteriolytic properties of the purified enzymes were compared with those of hen egg-white lysozyme.

The hydrolysis of bacterial cell walls and oligosaccharides by the lysozyme from patients with leukaemia has been investigated in mixed aqueous and organic

[401] R. W. Gordon, D. J. Manners, and J. R. Stark, *Carbohydrate Res.*, 1975, **42**, 125.
[402] G. Dunn and D. J. Manners, *Carbohydrate Res.*, 1975, **39**, 283.
[403] G. Mintz, D. R. Herbold, and L. Glaser, *Analyt. Biochem.*, 1975, **66**, 272.
[404] D. E. Ryono and G. M. Loudon, *J. Amer. Chem. Soc.*, 1976, **98**, 1889.
[405] G. M. Loudon and D. E. Ryono, *J. Amer. Chem. Soc.*, 1976, **98**, 1900.
[406] W. C. Trentini and R. G. E. Murray, *Canad. J. Microbiol.*, 1975, **21**, 164.
[407] T. Yoshimoto, S. Hayashida, M. Tobiishi, K. Kado, and D. Tsuru, *J. Biochem.* (*Japan*), 1975, **78**, 253.

solvents and in solutions of high salt concentrations to see whether direct *X*-ray crystallography of the productive enzyme–substrate complex is feasible.[408] The lysozyme–substrate complex was shown to be stable indefinitely in mixed solvents and to form at the low temperatures required for crystallographic studies.

The free energy, enthalpy, and steady-state kinetics for the reaction of turkey egg-white lysozyme with *N*-acetylchito-oligosaccharides have been determined.[409] These oligosaccharides bind in the same way to turkey and hen egg-white lysozymes, except for the interactions involving glycine-101 and aspartic acid-101, respectively, in the enzymes. The essential identity of the active site of both enzymes allowed the thermodynamic properties associated with the formation of the two hydrogen-bonds between aspartic acid-101 and the substrate to be estimated. Changes occurring in the u.v. spectra of turkey and hen egg-white lysozymes with pH and on binding oligosaccharides have been examined.[410]

Hen and quail egg-white lysozymes have been purified to homogeneity by affinity chromatography on a lysate of the cell walls of *Micrococcus lysodeikticus* coupled to agarose.[411] The importance of tryptophanyl residues in the binding of substrates by lysozymes was discussed.

The specific heat of hen egg-white lysozyme as a function of the water-content has been determined.[412] Measurements of the specific heat were also carried out on aqueous solutions of hen egg-white lysozyme at different concentrations in order to obtain a value for the partial specific heat at infinite dilution, and the observed and calculated values were compared. The heats of dilution and saccharide-binding for hen egg-white lysozyme have been measured, and values for the associated thermodynamic parameters were then calculated.[413] The results are consistent with head-to-tail contact in the self-association of lysozyme. Glutamic acid-35, tryptophan-62, and other amino-acids are involved in intermolecular contact.

The real-space refinement procedure of Diamond (*Acta Cryst.*, 1971, **A27**, 436) has been applied to the structure of hen egg-white lysozyme.[414] The next stage of refinement of the atomic co-ordinates of lysozyme, known as energy refinement, has also been reported.[415]

Details of the conformation of the triclinical form of hen egg-white lysozyme have been reported; the triclinical and tetragonal crystal forms were compared using appropriately weighted difference maps at 2.5 Å resolution.[416] Another model of the triclinical structure was obtained using a real-space refinement procedure. Differences between the conformations of the two forms occur at regions on the surface involved in intermolecular contacts. Regeneration of the enzymic activity of the reduced peptide 1-127 from hen egg-white lysozyme

[408] P. Douzou, G. Hui Bon Hoa, and G. A. Petsko, *J. Mol. Biol.*, 1975, **96**, 367.
[409] S. K. Banerjee and J. A. Rupley, *J. Biol. Chem.*, 1975, **250**, 8267.
[410] T. Imoto, L. J. Andrews, S. K. Banerjee, A. Shrake, L. S. Forster, and J. A. Rupley, *J. Biol. Chem.*, 1975, **250**, 8275.
[411] T. Yoshimoto and D. Tsuru, *J. Biochem. (Japan)*, 1974, **76**, 887.
[412] J. Suurkuusk, *Acta Chem. Scand. (B)*, 1974, **28**, 409.
[413] S. K. Banerjee, A. Pogolotti, and J. A. Rupley, *J. Biol. Chem.*, 1975, **250**, 8260.
[414] R. Diamond, *J. Mol. Biol.*, 1974, **82**, 371.
[415] M. Levitt, *J. Mol. Biol.*, 1974, **82**, 393.
[416] J. Moult, A. Yonath, W. Traub, A. Smilansky, A. Podjarny, D. Rabinovich, and A. Saya, *J. Mol. Biol.*, 1976, **100**, 179.

showed that the carboxy-terminal dipeptide arginyl–leucine is not essential for the molecule to assume its natural three-dimensional structure.[417]

Laser Raman spectroscopy has been used to obtain spectra of aqueous solutions and of two crystalline forms of hen egg-white lysozyme.[418]

The c.d. spectra of hen egg-white lysozyme and derivatives having tryptophan-62 or -108 or both selectively oxidized have been measured as a function of pH.[419] Since neither of these tryptophanyl residues is chiefly responsible for the positive rotational strength in the region 280—300 nm and derivatization had little effect on the spectrum in the region 200—230 nm, it was necessary to reinterpret the c.d. spectra of lysozymes and α-lactalbumins.

The titration curve of ultrasonic absorption at 2.82 MHz in aqueous solutions of hen egg-white lysozyme has been analysed theoretically.[420, 421] The luminescence of hen egg-white lysozyme at 77 K has been compared with that of α-lactalbumin.[422]

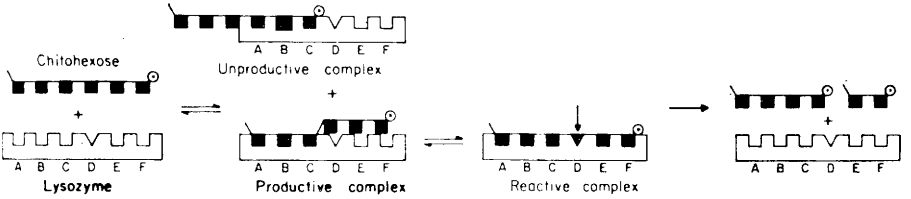
Denatured hen egg-white lysozyme and denatured bovine α-lactalbumin have been compared using difference spectroscopy, viscometry, polarimetry, and o.r.d. spectroscopy.[423] Treatment of lysozyme with lithium perchlorate, lithium chloride, guanidinium hydrochloride, or urea produced three denatured forms that are not members of a simple linear progression from the natural state to the completely disordered state. The study also indicated that the backbone conformations of lysozyme and α-lactalbumin are similar.

Flow and relaxation methods have been used to study the kinetics of oligosaccharide-binding to hen egg-white lysozyme and of the lysozyme-catalysed hydrolysis of chitohexaose.[424] In contrast to previous work, evidence for 'stable', productive lysozyme–substrate complexes was presented and the mechanism proposed (Scheme 3) accounts for differences in the efficiencies of the lysozyme-catalysed hydrolyses of chito-pentaose and -hexaose.

The binding of di-*N*-acetylchitobiose to hen egg-white lysozyme has been studied by n.m.r. spectroscopy and temperature-jump methods, which indicated a two-step mechanism for binding; values for the individual rate constants were determined.[425] The n.m.r. data characterize the environment experienced by the inhibitor at each stage of binding, thereby providing a dynamic, three-dimensional picture of the interaction.

Di-*N*-acetylchitobiose and 2-acetamido-2-deoxy-D-glucose are bound as the β-anomers in triclinical crystals of hen egg-white lysozyme and binding causes some of the lysozyme atoms to shift their positions.[426] Attempts to bind tri-*N*-acetylchitotriose in the crystals were not successful.

[417] E. R. Johnson, K.-J. Oh, and D. B. Wetlaufer, *J. Biol. Chem.*, 1976, **251**, 3154.
[418] L. Genzel, F. Keilmann, T. P. Martin, G. Winterling, Y. Jacoby, H. Frölich, and M. W. Makinen, *Biopolymers*, 1976, **15**, 219.
[419] F. Tanaka, L. S. Forster, P. K. Pal, and J. A. Rupley, *J. Biol. Chem.*, 1975, **250**, 6977.
[420] H. Kanda, N. Ookubo, H. Nakajima, Y. Suzuki, M. Minato, T. Ihara, and Y. Wada, *Biopolymers*, 1976, **15**, 785.
[421] H. Kanda, N. Ookubo, H. Nakajima, Y. Suzuki, M. Minato, T. Ihara, and Y. Wada, *Biopolymers*, 1976, **15**, 1641.
[422] J. N. Miller and L. A. King, *Biochim. Biophys. Acta*, 1975, **393**, 435.
[423] R. N. Sharma and C. C. Bigelow, *J. Mol. Biol.*, 1974, **88**, 247.
[424] E. Holler, J. A. Rupley, and G. P. Hess, *Biochemistry*, 1975, **14**, 2377.
[425] J. H. Baldo, S. E. Halford, S. L. Patt, and B. D. Sykes, *Biochemistry*, 1975, **14**, 1893.
[426] K. Kurachi, L. C. Sieker, and L. H. Jensen, *J. Mol. Biol.*, 1976, **101**, 11.



Scheme 3 *Graphical representation of the formation of the two main lysozyme–chitosaccharide complexes considered in the analysis of the data. The unproductive complex involves binding of the reducing end of the substrate (a) to site C of the enzyme and the adjacent pyranose rings bind to sites B and A and then protrude into the solvent. The productive complex involves binding of the non-reducing end of the substrate (b) to site A of lysozyme and the adjacent pyranose rings bind to sites B and C and then protrude into the D—F region of the molecule. The chair conformation of the saccharide is*



indicated by (c) and the half-chair conformation by (d). The reactive complex involves stabilization of the half-chair conformation of the pyranose ring which makes contact with site D by the geometry of this binding site.

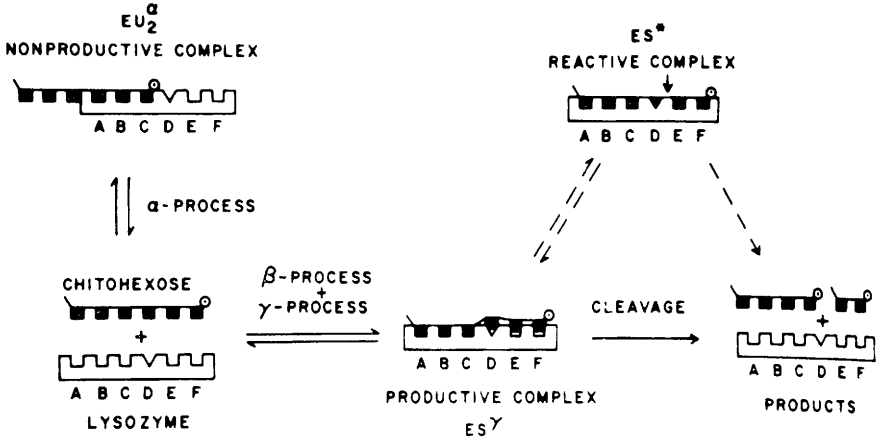
The binding of tri-*N*-acetylchitotriose to hen egg-white lysozyme has been studied by rapid-reaction kinetic methods that use the fluorescence of tryptophanyl residues to observe the formation of transients.[427] The results are consistent with the rapid formation of an initial complex, which perturbs the environment of tryptophan-62, followed by slower rearrangement of the complex, which perturbs the environment of tryptophan-108. The interaction of the ligand and lysozyme may be visualized as involving the initial formation of a complex across the top of the cleft of the active site, followed by the ligand 'diving' into the cleft.

Studies of the binding of an inhibitor to a deactivated form of hen egg-white lysozyme have reinforced the view that distortion of the sugar residue at site D is important in catalysis by lysozyme.[428] *X*-Ray diffraction has been used to study the binding of tetra-*N*-acetylchitotetraono-1,5-lactone (a transition-state analogue) in crystals of hen egg-white lysozyme.[429] This lactone binds to sites A—D, with the sugar residues at sites A, B, and C in positions similar to those occupied by tri-*N*-acetylchitotriose. An electron-density map for site D indicated that the lactone ring adopts a sofa (or boat) conformation having the hydroxymethyl group in axial orientation; an orientation slightly different from that proposed previously. These studies support the view that ring-strain plays a part in the reactions catalysed by lysozyme.

The effects of urea and guanidinium hydrochloride on the binding of bacterial substrates and inhibitors (*e.g.* 2-acetamido-2-deoxy-D-glucose and tetra-*N*-acetylchitotetraose) to hen egg-white lysozyme have been examined at 20 and 40 °C.[430]

[427] S. E. Halford, *Biochem. J.*, 1975, **149**, 411.
[428] C. R. Beddell, C. C. F. Blake, and S. J. Oatley, *J. Mol. Biol.*, 1975, **97**, 643.
[429] L. O. Ford, L. N. Johnson, P. A. Machin, D. C. Phillips, and R. Tjian, *J. Mol. Biol.*, 1974, **88**, 349.
[430] J. Saint-Blancard, M. Allary, S. Hartmann, D. Caquet, J. Berthou, and P. Jollès, *Biochimie*, 1975, **57**, 1163.

The location and occupation of the binding sites for Mn^{2+}, Co^{2+}, and Gd^{3+} ions in triclinical crystals of hen egg-white lysozyme have been determined accurately; the binding sites for Mn^{2+}ions in the di-*N*-acetylchitobiose–lysozyme complex did not differ significantly from those in the native protein, although fewer sites were occupied.[431] The binding of Cu^{2+} ions to aspartic acid-52 of hen egg-white lysozyme has also been studied.[432]



Scheme 4 *Schematic representation of the enzyme forms important for the lysozyme-catalysed cleavage of chitohexaose. The non-productive complexes* (EU_2^a) *formed in the α-process through binding of* (D-Glc*N*Ac*p*)$_3$ *or larger oligosaccharides are similar and involve the ABC sites of the enzyme with the reducing-end unit* (*a*) *at site C. Productive complexes formed in the β-process and the γ-process are observed with* (D-Glc*N*Ac*p*)$_4$ *and higher oligomers. The structures of these complexes are believed to be like those observed in the crystal for the disaccharides* D-Glc*N*Ac*p* → D-Glc*p and* D-Glc*N*Ac*p* → D-Xyl. *Thus, in productive complexes saccharide is bound at the ABC sites, as in the non-productive complexes, but with the non-reducing end group* (*b*) *at site A and with the three saccharide units at the reducing end extending into and partially*



filling the DEF region. It is considered likely that the contacts between enzyme and saccharide in the DEF region are not fully developed in ES^Y. *Full development of these contacts would come in a possible* (*but unobserved*) *reaction to give a reactive complex* (*ES**) *or alternatively the substrate might move fully into the cleft in the same process in which the glycosidic bond is broken.*

The reason for the biphasic lytic action of small concentrations of lysozyme on bacteria has been investigated.[433] Temperature-jump and stop-flow methods have been used to study the effect of temperature on the initial steps in the reaction of hen egg-white lysozyme with *N*-acetylchito-oligosaccharides.[434] The processes

[431] K. Kurachi, L. C. Sieker, and L. H. Jensen, *J. Biol. Chem.*, 1975, **250**, 7663.
[432] V. I. Teichberg, N. Sharon, J. Moult, A. Smilansky, and A. Yonath, *J. Mol. Biol.*, 1974, **87**, 357.
[433] I. A. Cherkasov, N. A. Kravchenko, P. E. Pavlovskii, and L. P. Bragina, *Biochemistry* (*U.S.S.R.*), 1974, **39**, 1087.
[434] S. K. Banerjee, E. Holler, G. P. Hess, and J. A. Rupley, *J. Biol. Chem.*, 1975, **250**, 4355.

depicted in Scheme 4 were proposed, based on the results of isotopic substitution, fluorescence spectroscopy, and thermodynamic calculations.

The role of tryptophan-62 in reactions catalysed by lysozyme has been investigated.[435] Studies on the regeneration of a reduced form of hen egg-white lysozyme by glutathione have yielded information on the folding mechanism of the reduced enzyme.[436] The structure of the reactive site surrounding the two central disulphide linkages of lysozyme has been investigated.[437]

The extent of decrease in immunochemical reactivity on succinylation of hen egg-white lysozyme, in conjunction with the results of conformational analysis, has indicated that lysine-33, -96, and -116 form part of the antigenic structure.[438]

Goat antibodies specific for hen egg-white lysozyme, the loop-region (residues 60—83), and other regions reacted with the loop-region of the intact enzyme.[439] Other egg-white lysozymes interfered in this reaction; for example, quail lysozyme was as reactive as hen lysozyme in the anti-lysozyme system but it was much less reactive with anti-loop antibodies, whereas turkey lysozyme was as reactive as hen lysozyme with anti-loop antibodies but it differed in its reactivity with anti-lysozyme. Thus, it appears that the loop-region of hen egg-white lysozyme is the most reactive of all the loop-regions, and the results were discussed in terms of the amino-acid sequences of these lysozymes.

The conformation, enzymic activity, and immunochemistry of hen egg-white lysozyme with two reduced carboxy-groups have been reported. It appears that aspartic acid-119 and the carboxy-terminal leucine residues do not form part of an antigenic structure of this enzyme.[440] Related studies of two disulphide peptides (residues 64—80 and 76—94) have indicated that the antigenic site in this region of lysozyme includes tryptophan-62 or -63 or both and lysine-96 or -97 or both.[441] The two disulphide peptides bring both of these regions into a single reactive site, so that they are essential in maintaining the reactivity of the site.

The effect of anhydrous hydrogen fluoride on the enzymic activity of hen egg-white lysozyme has been investigated.[442] One of the fractions recovered (by gel filtration) after treatment of lysozyme with anhydrous hydrogen fluoride retained the enzymic activity and was indistinguishable from the original enzyme.

The transformation of ^{3}H- or ^{131}I-labelled hen egg-white lysozyme following intestinal adsorption *in vitro*,[443] and the distribution in the tissues and the degradation of ^{131}I-labelled lysozyme by the kidneys [444] have been investigated.

The effect of hen egg-white lysozyme on the binding of DNA by the membranes of *E. coli* cell walls has been investigated.[445] The chromosomes of *E. coli* showed

[435] T. Imoto, M. Fujimoto, and K. Yagishita, *J. Biochem.* (*Japan*), 1974, **76**, 745.
[436] W. L. Anderson and D. B. Wetlaufer, *J. Biol. Chem.*, 1976, **251**, 3147.
[437] M. Z. Atassi, C.-L. Lee, and A. F. S. A. Habeeb, *Immunochemistry*, 1976, **13**, 7.
[438] C.-L. Lee, M. Z. Atassi, and A. F. S. A. Habeeb, *Biochim. Biophys. Acta*, 1975, **400**, 423.
[439] M. Fainaru, A. C. Wilson, and R. Arnon, *J. Mol. Biol.*, 1974, **84**, 635.
[440] M. Z. Atassi, A. M. Suliman, and A. F. S. A. Habeeb, *Biochim. Biophys. Acta*, 1975, **405**, 452.
[441] C.-L. Lee and M. Z. Atassi, *Biochim. Biophys. Acta*, 1975, **405**, 464.
[442] S. Aimoto and Y. Shimonishi, *Bull. Chem. Soc. Japan*, 1975, **48**, 3293.
[443] T. Yuzuriha, K. Katayama, and T. Fujita, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 1309.
[444] T. Yuzuriha, K. Katayama, and T. Fujita, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 1315.
[445] S. Silberstein and M. Inouye, *Biochim. Biophys. Acta*, 1974, **366**, 149.

three distinct sedimentation behaviours that depend on the concentration of lysozyme used for lysis of the cell walls. The binding of lysozyme with D-glucose and Sephadex has been investigated.[446]

Lysozyme from plaice (*Pleuronectes platessa*) serum has been purified by chromatography on chitin-coated and carboxymethyl-celluloses.[447] The enzyme, which was homogeneous on examination by polyacrylamide gel electrophoresis, has a molecular weight identical to that of hen egg-white lysozyme.

The activities of lysozyme in whole and in fractionated haemolymphs of the molluscs *Crassostrea virginica* and *Mercenaria mercenaria* have been measured; lysozyme released from haemolymph cells was detected in the sera of both species and may play a role in the defence mechanism of the cells.[190]

Some features of the primary structure[342] and the complete amino-acid sequence[343] of the lysozyme from a *Chalaropsis* species have been reported. The effects of body fluids and macromolecules on the lysis of group A Streptococci by *Streptomyces albus* lysozyme have been studied.[448] The purification and characterization of *S. albus* lysozyme ('*endo-N*-acetylmuramidase'), which degrades the cells of group A and other β-haemolytic Streptococci, have been reported.[449]

Active immobilized derivatives of lysozyme have been prepared by reaction of the enzyme with an activated, cross-linked poly(4-methacryloxybenzoic acid)[292] and with alginic acid cyclic imidocarbonate (this derivative displayed reversible insolubility–solubility characteristics with pH).[450]

exo-α-1,2-D-Mannanases

Flavobacterium dormitator growing on a D-mannan synthesized an *exo*-α-1,2-D-mannanase; the purified enzyme (mol. wt. 9×10^5, pH optimum 7.0, K_m for yeast mannan 0.16% w/v) was stable at pH 6.0, was not activated by Zn^{2+} ions, and was highly specific for the α-(1 → 2)-linkages in yeast mannan.[451]

endo-α-1,6-D-Mannanases

A *Bacillus* species growing on an unbranched (1 → 6)-linked α-D-mannan secreted an *endo*-1,6-D-mannanase; the purified enzyme consists of a single protein chain (mol. wt. 1.31×10^5), is unusually stable to heat, and appears to be highly extended (*i.e.* it possesses very little α-helicity and a high proportion of β-structure).[452] This mannanase acted on unbranched α-(1 → 6)-linked D-mannans to give D-mannose and α-(1 → 6)-linked D-mannobiose, with the intermediate formation of D-manno-oligosaccharides. The smallest substrate for the enzyme is α-(1 → 6)-linked D-mannotriose, although the reduced trisaccharide inhibited the enzyme. The binding site appears to encompass 6—8 D-mannosyl residues and Ca^{2+} ions are required to activate fully the enzyme.

[446] A. G. Ogston, W. H. Sawyer, and D. J. Winzor, *Proc. Austral. Biochem. Soc.*, 1975, **8**, 23.
[447] T. C. Fletcher and A. White, *Comp. Biochem. Physiol.*, 1976, **55B**, 207.
[448] I. Ginsburg, *Infection and Immunity*, 1975, **11**, 869.
[449] B. Heymer and W. Schmidt, *Microbios*, 1975, **12**, 51.
[450] M. Charles, R. W. Coughlin, and F. X. Hasselberger, *Biotechnol. and Bioeng.*, 1974, **16**, 1553.
[451] S. Yamamoto and S. Nagasaki, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1981.
[452] T. Nakajima, S. K. Maitra, and C. E. Ballou, *J. Biol. Chem.*, 1976, **251**, 174.

Mannanases (Miscellaneous)

Germinating lettuce (*Lactuca sativa*) seeds synthesize a β-D-mannanase that hydrolyses the D-mannan in the endosperm wall.[453]

The β-D-mannanase activities in germinating seeds of lucerne, guar, carob, and soybean could be separated from the accompanying α-D-galactosidase activities only with difficulty.[125] The role of the β-D-mannanases in degrading the galactomannan in cotyledon embryos was discussed.

An *exo*-D-mannanase activity that liberates D-mannose from the polysaccharides in grape juice is present in 'Pectawamorin P10X'.[299]

Mycodextranases

Mycodextranase, an extracellular *endo*-α-1,4-D-glucanase synthesized by *Penicillium melinii*, has been identified as a glycoprotein containing D-glucose and D-mannose in the molar ratio 2 : 1.[454] At least 80% of the carbohydrate was released on treatment of the enzyme with alkaline borohydride, with concomitant destruction of serine and threonine residues. D-Mannose residues are involved in the glycopeptide linkages. Smith degradation, methylation analysis, and digestion with glycoside hydrolases indicated that the carbohydrate side-chains in mycodextranase contain D-mannose, 2-*O*-α-D-glucopyranosyl-D-mannose, and α-D-mannopyranosyl-(1 → 2)-α-D-glucopyranosyl-(1 → 2)-D-mannose. Each enzyme molecule possesses 25 side-chains, which are attached in clusters to a few threonine-rich regions in the protein chain, rather than being separated by non-glycosylated regions.

Neoagarases

The degradation of neoagaro-tetraose and -biose by *Cytophaga flevensis* has been investigated.[455] This organism synthesizes an enzyme that hydrolyses the tetrasaccharide to neoagarobiose, which is then cleaved to D-galactose and 3,6-anhydro-L-galactose. Partially purified preparations of the enzyme contained both neoagarotetra-ase (pH optimum 7.0) and neoagarobiase (pH optimum 6.75) activities. Although both activities were inhibited by Ag^{+}, Hg^{2+}, and Zn^{2+} ions and by 4-chloromercuribenzoate, the substrate specificities indicated that they are distinct species.

A neoagarobiase activity isolated from *Pseudomonas atlantica* hydrolysed 4-nitrophenyl α-D-galactopyranoside and neoagarotetraose, and was stimulated by Na^{+} ions.[148]

Oligo-1,6-D-glucosidases

Amino-acid sequences around the essential carboxylic acid group in the active sites of the sucrose α-D-glucohydrolase:oligo-1,6-glucosidase (sucrase–isomaltase) complex of rabbit small intestines have been determined by selective labelling with [^{3}H]conduritol-B epoxide.[456]

[453] P. Halmer, J. D. Bewley, and T. A. Thorpe, *Nature*, 1975, **258**, 716.
[454] A. L. Rosenthal and J. H. Nordin, *J. Biol. Chem.*, 1975, **250**, 5295.
[455] H. J. van der Meulen and W. Harder, *J. Microbiol. Serol.*, 1976, **42**, 81.
[456] A. Quaroni and G. Semenza, *J. Biol. Chem.*, 1976, **251**, 3250.

The role of oligo-1,6-D-glucosidase in the metabolism of exogenous and endogenous carbohydrates by *Aerobacter aerogenes*, *E. coli*, and *Streptococcus mutans* has been discussed.[272]

Pectate, Pectin, and Poly-D-galacturonate Lyases

The pectin lyase from *Aspergillus japonicus* has been used in determining the structure of a polysaccharide of plant origin,[457] and cellular and environmental factors affecting the synthesis of polygalacturonate lyase by *Bacillus subtilis*[458] and *Fusarium oxysporum*[459] have been discussed.

The pectolytic enzymes synthesized by *Phytophthora parasitica* are pectin lyases rather than polygalacturonases.[460]

The action of a storage rot pathogen (*Phoma betae*) on harvested sugar beets (*Beta vulgaris*) depends, in part, on the synthesis of an *endo*-poly-D-galacturonate lyase.[461]

Optimal conditions for the synthesis of pectin lyase by *Rhizoctania solani* have been sought,[317] and pectate lyase has been found in *Sphaeropsis malocum* growing on apple tissues.[318]

Pentosanases

The temperature optimum (40 °C) and thermal stabilities of pentosanases used in the brewing industry have been compared with those of other endogenous polysaccharide hydrolases and β-D-glucosidase.[170]

Poly-D-galacturonases

The action of a poly-D-galacturonase on a triazinyl derivative of pectin has been investigated,[462] and *endo*-poly-D-galacturonase has been used in determining the structure of a polysaccharide of plant origin.[457]

An enzyme that hydrolyses pectic acid has been found in several phytophagous bugs.[121]

A poly-D-galacturonase activity in extracts of citrus oranges (*Citrus sinensis*) has been separated by gel electrophoresis.[307]

One of two pectolytic enzymes present in ripe D'Anjou pears is a poly-D-galacturonase that randomly cleaves pectate chains.[463] The enzyme (pH optimum 4.5) was separated from the accompanying *exo*-polygalacturonase by gel filtration.

The activity of poly-D-galacturonase in tomatoes stored at various temperatures has been determined; it is low in mature green fruit but increased rapidly at *ca.* 25 °C, although this pattern was not observed at other temperatures.[464] It was concluded that the storage-life of tomatoes can be extended by keeping them at 33 °C for several days, since the poly-D-galacturonase activity is then

[457] T. Kikuchi and H. Sugimoto, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 87.
[458] W. M. Kurowski and J. A. Dunleavy, *European J. Appl. Microbiol.*, 1976, **2**, 103.
[459] L. Ferraris, A. Garibaldi, and A. Matta, *Phytopathol. Z.*, 1974, **81**, 1.
[460] H. C. Dube and H. M. Gour, *Current Sci.*, 1975, **44**, 134.
[461] W. M. Bugbee, *Canad. J. Botany*, 1975, **53**, 1347.
[462] C. S. Lee and K. Maekawa, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 785.
[463] R. Pressey and J. K. Avants, *Phytochemistry*, 1976, **15**, 1349.
[464] N. Ogura, H. Nakagawa, and H. Takehana, *J. Agric. Chem. Soc. Japan*, 1975, **49**, 271.

suppressed. An *endo*-poly-D-galacturonase isolated from ripening tomato fruit degraded pectic acid, but not pectin, and did not possess eliminase activity.[465]

The synthesis of pectolytic enzymes by *Alternalia* species during the pathogenesis of tomato fruit [309] and by *Aspergillus carbonarius* [466] has been investigated.

Two poly-D-galacturonases have been obtained from cultures of *Botrytis cinerea*.[467] One of them (pH optimum 4.5, temperature optimum 50 °C) hydrolysed roughly 40% of the glycosidic linkages in sodium polypectate, but it did not hydrolyse oligogalacturonides of d.p. <5, whereas the other (pH optimum 4.0, temperature optimum 45 °C) hydrolysed sodium polypectate (89%) and oligogalacturonides of d.p. 2—4. Tetranitromethane and 4-chloromercuribenzoate inhibited both activities, although cysteine reactivated the latter.

Two extracellular poly-D-galacturonases have been isolated from culture fluids of *Botrytis cinerea*.[468] One of them (mol. wt. 3.7×10^4) specifically hydrolysed sodium polypectate, whereas the other (mol. wt. 6.9×10^4) hydrolysed sodium polypectate and pectin, but was accompanied by pectinesterase activity.

Conditions affecting the *in vitro* synthesis of poly-D-galacturonase by *Fusarium oxysporum* have been studied,[459] and the enzyme has been isolated by ion-exchange chromatography.[469] The preparation contained two distinct 'isoenzymes' that behaved as charge isomers on polyacrylamide gel electrophoresis. Some properties of the isoenzymes (mol. wt. 3.7×10^4), which contain covalently linked carbohydrate residues, were compared.

The synthesis of an extracellular poly-D-galacturonase by *Pyrenochaeta lycopersici* is regulated under growth-limiting conditions.[470] The role of pectolytic enzymes during invasion of soybeans by *Rhizobium japonicum* has been investigated.[316]

Optimal conditions for the synthesis of poly-D-galacturonase by *Rhizoctonia solani* have been sought,[317] and poly-D-galacturonase activity has been found in cultures of *Sphaeropsis malorum* growing on apple tissues.[318]

exo-Poly-D-galacturonases

The *exo*-poly-D-galacturonases extracted from carrots and peaches have different molecular weights, pH optima, and responses to cations.[471] Neither enzyme could completely hydrolyse citrus pectate and polygalacturonates, although the extents of degradation achieved were similar. The rate of hydrolysis and extent of substrate-binding increased with increasing molecular weight of the substrate, but the enzyme from peaches exhibited greater affinity for substrates.

One of two pectate-hydrolysing enzymes present in ripe D'Anjou pears is an *exo*-poly-D-galacturonase that catalyses the step-wise removal of monosaccharides from the non-reducing end of the substrate.[463] The enzyme (pH optimum 5.5), which is activated by Ca^{2+} and Sr^{2+} ions, is most active towards large substrate molecules.

[465] W. J. Hunter and G. H. Elkan, *Phytochemistry*, 1974, **13**, 2725.
[466] K. R. Sreekantiah, S. A. Jaleel, D. Narayanarao, and R. M. Raghavendra Rao, *European J. Appl. Microbiol.*, 1975, **1**, 173.
[467] H. Urbanek and J. Zalewska-Sobczak, *Bull. Acad. polon. Sci., Sér. Sci. Biol.*, 1975, **23**, 669.
[468] J. Zalewska-Sobczak and H. Urbanek, *Bull. Acad. polon. Sci., Sér. Sci. Biol.*, 1975, **23**, 663.
[469] L. L. Strand, M. E. Corden, and D. L. MacDonald, *Biochim. Biophys. Acta*, 1976, **429**, 870.
[470] P. W. Goodenough and R. J. Kempton, *Phytopathol. Z.*, 1974, **81**, 78.
[471] R. Pressy and J. K. Avants, *Phytochemistry*, 1975, **14**, 957.

The enzyme in 'Pectawamorin P10X' that releases D-galacturonic acid from the polysaccharides in the juice and skin of grapes is probably an *exo*-poly-D-galacturonase.[299]

An *exo*-poly-D-galacturonase that hydrolyses D-galacturonans at the non-reducing end has been isolated from culture filtrates of *Aspergillus niger*.[472] The purified enzyme (activity and stability optimum pH 5.2) degraded bis-(D-galacturonic acid), 4-nitrophenyl α-D-galactopyranosiduronic acid, and oligogalacturonides containing terminal, non-reducing residues of 4-deoxy-α-L-*threo*-hex-4-enopyranosyluronic acid.

exo-Poly-D-galacturonase activity has been found in cultures of *Sphaeropsis malorum* growing on apple tissues.[318]

Pullulanases

Intracellular and extracellular pullulanases accelerated the hydrolysis of waxy corn starch by the glucoamylase of a *Rhizopus* species, but they are less active towards non-waxy corn starch.[370]

A strain of *Bacillus cereus* synthesizes a pullulanase and a β-amylase, which together convert starch into maltose in high yield.[296] The homogeneous pullulanase was obtained by fractional precipitation, adsorption onto starch and Celite, and gel filtration.[297] The purified enzyme (pH optimum 6.0—6.5, mol. wt. $1.10 \pm 0.20 \times 10^5$) released maltose, maltotriose, and maltotetraose from β-limit dextrin and maltotriose from pullulan, but it did not release amylose-like substances from amylopectin. The enzymic activity was inhibited by mercuric chloride and 4-chloromercuribenzoate, although the activity in the latter instance could be restored by cysteine.

A study of the metabolism of exogenous and endogenous carbohydrates by *E. coli* showed that pullulanase and other enzymes are induced by maltodextrins.[272] The pullulanase, which appears to be bound to the cell wall, has properties similar to those of *A. aerogenes* pullulanase. It is suggested that *E. coli* uses pullulanase for debranching extracellular polysaccharides.

Pullulanase was converted into an active immobilized form when it reacted with a copolymer of acrylic acid and acrylamide.[298]

Sucrose α-D-Glucohydrolases

Some of the amino-acid sequences surrounding the essential carboxylic acid group in the active sites of the sucrose α-D-glucohydrolase:oligo-1,6-D-glucosidase (sucrase–isomaltase) complex of rabbit small intestines have been determined by selective labelling with [^{3}H]conduritol-B epoxide.[456]

The rate of synthesis of sucrose α-D-glucohydrolase is decreased in the small intestines of thyroidectomized and adrenalectomized rats.[64]

αα-Trehalases

The release of αα-trehalase from the vesicles of brush-border membranes in human intestines by various enzymes, especially pancreatic proteases, has been studied.[81]

[472] K. Heinrichová and Ľ. Rexová-Benková, *Biochim. Biophys. Acta*, 1976, **422**, 349.

αα-Trehalases (each of mol. wt. 9.4×10^4, p*I* 4.8) have been isolated from human and porcine kidneys by extensive gel chromatography, ion-exchange chromatography, and preparative gel electrophoresis.[473] The effects of *pseudo*-phloretins and temperature on the enzymes' kinetics were studied; the effect of pH on V_{max} suggested that carboxylic acid and imidazole groups are located at the active sites. The enzymes interacted with immobilized concanavalin A.

An αα-trehalase solubilized with Triton X-100 and butanol from microvillous membranes of the intestinal mucosa of rats has been purified by gel filtration and ion-exchange chromatography to yield a fraction (mol. wt. 9.6×10^4 in the presence of sodium dodecyl sulphate) that was homogeneous on polyacrylamide gel electrophoresis.[474] The enzyme (pH optimum 5.5—5.7), which shows a high specificity for αα-trehalose (K_m 5.4 mmol l^{-1}), was inhibited by 4-chloromercuribenzoate, mercuric chloride, and tris(hydroxymethyl)aminomethane.

Free and bound forms of the αα-trehalase in honey-bee (*Apis mellifera*) thorax have been purified to electrophoretic homogeneity.[475] Although both forms possess identical specificities, inhibition characteristics, pH optima (both biphasic at pH 3.5 and 6.5), molecular weights (6.5×10^4), and p*I* values (5.1), *etc.*, they appear to contain different subunits.

Xylanases (Miscellaneous)

The level of xylanase activity (pH optimum 7.1, temperature optimum 31 °C) present in cell-free filtrates of sheep-rumen liquor changes during the feeding cycle.[476] Many reagents, particularly heavy-metal ions and phenols, inhibited this activity, which was enhanced by reducing agents. No activity towards disaccharides or glycosides was found. The crude xylanase was degraded by proteolytic enzymes in rumen liquor, but was stable after purification. The preparation 'Pectawamorin P10X' released D-xylose from the polysaccharides in grape skin, presumably due to the presence of an *exo*-xylanase.[299]

Xylanases occur in the multicomponent hemicellulase system of *Aspergillus awamori*; the purification and molecular properties of the xylanases were described.[361] The most active of three xylanases from *A. niger* converted D-xylo-oligosaccharides into D-xylose and D-xylobiose.[301] Some of the xylanases exhibited arabinanase or glycosylating activities or both.

Recommendations have been made for selecting strains of a *Basidiomycetes* species that synthesize high levels of xylanase.[477]

An extracellular *endo*-hemicellulase (pH optimum 5.1, temperature optimum 80 °C) isolated from cultures of the fungal plant pathogen *Ceratocystis paradoxa* has been purified by isoelectric focusing, *etc.*[478] The homogeneous protein, which contained traces of a β-D-fructofuranosidase activity, randomly degraded hemicellulases to D-xylose, arabinoxylo-oligosaccharides (d.p. 3—6), and β-(1 → 4)-linked D-xylo-oligosaccharides (d.p. 2—5). D-Xylotriose is the

[473] J. Labat, F. Baumann, and J. E. Courtois, *Biochimie*, 1974, **56**, 805.
[474] K. Sasajima, T. Kawachi, S. Sato, and T. Sugimura, *Biochim. Biophys. Acta*, 1975, **403**, 139.
[475] B. G. Talbot, J. G. Muir, and R. E. Huber, *Canad. J. Biochem.*, 1975, **53**, 1106.
[476] I. M. Morrison, *Carbohydrate Res.*, 1976, **47**, 129.
[477] M. Kubačková, Š. Karácsony, and J. Váradi, *Folia Microbiol.*, 1975, **20**, 29.
[478] R. F. H. Dekker and G. N. Richards, *Carbohydrate Res.*, 1975, **42**, 107.

smallest D-xylo-oligosaccharide that is attacked at a significant rate; it yields D-xylose and D-xylobiose.

Conditions for the synthesis of a xylanase by one (*Malbranchea pulchella*) of several xylanase-producing thermophilic fungi have been improved.[479] The enzyme (pH optimum 6—8, temperature optimum 55 °C) is thermally stable and hydrolysed D-xylan principally to D-xylose (93%). Xylanases from several fungi (*Aspergillus niger*, *Chaetomium trilaterale*, *Trichoderma viride*, and *M. pulchella*) have been tested for their ability to hydrolyse the oligosaccharides remaining after D-xylan had been degraded with a xylanase from a *Streptomyces* species.

The xylanase system of a *Streptomyces* species converted hardwood xylan principally into a mixture of D-xylose and D-xylobiose; crystalline D-xylobiose was obtained following the enzymic removal of D-xylose.[480]

A xylanase has been isolated from a wood-rot fungus *Trametes hirsuta*,[481] and the subunits of a xylanase from *Trichoderma viride* have been fractionated by preparative electrophoresis on polyacrylamide gel.[482]

Carbohydrate Dehydrogenases

D-Arabinose Dehydrogenase.—D-Arabinose dehydrogenase (isolated from a *Pseudomonas* species growing on D-arabinose) has been used in specific micro-assays of D-arabinose and L-fucose.[483]

Carbohydrate Isomerases

Arabinose Isomerases.—The activity of a D-arabinose isomerase (L-fucose isomerase) obtained from *Klebsiella aerogenes* was inhibited by tris and its analogues, which had no action on the L-arabinose isomerase present.[484]

D-Glucose Isomerases.—Attention has been drawn to some of the problems encountered in the enzymic transformation of starch into D-fructose syrups.[33]

D-Glucose isomerase has been converted into active immobilized forms by adsorption onto Duolite A7 (a phenol–formaldehyde resin),[485] by entrapment in cellulose fibres,[155] and by adsorption onto the internal surface of controlled-pore alumina.[486] The possibility of using an immobilized form of D-glucose isomerase in the continuous production of D-fructose from D-glucose has been investigated.[487]

Carbohydrate Oxidases

Cellobiose Oxidases.—The synthesis of an extracellular cellobiose oxidase (cellobiose dehydrogenase, cellobiose: quinone oxidoreductase) parallels that of the cellulase activity when *Chrysoporium lignorum* and *Polyporus versicolor*

[479] M. Matsuo, T. Yasui, and T. Kobayashi, *J. Agric. Chem. Soc. Japan*, 1975, **49**, 263.
[480] I. Kusakabe, T. Yasui, and T. Kobayashi, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1355.
[481] M. Kubačková and Š. Karácsonyi, *Folia Microbiol.*, 1976, **21**, 28.
[482] S. Hashimoto and M. Funatsu, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 635.
[483] K. Yamanaka, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2227.
[484] K. Yamanaka and K. Izumori, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 439.
[485] Y. Yokote, K. Kimura, and H. Samejima, *Stärke*, 1975, **27**, 302.
[486] R. A. Messing and A. M. Filbert, *J. Agric. Food Chem.*, 1975, **23**, 920.
[487] L. Zittaz, P. B. Poulsen, and S. H. Hemmingsen, *Stärke*, 1975, **27**, 236.

grow on cellulosic substrates.[312] The oxidase (pH optimum 4.5—5.0) from *C. lignorum* catalyses a reaction in which quinone is reduced to the corresponding phenol and cellobiose is oxidized to cellobionic acid, probably by way of cellobiono-1,5-lactone. Lactose is oxidized by the enzyme at a lower rate, but no other mono- or di-saccharides served as substrates.

An oxidase that participates in the degradation of cellulose has been found in culture filtrates of *Sporotrichum pulverulentum*.[321] The action of the 'cellulose oxidase' considerably enhanced the rate of hydrolysis of cellulose by *endo*- and *exo*-D-glucanases, and an overall sequence involving these cellulases was suggested.

D-Galactose Oxidases.—E.s.r. spectroscopic measurements have indicated that trivalent copper is probably an intermediate in reactions catalysed by D-galactose oxidase.[488]

D-Glucose Oxidases.—The radical produced aerobically in the reaction catalysed by D-glucose oxidase reduced oxidized Cytochrome C, and additional evidence was presented for the formation of hydrogen peroxide in the enzyme-catalysed reaction.[489]

The D-glucose oxidase from *Aspergillus niger* binds concanavalin A to form an enzymically active precipitate, which is dissociated by methyl α-D-glucopyranoside.[490] The adsorption, desorption, and activity of this D-glucose oxidase on selected clays followed the pattern exhibited by other proteins, with maximum adsorption onto the clay occurring at, or below, the isoelectric point.[491]

D-Glucose oxidase has been immobilized, with retention of activity, by reaction with agarose cyclic imidocarbonate,[492] by covalent attachment to derivatives of attapulgites, Kieselguhr, *etc.*,[493] alkylamino [494] and diazonium [495] derivatives of glass, and vanacryl and covanacryl (polyaldehyde) polymers,[88] and by entrapment in polyacrylamide gel.[496, 497] D-Glucose oxidase has been immobilized together with catalase (by adsorption within controlled-pore titania) [498] and with alkaline phosphatase (by adsorption on platinum within a nylon net).[499]

Carbohydrate Transferases

Cyclodextrin D-Glucanotransferases.—The 'neutral' cyclodextrin D-glucanotransferase from an alkaliphilic *Bacillus* species has been purified by adsorption onto starch, ion-exchange chromatography, gel filtration, and polyacrylamide gel electrophoresis.[500] The enzyme (mol. wt. 8.5—8.8 × 10^4 in the presence of sodium dodecyl sulphate) is most active at pH 7 and 50 °C, is thermally stable, and converts starches and glycogens principally into cyclohepta-amylose.

[488] G. R. Dyrkacz, R. D. Libby, and G. A. Hamilton, *J. Amer. Chem. Soc.*, 1976, **98**, 626.
[489] K. Kovács, B. Hanusz, and B. Matkowics, *Enzyme*, 1975, **20**, 123.
[490] D. T. Dorai, S. Bishayee, and B. K. Bachhawat, *Indian J. Biochem. Biophys.*, 1975, **12**, 4.
[491] H. W. Morgan and C. T. Cooke, *Canad. J. Microbiol.*, 1976, **22**, 684.
[492] R. A. Valyulis, A. A. Glemzha, and V. V. Trakimene, *Biochemistry* (*U.S.S.R.*), 1975, **40**, 765.
[493] P. F. Greenfield, J. R. Kittrell, and R. L. Laurence, *Analyt. Biochem.*, 1975, **65**, 109.
[494] K. Mosbach, B. Danielsson, A. Borgerud, and M. Scott, *Biochim. Biophys. Acta*, 1975, **403**, 256.
[495] W. Dritschilo and M. K. Weibel, *Biochem. Med.*, 1974, **9**, 32.
[496] B. Atkinson and D. E. Lester, *Biotechnol. and Bioeng.*, 1974, **16**, 1299.
[497] B. Atkinson and D. E. Lester, *Biotechnol. and Bioeng.*, 1974, **16**, 1321.
[498] R. A. Messing, *Biotechnol. and Bioeng.*, 1974, **16**, 897.
[499] G. G. Guilbault and M. Najo, *Analyt. Chim. Acta*, 1975, **78**, 69.
[500] N. Nakamura and K. Horikoshi, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 1785.

A purified extracellular cyclodextrin D-glucanotransferase (pH optimum 4.6, p*I* 5.4, mol. wt. 8.8×10^4) from an alkaliphilic *Bacillus* species has been shown to be a single homogeneous protein by polyacrylamide gel electrophoresis and ultracentrifugation.[501] The K_m values for cyclohexa-, cycloheptа-, and cyclo-octa-amyloses at a constant concentration of sucrose are 5.88, 0.39, and 0.25 mmol l^{-1}, respectively. The enzyme converted starch, amylopectin, glycogen, and amylopectin β-limit dextrin into cyclodextrins.

A new trisaccharide, α-D-glucopyranosyl-(1 → 4)-α-D-glucopyranosyl-(1 → 2)-D-glucose, was formed by the transglycosylation reaction catalysed by the cyclodextrin D-glucanotransferase from *B. megaterium* in a system containing kojibiose and soluble starch.[502] Glycosylation occurred at O-4 of the non-reducing residue of kojibiose.

The transglycosylation reactions catalysed by cyclodextrin D-glucano-transferases from *B. megaterium* and *B. macerans* have been shown to differ towards such acceptors as D-glucose, maltose, maltotriose, and sucrose.[503] It was suggested that cyclodextrin D-glucanotransferase may transfer D-glucosyl residues from starch, as well as from cyclodextrins, to an acceptor.

Glycopeptide-linkage Hydrolases

***N*-Acetylmuramoyl-L-alanine Amidases.**—A sensitive fluorometric assay for *N*-acetylmuramoyl-L-alanine amidase uses as the substrate *B. subtilis* cell walls labelled with fluorescamine.[403]

4-L-Aspartyl-β-D-glucosylamine Amidohydrolases.—4-L-Aspartyl-β-D-glucosyl-amine amidohydrolase isolated from the livers of patients with aspartyl-glucosaminuria – the enzymic activity is significantly reduced in this disease – has an identical molecular weight and pH optimum as, and does not inhibit, the normal enzyme.[501]

L-Seryl/L-Threonyl-α-D-2-acetamido-2-deoxygalactosidases.—A glycosidase that is able to act on the *O*-glycosidic linkage between 2-acetamido-2-deoxy-α-D-galacto-pyranosyl and serine or threonine residues in glycopeptides has been detected in a commercial preparation of *Clostridium perfringens* (*C. welchii*) and in culture filtrates of *Diplococcus pneumoniae*.[218]

A purified extracellular enzyme from *D. pneumoniae* specifically hydro-lysed 2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-glucopyranosyl units attached to either threonine or serine residues.[219]

Miscellaneous.—*endo*-β-D-2-Acetamido-2-deoxyglucanase H from *Streptomyces griseus* completely hydrolysed the unit A (D-mannose → 2-acetamido-2-deoxy-D-glucose) glycopeptides of thyroglobulin, but did not act on a modified unit B (a complex heteropolysaccharide) free from side-chains.[221] This and other evidence indicated that this enzyme and an *endo*-β-D-2-acetamido-2-deoxy-glucanase D from *Diplococcus pneumoniae* have different specificities: the

[501] N. Nakamura and K. Horikoshi, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 935.
[502] S. Chiba, S. Okada, S. Kitahata, and T. Shimomura, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 2353.
[503] S. Kitahata and S. Okada, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 2185.
[504] H. Savolainen, *Biochem. J.*, 1976, **153**, 749.

H enzyme generally hydrolysed glycopeptides containing D-mannosyl → 2-acetamido-2-deoxy-D-glucose units, whereas the D enzyme hydrolysed glycopeptides free from side-chains in the heteropolysaccharide unit. Both enzymes recognize structural differences in the α-D-mannosyl residues of oligo-D-mannosyl cores of glycoproteins.

Proteinases

Acid Proteases and Chymosins.—'Acid' proteases and chymosin from *Mucor michei* have been shown to contain carbohydrate residues, since they bind concanavalin A.[505]

Deoxyribonucleases and Ribonucleases

Deoxyribonucleases.—Glycopeptides corresponding to the sequences 18—19, 18—20, and 18—22 of deoxyribonuclease I have been synthesized.[506]

Ribonucleases.—Immobilized forms of ribonucleases I and II were obtained when the enzymes reacted with agarose cyclic imidocarbonate.[507]

Phosphatases

Acid Phosphatases.—The molecular properties of the multiple forms of an acid phosphatase from horse liver have been studied.[508]

Affinity electrophoresis and crossed immunoaffinoelectrophoresis with concanavalin A showed that an acid phosphatase from malted barley is a glycoprotein.[509] The enzyme contains residues of D-glucose or D-mannose or both and retained its enzymic activity after binding concanavalin A. The repressible, non-specific acid phosphatase from *Schizosaccharomyces pombe* has been purified to homogeneity (by ultracentrifugation, electrophoresis, and chromatography).[510] The enzyme (mol. wt. 3.81×10^5 by gel filtration, 3.85×10^5 by sucrose density-gradient centrifugation) is dissociated in the presence of both urea and sodium dodecyl sulphate into subunits (mol. wt. 1.04×10^5). D-Galactose and D-mannose account for 66% of the total weight of the enzyme, which also contains 2-amino-2-deoxy-D-glucose (1.65%). The acid phosphatase possesses a broad substrate specificity, does not show diesterase activity, and is competitively inhibited by phosphate and sulphate anions.

Alkaline Phosphatases.—The properties of an alkaline phosphatase cell-wall complex of *Pseudomonas aeruginosa* have been studied *in vitro*.[511]

Sulphatases

Arylsulphatases.—Incubation of normal human fibroblasts with chloroquine at physiological pH released arylsulphatase A activity into the medium.[180] The

[505] W. S. Rickert and P. A. McBride-Warren, *Canad. J. Biochem.*, 1976, **54**, 120.
[506] H. G. Garg and R. W. Jeanloz, *J. Org. Chem.*, 1976, **41**, 2480.
[507] K. Ohgi, T. Nishimura, and M. Irie, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2739.
[508] Z. Wasyl, *Acta Biochim. Polon.*, 1975, **22**, 201.
[509] T. C. Bøg-Hansen, C. H. Brogren, and I. McMurrough, *J. Inst. Brewing*, 1974, **80**, 443.
[510] G. Dibenedetto and I. Cozzani, *Biochemistry*, 1975, **14**, 2847.
[511] D. F. Day and J. M. Ingram, *Canad. J. Microbiol.*, 1975, **21**, 9.

uptake of arylsulphatase A by fibroblasts that are deficient in the enzyme – from a patient with metachromatic leukodystrophy – was completely inhibited by prior treatment of the cells with chloroquine, indicating that chloroquine competes with arylsulphatase A in binding to the cell membrane, *etc.*

The purification of an arylsulphatase A from sheep brain has been accomplished by affinity chromatography on immobilized concanavalin A and pH-dependent polymerization and depolymerization of the enzyme, which is a glycoprotein containing D-glucose and D-mannose (25% total) and sialic acid (0.5%).[512]

An enzyme electrode for the assay of sulphate ion is based on inhibition of the hydrolysis of 4-nitrocatechol sulphate by arylsulphatase, which is used in an immobilized form on a platinum electrode.[513]

The sulphatase 'A' (mol. wt. 1.07×10^5) from bovine liver is a glycoprotein containing D-galactose (8), D-mannose (14), 2-amino-2-deoxy-D-glucose (18), and terminal, non-reducing 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (8 moles mole^{-1}), and traces of L-fucose and D-glucose.[514] The implications of studies of this enzyme to metachromatic leukodystrophy were discussed.

Miscellaneous Enzymes

Dextransucrases.—Sucrose induced dextransucrase activity in *Leuconostoc mesenteroides*, and the enzyme was detected both in the culture supernatant and in the cells.[515] The extracellular enzyme gave a number of active bands on examination by disc electrophoresis, whereas the intracellular enzyme gave only two bands. Moreover, the multiple forms of the extracellular enzyme (monomer mol. wt. 4.2×10^4) behaved as oligomeric isoenzymes on gel filtration, whereas the intracellular enzymes (mol. wt. 7.4×10^4) carry different charges. The isoenzymes also have different pH and temperature optima and K_m values. The kinetic properties of an extensively purified dextransucrase from *L. mesenteroides* have been investigated.[516] The content of α-(1 → 6)-D-glucosidic linkages in the resulting dextran increased as the purity of the enzyme increased. Dextrans with a higher proportion of other D-glucosidic linkages were obtained when an aliquot from the culture fluid was added to the purified enzyme, and it appears that Ca^{2+} and other divalent cations induce the 'branching factor'.

The dextransucrase activity (mol. wt. 9.4×10^4, pH optimum 5.5) from supernatants of cell-free cultures of *Streptococcus mutans* has been purified (1500-fold) by fractional precipitation, chromatography on hydroxyapatite, and isoelectric focusing (p*I* 4.0).[517] Electrophoresis on polyacrylamide gel showed that the preparation contained two enzymes having appreciably different specific activities. The dextransucrase possesses a broad temperature optimum (34—42 °C), is active over a narrow range of pH, and is competitively inhibited by D-fructose.

[512] K. A. Balasubramanian and B. K. Bachawat, *Biochim. Biophys. Acta*, 1975, **403**, 113.
[513] T. Cserfalvi and G. G. Guilbault, *Analyt. Chim. Acta*, 1976, **84**, 259.
[514] E. R. B. Graham and A. B. Roy, *Biochim. Biophys. Acta*, 1973, **329**, 88.
[515] M. Kobayashi and K. Matsuda, *Biochim. Biophys. Acta*, 1974, **370**, 441.
[516] Y. Tsumuraya, N. Nakamura, and T. Kobayashi, *Agric. and Biol. Chem.* (*Japan*), 1976, **40**, 1471.
[517] A. M. Chludzinski, G. R. Germaine, and C. F. Schachtele, *J. Bacteriol.*, 1974, **118**, 1.

Esterases.—Affinity electrophoresis and crossed immunoaffinoelectrophoresis with concanavalin A showed that an esterase from malted barley is a glycoprotein containing residues of D-glucose or D-mannose or both.[509] The implications of microheterogeneity in the carbohydrate residues were discussed. The enzyme retained its activity after binding concanavalin A.

α-Lactalbumins.—Re-examination of the c.d. spectrum of a lysozyme, particularly the contribution of the tryptophanyl residues, has required the c.d. spectra of α-lactalbumins to be reinterpreted.[419]

Ethoxyformylation of histidine-32 in human α-lactalbumin inactivated the associated lactose synthase, although incubation with hydroxylamine restored full activity.[518]

The luminescence of bovine α-lactalbumin at 77 °K has been compared with that of lysozyme.[422] α-Lactalbumin has a fluorescence spectrum that shows vibrational fine structure, an abnormal phosphorescence spectrum, a high fluorescence : phosphorescence ratio, and an abnormal phosphorescence decay; these properties are due largely to the proximity of tryptophanyl residues to the disulphide linkages.

The role of bovine α-lactalbumin in reactions catalysed by bovine-milk D-galactosyltransferase has been studied in detail.[519] Bovine α-lactalbumin appears to regulate the lactose synthase system by reversible association with a complex containing D-galactosyltransferase, Mn^{2+} ion, and UDP-D-galactose, which has a higher affinity than α-lactalbumin for various monosaccharides.[520] Dissociation constants for the complex were determined at several temperatures and salt concentrations. 2-Acetamido-2-deoxy-D-glucose did not bind to bovine α-lactalbumin, whereas it is bound to lysozyme under similar conditions, implying that α-lactalbumin does not retain a binding site for this sugar.[521]

The reactions of bovine α-lactalbumin with *N*-bromosuccinimide and 2-hydroxy-5-nitrobenzyl bromide have led to a better understanding of the interaction of α-lactalbumin with the lactose synthase system and of the conformation of α-lactalbumin.[522] Comparison of the properties of denatured forms of bovine α-lactalbumin and of hen egg-white lysozyme showed that different regions of the molecules unfold in response to denaturants.[423] Amino-acid analyses confirmed that the compositions of the backbones of the two molecules are similar.

α-Lactalbumin has been immobilized by reaction with agarose cyclic imidocarbonate.[523]

Lactose Synthases.—2-Diazoacetamido-2-deoxy-D-glucose accepted the D-galactosyl residue transferred in a reaction catalysed by lactose synthase.[521] Physicochemical studies suggested that the amino-sugar derivative makes contact with the A protein, rather than with α-lactalbumin, in the lactose synthase complex.

[518] M. Schindler, N. Sharon, and J.-P. Prieels, *Biochem. Biophys. Res. Comm.*, 1976, **69**, 167.
[519] J. E. Bell, T. A. Beyer, and R. L. Hill, *J. Biol. Chem.*, 1976, **251**, 3003.
[520] J. T. Powell and K. Brew, *J. Biol. Chem.*, 1975, **250**, 6337.
[521] A. E. Burkhardt, S. O. Russo, C. G. Rinehardt, and G. M. Loudon, *Biochemistry*, 1975, **14**, 5465.
[522] J. E. Bell, F. J. Castellino, I. P. Trayer, and R. L. Hill, *J. Biol. Chem.*, 1975, **250**, 7579.
[523] C. R. Geren, S. C. Magee, and K. E. Ebner, *Arch. Biochem. Biophys.*, 1976, **172**, 149.

Reversible inactivation of human lactose synthase can be achieved by modifying histidine-32 in human α-lactalbumin.[518]

There is a single binding site for UDP-D-galactose on a 'lactose synthase'–Mn^{2+} ion complex derived from bovine lactose synthase.[520] Interactions of the complex with bovine α-lactalbumin were also studied.

The active site of lactose synthase lies on the inner surface of the Golgi membrane of rats, and the significance of this topography in the synthesis of *N*-acetylneuraminyl-lactose and in the secretion of milk sugars was discussed.[524]

Monophenol Mono-oxygenases.—Monophenol mono-oxygenase from *Neurospora crassa* is a glycoprotein.[525] The carbohydrate residue responsible for binding concanavalin A does not appear to be located at the active site, since the resulting complex is enzymically active and serves as an immobilized form of the enzyme. Immobilization was also achieved by reaction of the enzyme with agarose cyclic imidocarbonate.

Pectinesterases.—The pectinesterase activities in tomatoes stored at different temperatures have been determined.[464] The level of activity is low in mature green fruit but increased rapidly at *ca.* 20 °C – but not at either 4 or 33 °C. The storage-life of tomatoes kept at 33 °C for several days is increased, owing to suppression of the polygalacturonase activity. Multiple forms of the enzyme are responsible for the pectinesterase activity of tomatoes; one of the forms has been characterized.[526]

The pectinesterase activity isolated from culture fluids of *Botrytis cinerea* was associated with a poly-D-galacturonase fraction, even after gel filtration and ion-exchange chromatography.[468] Pectinesterase activity has been detected in cultures of *Sphaeropsis malorum* growing on apple tissues.[318]

Peroxidases.—Binding of peanut-cell peroxidase by immobilized concanavalin A indicated that the enzyme is a glycoprotein.[527]

Index of Enzymes Referred to in Chapter 6

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
α-D-2-Acetamido-2-deoxy-galactosidase	2-Acetamido-2-deoxy-α-D-galacto-side acetamidodeoxygalacto-hydrolase	3.2.1.49	337
endo-α-D-2-Acetamido-2-deoxy-galactanase			362
endo-β-D-2-Acetamido-2-deoxy-galactanase			362
β-D-2-Acetamido-2-deoxy-galactosidase	2-Acetamido-2-deoxy-β-D-galacto-side acetamidodeoxygalacto-hydrolase	3.2.1.53	337
α-D-2-Acetamido-2-deoxy-glucosidase	2-Acetamido-2-deoxy-α-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.50	337
β-D-2-Acetamido-2-deoxy-glucosidase	2-Acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase	3.2.1.30	337

[524] N. J. Kuhn and A. White, *Biochem. J.*, 1975, **148**, 77.
[525] S. C. Froehner and K.-E. Eriksson, *Acta Chem. Scand.* (*B*), 1975, **29**, 691.
[526] O. Markovič, *Coll. Czech. Chem. Comm.*, 1974, **39**, 908.
[527] R. B. van Huystee, *Canad. J. Botany*, 1976, **54**, 876.

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
endo-β-D-2-Acetamido-2-deoxyglucanase	*see* Chitinase	3.2.1.14	363
β-D-2-Acetamido-2-deoxyhexosidase	2-Acetamido-2-deoxy-β-hexoside acetamidodeoxyhexohydrolase	3.2.1.52	337
Acid phosphatase	Orthophosphoric-monoester phosphohydrolase (acid optimum)	3.1.3.2	403
Acid protease			403
Alkaline phosphatase	Orthophosphoric-monoester phosphohydrolase (alkaline optimum)	3.1.3.1	403
endo-α-D-2-Amino-2-deoxygalactanase			363
Agarase	Agarose 3-glucanohydrolase	3.2.1.81	363
Alginase *		[3.2.1.16]	364
Alginate lyase	Poly(1,4-β-D-mannuronide lyase)	4.2.2.3	364
α-Amylase	1,4-α-D-Glucan glucanohydrolase	3.2.1.1	364
β-Amylase	1,4-α-D-Glucan maltohydrolase	3.2.1.2	372
Amylo-1,6-D-glucosidase	Dextrin 6-α-glucosidase	3.2.1.33	373
Arabinanase			373
α-1,3-D-Arabinofuranosidase			342
α-L-Arabinofuranosidase	α-L-Arabinofuranoside arabinohydrolase	3.2.1.55	342
D-Arabinose dehydrogenase	D-Arabinose: NAD^+ 1-oxidoreductase	1.1.1.116	400
D-Arabinose isomerase	D-Arabinose ketol-isomerase	5.3.1.3	400
L-Arabinose isomerase	L-Arabinose ketol-isomerase	5.3.1.4	400
α-D-Arabinosidase			342
α-L-Arabinosidase			342
Arylsulphatase	Arylsulphate sulphohydrolase	3.1.6.1	403
Carbohydrate dehydrogenases			400
Carbohydrate isomerases			400
Carbohydrate oxidases			400
Carbohydrate transferases			401
Cellobiose oxidase			400
Cellulase	1,4-(1,3; 1,4)-β-D-Glucan 4-glucohydrolase	3.2.1.4	374
Chitinase	Poly[1,4-β-(2-acetamido-2-deoxy-D-glucoside)] glycanohydrolase	3.2.1.14	376
Chitosanase			376
Chymosin		3.4.23.4	403
Cyclodextrin D-glucanotransferase	1,4-α-D-Glucan-4-α-(1,4-α-glucano)transferase (cyclizing)	2.4.1.19	401
Deoxyribonuclease			403
Deoxyribonuclease I	Deoxyribonucleate 3′-oligonucleotidohydrolase	3.1.4.5	403
Dermatan sulphate lyase			377
Dextranase	1,6-α-D-Glucan 6-glucanohydrolase	3.2.1.11	377
Dextransucrase	Sucrose:1,4-α-D-glucan 4-α-glucosyltransferase	2.4.1.5	404
β-1,4-*N*,6-*O*-Diacetylmuramidase			378

* These entries are now deleted from tables of Enzyme Nomenclature: they are retained in this Report, since they are still used by some workers; see Vol. 7, p. 201 of this series.

Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
Esterase			405
β-D-Fructofuranosidase	β-D-Fructofuranoside fructohydrolase	3.2.1.26	343
L-Fucose isomerase	*see* D-Arabinose isomerase	5.3.1.3	400
α-D-Fucosidase	α-D-Fucoside fucohydrolase		343
α-L-Fucosidase	α-L-Fucoside fucohydrolase	3.2.1.51	343
β-D-Fucosidase *	β-D-Fucoside fucohydrolase	[3.2.1.38]	343
β-D-Galactanase			378
endo-β-D-Galactanase			378
D-Galactose oxidase	D-Galactose: oxygen 6-oxidoreductase	1.1.3.9	401
α-D-Galactosidase	α-D-Galactoside galactohydrolase	3.2.1.22	345
β-D-Galactosidase	β-D-Galactoside galactohydrolase	3.2.1.23	345
β-D-Galactosidases (glycosphingolipid-specific)			345
β-D-Galactosphingosidase			345
β-D-Galactosylceramidase	D-Galactosyl-*N*-acylsphingosine galactohydrolase	3.2.1.46	345
D-Galactosyl-D-galactosyl-D-glucosylceramidase	D-Galactosyl-D-galactosyl-D-glucosylceramide galactohydrolase	3.2.1.47	345
β-D-Galactosyl-D-glucosylceramidase			345
endo-α-1,3-D-Glucanase	1,3-(1,3; 1,4)-α-D-Glucan-3-glucanohydrolase	3.2.1.59	378
endo-β-1,3-D-Glucanase	1,3-β-D-Glucan glucanohydrolase	3.2.1.39	379
endo-α-1,4-D-Glucanase			380
endo-β-1,4-D-Glucanase			380
endo-β-1,6-D-Glucanase	1,6-β-D-Glucan glucanohydrolase	3.2.1.75	380
exo-β-1,3-D-Glucanase	1,3-β-D-Glucan glucohydrolase	3.2.1.58	379
exo-β-1,4-D-Glucanase	1,4-β-D-Glucan glucohydrolase	3.2.1.74	380
exo-β-1,6-D-Glucanase			381
D-Glucanases (miscellaneous)			381
Glucoamylase	1,4-α-D-Glucan glucohydrolase	3.2.1.3	382
D-Glucose isomerase	D-Glucose ketol-isomerase	5.3.1.18	400
D-Glucose oxidase	β-D-Glucose: oxygen 1-oxidoreductase	1.1.3.4	401
α-D-Glucosidase	α-D-Glucoside glucohydrolase	3.2.1.20	353
β-D-Glucosidase	β-D-Glucoside glucohydrolase	3.2.1.21	353
exo-β-1,3-D-Glucosidase	*see* 1,3-β-D-Glucan glucohydrolase	3.2.1.58	379
exo-β-1,4-D-Glucosidase	*see* 1,4-β-D-Glucan glucohydrolase	3.2.1.74	380
α-D-Glucosylceramidase	D-Glucosyl-*N*-acylsphingosine glucohydrolase	3.2.1.45	353
β-D-Glucuronidase	β-D-Glucuronide glucuronohydrolase	3.2.1.31	356
Glycanase			384
Glycopeptidases			402
Glycopeptide-linkage hydrolases			402
Glycopeptide-linkage hydrolases (miscellaneous)			402

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Trivial name and name used in this volume	*Systematic name*	*E.C. No.*	*Page*
β-D-2-Glycylamido-2-deoxy-glucosidase			358
Heparin hydrolase *		[3.2.1.19]	384
Heparin lyase	Heparin lyase	4.1.2.7	385
Heparan sulphate lyase	Heparan sulphate lyase	4.2.2.8	385
Hyaluronate lyase	Hyaluronate lyase	4.2.2.1	385
Hyaluronidase	Hyaluronate 4-glycanohydrolase	3.2.1.35	385
α-L-Iduronidase	α-L-Iduronide iduronohydrolase	3.2.1.76	358
Isoamylase	Glycogen 6-glucanohydrolase	3.2.1.68	387
Keratan sulphate hydrolase			387
α-Lactalbumin	*see*	2.4.1.22	405
Lactose synthase	UDP-Galactose: D-4-β-galactosyl-transferase	2.4.1.22	405
Lactosylceramidase			345
Laminarinase	1,3-(1,3; 1,4)-β-D-Glucan 3(4)-glucanohydrolase	3.2.1.6	387
Lichenanase	1,3-1,4-β-D-Glucan 4-glucano-hydrolase	3.2.1.73	387
Limit dextrinase			387
Lysozyme	Mucopeptide *N*-acetyl-muramylhydrolase	3.2.1.17	388
D-Mannanases (miscellaneous)			395
exo-α-1,2-D-Mannanase			394
endo-α-1,6-D-Mannanase			394
α-D-Mannosidase	α-D-Mannoside mannohydrolase	3.2.1.24	359
Monophenol monooxygenase	Monophenol dihydroxyphenyl-alanine : oxygen oxidoreductase	1.14.18.1	406
Mycodextranase			395
Neuraminidase	Acylneuraminyl hydrolase	3.2.1.18	360
Neoagarase			395
Oligo-1,6-D-glucosidase	Dextrin 6-α-glucanohydrolase	3.2.1.10	395
Pectate lyase	Poly(1,4-α-D-galacturonide) lyase	4.2.2.2	396
Pectinesterase	Pectin pectylhydrolase	3.1.1.11	406
Pectin lyase	Poly(methoxygalacturonide) lyase	4.2.2.10	396
Pentosanase			396
Peroxidase	Donor: hydrogen-peroxidase oxidoreductase	1.11.1.7	406
Phosphatases			403
Poly-D-galacturonase	Poly(1,4-α-D-galacturonide) glycanohydrolase	3.2.1.15	396
endo-Poly-D-galacturonase	Poly(1,4-α-D-galacturonide) galacturonohydrolase	3.2.1.67	397
Poly-D-galacturonate lyase			396
exo-Poly-D-galacturonate lyase			396
Proteases	*see* Proteinases		403
Proteinases			403
Pullulanase	Pullulan 6-glucanohydrolase	3.2.1.41	398
Ribonuclease			403
Ribonuclease II	Ribonucleate 3′-oligonucleotido-hydrolase	3.1.4.23	403
L-Seryl/L-threonyl-α-D-2-acet-amido-2-deoxy-galactosidase			402

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Sialidase *			360
Sucrose α-D-glucohydrolase	Sucrose α-D-glucohydrolase	3.2.1.48	398
Sulphatase			403
αα-Trehalase	αα-Trehalose glucohydrolase	3.2.1.28	398
Xylanase (miscellaneous)			399
β-D-Xylosidase			362

* Sialidase is a term generally used to include neuraminidase and unspecified acylneuraminyl hydrolases. Since there is little evidence for the existence of acylneuraminyl hydrolases that do not cleave 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonyl residues, use of the term neuraminidase is preferred.

7
Glycolipids and Gangliosides

BY R. J. STURGEON

Introduction

Several chapters in a book on the biochemical analysis of membranes have dealt with the isolation and chemical analysis of membrane proteins, lipids, and carbohydrates.[1] An improved procedure reported for the isolation of cerebrosides is suitable for large-scale preparations.[2] H.p.l.c. on totally porous silica beads has been used in separations of the glycolipids from human erythrocytes; ceramide di- and tri-hexosides were each separated into fractions that differed in the compositions of the fatty acids and long-chain bases.[3] The bovine-brain gangliosides G_{M_1}, $G_{D_{1a}}$, $G_{D_{1b}}$, and G_{T_1} have also been obtained in a high degree of purity using a similar system.[4] Ascending dry-column chromatography has been used in the isolation of glycolipids,[5] and fully benzoylated derivatives of D-galactosylceramides have been separated by isocratic h.p.l.c.[6] A new micro-method for determining the *N*-acylneuraminic acid content of erythrocyte membranes avoids many of the difficulties encountered with earlier methods.[7] The erythrocyte membranes are collected directly on filters, and the neuraminic acids released from them *in situ* with acid are assayed with the periodate–thiobarbituric acid reagent. A D-galactosylceramide containing a spin-label covalently attached near the methyl-end of the fatty-acid chain has been used to investigate the behaviour of glycosphingolipids in lipid bilayers.[8] The glycolipid was distributed randomly in fluid lipid bilayers, but tended to be excluded from regions rich in phosphatidylserine because of Ca^{2+}-induced, lateral phase separation. Small changes in the chemical structures of anionic detergents have been shown to affect the hydrolysis of the ganglioside G_{M_1} and of lactosylceramide by rabbit-brain β-galactosidase.[9]

Animal Glycolipids and Gangliosides

An article on the biosynthesis of glycosphingolipids and tumourigenesis has reviewed the role of the Golgi apparatus in the synthesis of specific receptor

[1] 'Biochemical Analysis of Membranes', ed. A. H. Maddy, Chapman and Hall Ltd., London, 1976.
[2] N. S. Radin, *J. Lipid Res.*, 1976, **17**, 290.
[3] S. Ando, M. Isobe, and Y. Nagai, *Biochim. Biophys. Acta*, 1976, **424**, 98.
[4] T. Momoi, S. Ando, and Y. Nagai, *Biochim. Biophys. Acta*, 1976, **441**, 488.
[5] C. V. Viswanathan and A. Hayashi, *J. Chromatog.*, 1976, **123**, 243.
[6] R. H. McCluer and J. E. Evans, *J. Lipid Res.*, 1976, **17**, 412.
[7] R. Schauer, A. P. Corfield, M. Wember, and D. Danon, *Z. physiol. Chem.*, 1975, **356**, 1727.
[8] F. J. Sharom and C. W. M. Grant, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 1501.
[9] J. W. Callahan and J. Gerrie, *J. Neurochem.*, 1976, **26**, 217.

molecules present at the surface of mammalian cells.[10] The chemical composition of brain myelin has been determined in two cases of multiple sulphatase deficiency (a variant form of metachromatic leukodystrophy).[11] The ganglioside pattern was abnormal in grey and white matter, where high proportions of the gangliosides G_{M_3}, G_{M_2}, and G_{D_3} were found. The brain of a patient with fucosidosis has been found to store glycosphingolipids, a related L-fuco-oligosaccharide, and a disaccharide.[12] The principal carbohydrates that accumulated were identified as an oligosaccharide having the sequence L-Fuc*p* → D-Gal*p* → D-Glc*NAcp* → D-Man*p* → [L-Fuc*p* → D-Gal*p* → D-Glc*NAcp* → D-Man*p*] → D-Man*p* → D-Glc*NAcp* and 2-acetamido-2-deoxy-6-*O*-L-fucopyranosyl-D-glucose. Smaller amounts of a related oligosaccharide, D-Gal*p* → D-Glc*NAcp* → D-Man*p* → [D-Gal*p*-D-Glc*NAcp* → D-Man*p*] → D-Man*p* → D-Glc*NAcp*, were isolated from the brain tissues of patients with G_{M_1}-gangliosidosis (Types I and II), where the principal storage material is known to be the ganglioside G_{M_1}. An oligosaccharide having a similar sequence, but which contains no D-galactosyl residues, was isolated from the brain tissues of a patient with a total deficiency of 2-acetamido-2-deoxy-β-D-hexosidase, *i.e.* the Sandhoff variant of G_{M_2}-gangliosidosis in which the principal storage materials are the ganglioside G_{M_2} and its asialo-derivative. The sialic acid contents of gangliosides in an average cell and in an average neuron have been used as an index of the relative mass of neuronal plasma membranes and their derivatives in neural tissues.[13]

Abnormalities in the patterns of brain gangliosides and fatty acids of adrenoleukodystrophy patients are thought to be independent of each other, one being a non-specific change and the other characteristic of adrenoleukodystrophy.[14] Gangliosides isolated from human kidneys have been partially characterized.[15] One of the gangliosides isolated was homogeneous, and was found to contain residues of L-fucose, D-galactose, D-glucose, 2-acetamido-2-deoxy-D-glucose, and *N*-acetylneuraminic acid (1 : 2 : 1 : 1 : 1); thus, it represents a new type of glycolipid structure having both neuraminic acid and L-fucose attached to the same carbohydrate chain. The structure of the carbohydrate moiety (1) of this ganglioside was established on the basis of methylation

D-Gal*p*-(1 → 4)-D-Glc*NAcp*-(1 → 3)-D-Gal*p*-(1 → 4)-D-Glc*p*-(1 → 1)-ceramide
3 3
↑ ↑
2 1
Neu*NAcp* L-Fuc*p*

(1)

analysis and partial hydrolysis with acid and with neuraminidase.[16] The ganglioside (1) can be regarded as an *N*-acetylneuraminyl derivative of the so-called 'X-antigen' previously isolated from human adenocarcinoma[17] and

[10] C. L. Richardson, S. R. Baker, D. J. Morré, and T. W. Keenan, *Biochim. Biophys. Acta*, 1975, **417**, 175.
[11] Y. Eto, C. Meier, and N. N. Herschkowitz, *J. Neurochem.*, 1976, **27**, 1071.
[12] G. C. Tsay and G. Dawson, *J. Neurochem.*, 1976, **27**, 733.
[13] H. H. Hess, N. H. Bass, C. Thalheimer, and R. Devarakonda, *J. Neurochem.*, 1976, **26**, 1115.
[14] M. Igarashi, D. Belchis, and K. Suzuki, *J. Neurochem.*, 1976, **27**, 327.
[15] H. Rauvala, *Biochim. Biophys. Acta*, 1976, **424**, 284.
[16] H. Rauvala, *F.E.B.S. Letters*, 1976, **62**, 161.
[17] H. J. Yang and S.-I. Hakomori, *J. Biol. Chem.*, 1971, **246**, 1192.

hog gastric mucosa.[18] An unusual glycolipid that accumulates in some human colon tumours has been separated from a monohexosylceramide fraction by acetylation and preparative t.l.c.; L-fucose was the only sugar found in the glycolipid, while the principal ceramide component was identified as *N*-palmitoyl-octadecasphingenine.[19] Methylation analysis and hydrolysis with an α-L-fucosidase indicated that the glycolipid is an α-L-fucopyranosyl-(1 → 1)-ceramide.

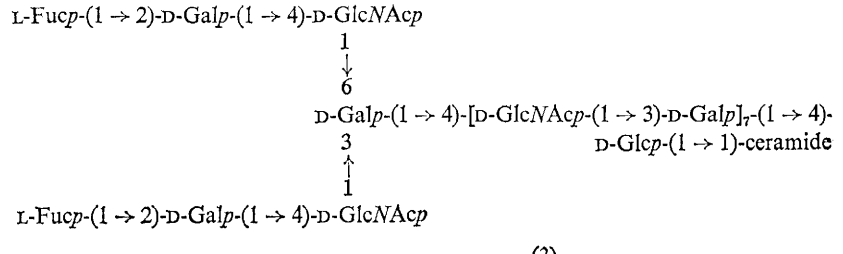
Significant increases have been found in the levels of gangliosides and *N*-acetylneuraminic acid in most human gastric tumours and in all colonic tumours, when comparison is made with the amounts in normal tissues.[20] The structures and quantitative distributions of the various gangliosides in human stomach and small and large intestinal mucosa have been reported; a disialo-tetraglycosylceramide (of unknown physiological significance) found in the gastrointestinal tract contains 2-amino-2-deoxy-D-glucose as one of the constituent sugars.[21] Linkage analysis of five principal gangliosides from human alimentary mucosa has been accomplished by a combination of methylation and g.l.c.–m.s.[22] Two of the gangliosides were identified as *N*-acetylneuraminyl-(2 → 3)-D-galactosyl-(1 → 4)-D-glucosyl-(1 → 1)-ceramide and *N*-acetylneuraminyl-*N*-acetylneuraminyl-(2 → 3)-D-galactosyl-(1 → 4)-D-glucosyl-(1 → 1)-ceramide. Both g.l.c.–chemical ionization m.s. and g.l.c.–electron impact m.s. were used to identify a D-glucosylsphingosine isolated from the spleens of patients with Gaucher's disease as D-glucopyranosyl-(1 → 1)-(2-amino-1,3-dihydroxy-octadec-4-ene).[23] The appearance of D-glucosyl-*N*-hexylsphingosine is accompanied by an appreciable reduction in the level of β-D-glucosidase during the growth of normal skin fibroblasts, while related enzymes (for example, α-D-glucosidase, β-D-galactosidase, and 2-acetamido-2-deoxy-β-D-glucosidase) exhibit either normal or elevated levels of activity.[24] This results in an increased level of D-glucosylceramide in inhibitor-treated cells, which can be used as a chemical model for Gaucher's disease. The total ganglioside content of various types of tumour of the central nervous system has been shown to be less than that of normal tissues, and a general increase in the specific activities of all acid hydrolases in the tumours was also noted.[25]

The haemolytic activity of staphylococcal α-toxin was inhibited by the ganglioside (2) (containing a 2-acetamido-2-deoxy-D-glucosyl residue) from

β-D-Gal*p*-(1 → 4)-β-D-Glc*NAc*p-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
3
↑
2
α-Neu*NAc*p

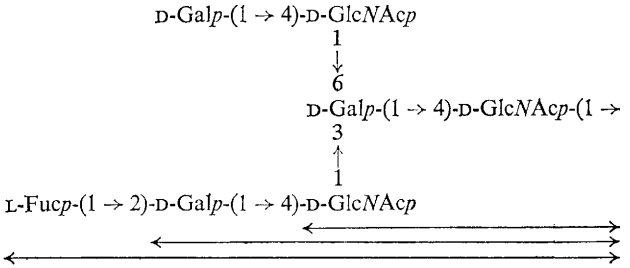
(2)

[18] B. L. Slomiany, A. Slomiany, and M. I. Horowitz, *European J. Biochem.*, 1975, **56**, 353.
[19] K. Watanabe, T. Matsubara, and S.-I. Hakomori, *J. Biol. Chem.*, 1976, **251**, 2385.
[20] A. Keränen, M. Lempinen, and K. Puro, *Clinica Chim. Acta*, 1976, **70**, 103.
[21] A. Keränen, *Biochim. Biophys. Acta*, 1975, **409**, 320.
[22] A. Keränen, *Biochim. Biophys. Acta*, 1976, **431**, 96.
[23] M. Oshima, *J. Biochem. (Japan)*, 1976, **80**, 53.
[24] K. R. Warren, I. A. Schafer, J. O. Sullivan, M. Petrelli, and N. S. Radin, *J. Lipid Res.*, 1976, **17**, 132.
[25] R. M. Aruna and D. Basu, *Indian J. Biochem. Biophys.*, 1976, **13**, 158.



(3)

human erythrocytes, but not by related glycolipids.[26] The ganglioside (2) may be a membrane receptor for the α-toxin. The macroglycolipid (3) isolated from human erythrocytes has been identified on the basis of chemical studies, although the possibility that it contains an asymmetric branch-structure cannot be excluded.[27] Human-erythrocyte glycoproteins free of glycolipids showed only weak blood-group A activity, and are, therefore, unlikely to be responsible for the antigenicity of the erythrocyte membrane.[28] A macroglycolipid fraction isolated from human erythrocytes has been shown to be responsible, in part, for the blood-group I activity of red-cells; three I-active, antigenic glycolipids are probably present.[29] They have similar structures at the reducing end of the chains, but differ in the length of the chain. The reactions with heterologous anti-I sera, following sequential degradation of the glycolipids with glycosidases, indicated that the antisera recognize different regions of the carbohydrate chains [see (4)] of the I-antigen complex. Surface-labelling of blood-group A variants,



The arrows indicate a structure recognized by different anti-I sera

(4)

and the reactivity of erythrocytes to antibodies directed against the blood-group H_3 variant and its degradation products, showed that complex variants of A or H determinants (A^c and A^d or H_3 and H_4) are either absent or significantly low in the erythrocytes of foetuses and the newborn, whereas they are present in appreciable amounts in the erythrocytes of adults.[30] By contrast, A determinants

[26] I. Kato and M. Naiki, *Infection and Immunity*, 1976, **13**, 289.
[27] A. Gardas, *European J. Biochem.*, 1976, **68**, 177.
[28] K. Yamato, S. Handa, and T. Yamakawa, *J. Biochem. (Japan)*, 1975, **78**, 1207.
[29] A. Gardas, *European J. Biochem.*, 1976, **68**, 185.
[30] K. Watanabe and S.-I. Hakomori, *J. Exp. Med.*, 1976, **144**, 644.

linked to simpler carbohydrates (A^a and A^b variants) are fully developed before birth and show no significant changes after birth. The precursor of the blood-group carbohydrates seems to be abundant in the erythrocytes of foetuses and the newborn, which are highly reactive towards antibodies directed against 2-acetamido-2-deoxy-β-D-glucosyl-(1 → 3)-β-D-galactosyl-(1 → 4)-β-D-glucosyl-(1 → 1)-ceramide. The reaction between antibodies directed against this precursor and glycolipids from human colon carcinoma was consistently higher than that between the antibodies and glycolipids from human intestinal mucosa. This and other evidence support the general concept that the process of ontogenesis of a blood-group carbohydrate chain occurs by step-by-step elongation and branching, and that the development of a carbohydrate chain is blocked in the process of oncogenesis.

The blood-group B-active glycosphingolipids B-I (5) and B-II (6) were degraded by α-D-galactosidase and by α-L-fucosidase; the assigned structures were based

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

(5)

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

(6)

on analysis of the glycosphingolipids and of methylated, degraded derivatives thereof by g.l.c.–m.s., as well as on haemagglutination studies.[31, 32] An H-active glycolipid fraction (7) was also isolated from B erythrocytes. It afforded the same

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
2
↑
1
α-L-Fuc*p*

(7)

methylated derivatives on methylation and acid hydrolysis as those derived from α-galactosidase-treated and fully methylated glycolipid B-I, and is serologically similar to the α-galactosidase-degraded glycolipids B-I and B-II. The glycolipid (7) is likely to be a precursor of B-I.

No evidence has been obtained for the presence of trihexosylceramide and globoside in blood-group p erythrocytes, although lactosylceramide and other complex glycolipids containing both sialic acid and 2-acetamido-2-deoxy-D-glucose accumulate.[33] Chemical analysis of the erythrocytes confirmed the

[31] P. Hanfland and H. Egge, *Chem. and Phys. Lipids*, 1975, **15**, 243.
[32] P. Hanfland, *Chem. and Phys. Lipids*, 1975, **15**, 105.
[33] J. Kóscielak, H. Miller-Podraza, R. Krauze, and B. Cedergren, *F.E.B.S. Letters*, 1976, **66**, 250.

results of immunological tests, which suggested that the blood-group p and p^k antigens are globoside and trihexosylceramide, respectively.[34]

The levels of haematoside and other glycolipids in plasma membranes of human lymphoblastoid cell line BR18 have been shown to differ from those in peripheral human-blood lymphocytes.[35] The biosynthesis of glycosphingolipids during discrete phases of the growth cycle has been studied using synchronized human KB cells.[36] Maximum incorporation of D-[^{14}C]galactose into glycosphingolipids occurred during the late phases of the growth cycle. The finding that the synthesis of glycosphingolipids occurs late in the cell cycle might be related to the observation that most glycolipid glycosyltransferases are glycoproteins, and are synthesized early in the cell cycle so that they can partake in glycosyltransferase reactions. The results also support the view that glycosphingolipids are incorporated during the final stages of synthesis of the cell membrane. CMP-sialic acid:lactosylceramide sialyltransferase was induced in HeLa cells by butyrate, which also produced morphological changes, including the extension of neurite-like processes, in the cells.[37] The induced enzyme is specific for lactosylceramide. The synthesis of haematoside *in vitro* from endogenous acceptors was also enhanced in cells growing in the presence of this short-chain fatty acid.

The amount of lactosylceramide that accumulates in the leucocytes of adults with Type 1 chronic non-neuropathic Gaucher's disease has been determined.[38] A number of gangliosides have been isolated from human leukaemic polymorphonuclear leucocytes; a combination of chemical and enzymic techniques was used to identify the principal ganglioside as *N*-acetylneuraminyl-D-galactosyl-2-acetamido-2-deoxy-β-D-glucosyl-β-D-galactosyl-β-D-glucosylceramide.[39] A closely related ganglioside was tentatively identified as *N*-acetylneuraminyl-D-galactosyl-2-acetamido-2-deoxy-β-D-glucosyl-[*N*-acetylneuraminyl]-β-D-galactosyl-β-D-glucosylceramide. Although the purified gangliosides were degraded with neuraminidase, treatment of intact cells with the enzyme did not alter the pattern of gangliosides, implying that either the carbohydrate chains of the gangliosides are not projecting from the surface of the membrane or the gangliosides are not located in the plasma membrane.

The amounts and structures of glycosphingolipids present in the plasma lipoproteins of normal subjects and of a patient homozygous for familial hypercholesterolaemia have been reported.[40] D-Glucosyl-, lactosyl- and trihexosylceramides and globoside were found to be associated with the lipoprotein fraction of human plasma.[41] Similar distributions of glycosphingolipids have been found in the serum lipoproteins of normal subjects and patients with hypo- and hyper-lipidaemias.[42] Two glycolipids containing L-fucose were synthesized

[34] D. M. Marcus, M. Naiki, and S. K. Kundu, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 3263.
[35] G. M. Levis, J. N. Karli, and N. J. Crumpton, *Biochem. Biophys. Res. Comm.*, 1976, **68**, 336.
[36] S. Chatterjee, C. C. Sweeley, and L. F. Velicer, *Biochem. Biophys. Res. Comm.*, 1973, **54**, 585.
[37] P. H. Fishman, R. M. Bradley, and R. C. Henneberry, *Arch. Biochem. Biophys.*, 1976, **172**, 618.
[38] C. Klibansky, Z. Ossimi, Y. Matoth, J. Pinkhas, and A. de Vries, *Clinica Chim. Acta*, 1976, **72**, 141.
[39] G. Dacremont and J. Hildebrand, *Biochim. Biophys. Acta*, 1976, **424**, 315.
[40] S. Chatterjee and P. O. Kwiterovich, *Lipids*, 1976, **11**, 462.
[41] F. A. J. T. M. van den Bergh and J. M. Tager, *Biochim. Biophys. Acta*, 1976, **441**, 391.
[42] G. Dawson, A. W. Kruski, and A. M. Scanu, *J. Lipid Res.*, 1976, **17**, 125.

when lacto-*N*-neotetraosylceramide and GDP-L-fucose were incubated with an L-fucosyltransferase from human serum.[43] The structure (7) is proposed for one of the glycolipids, whereas the other (8) has a single α-L-fucosyl residue attached at O-3 of the 2-acetamido-2-deoxy-β-D-glucosyl residue in the main chain.

β-D-Gal*p*-(1 → 4)-β-Glc*NAc*p-(1 → 3)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*-(1 → 1)-ceramide
3
↑
1
α-L-Fuc*p*

(8)

Fabry's disease (an X-linked, inborn error of the metabolism of neutral glycosphingolipids) is characterized by the accumulation of D-galactosyl-D-galactosyl-D-glucosylceramide in the small arteries, kidneys, and other tissues. This and three other neutral glycosphingolipids have been found mainly in two of the four fractions isolated from the serum lipoproteins of patients with Fabry's disease; the distribution of the excess lipid among the principal lipoprotein fractions was similar to that in a control group.[44] Measurements of the uptake of radiolabelled D-[6-^{3}H]galactosyl-(1 → 4)-α-D-galactosyl-(1 → 4)-β-D-glucosyl-(1 → 1)-ceramide by the lipoproteins in human sera showed that 24% of the bound glycolipid is associated with the low-density fraction, 47% with the high-density fraction, and 27% with the ultracentrifugal residue.[45] This distribution differs from that of the endogenous trihexosylceramide, suggesting that the glycolipid probably forms an integral part of the lipoprotein complexes *in vivo*. Exogenous ganglioside G_{M_1} was bound to the surface membrane of intact NCTC 2071 cells, where it served as a receptor for choleragen.[46] Although it is required for the responsiveness of intact cells to choleragen, the ganglioside does not play an essential role in the responsiveness of cell homogenates, presumably because the surface receptor can be by-passed.

Serotonin (5-hydroxytryptamine), but not acetylcholine, has been shown to bind to micelles of ox-brain gangliosides, to liposomes containing gangliosides, and to fetuin by means of the negatively charged *N*-acetylneuraminic acid residues.[47] The binding is reversible and can be blocked specifically by 7-methyltryptamine. The results of a ^{1}H n.m.r. spectroscopic study have indicated that there is a specific and strong interaction between serotonin and the gangliosides present in monkey brain.[48]

In addition to 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose, bovine spinal roots and sciatic nerve contain both *N*-acetyl- and *N*-glycolyl-neuraminic acids.[49] Two new gangliosides, provisionally named G_{5a} and G_{5b}, have been found in ox brain.[50] Ganglioside G_{5a} has a neutral

[43] T. Pacuszka and J. Kóscielak, *European J. Biochem.*, 1976, **64**, 499.
[44] J. T. R. Clarke, J. M. Stoltz, and M. R. Mulcahey, *Biochim. Biophys. Acta*, 1976, **431**, 317.
[45] J. T. R. Clarke and J. M. Stoltz, *Biochim. Biophys. Acta*, 1976, **441**, 165.
[46] J. Moss, P. H. Fishman, V. C. Manganiello, M. Vaughan, and R. O. Brady, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 1034.
[47] E. L. M. Ochoa and A. D. Bangham, *J. Neurochem.*, 1976, **26**, 1193.
[48] K. S. Krishnan and P. Balaram, *F.E.B.S. Letters*, 1976, **63**, 313.
[49] J. W. Fong, R. W. Ledeen, S. K. Kundu, and S. W. Brostoff, *J. Neurochem.*, 1976, **26**, 157.
[50] R. Ghidoni, S. Sonnino, G. Tettamanti, H. Wiegandt, and V. Zambotti, *J. Neurochem.*, 1976, **27**, 511.

glycosphingolipid core consisting of β-D-galactosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-galactosyl-(1 → 4)-β-D-galactosyl-(1 → 4)-β-D-glucosyl-(1 → 1)-ceramide, to which are attached residues of L-fucose at O-2 of the terminal D-galactosyl residue and of *N*-acetylneuraminic acid at O-3 of the internal D-galactosyl residue. Ganglioside G_{5b} is composed of a mixture of, at least, two isomeric disialogangliosides, each containing residues of *N*-acetyl- and *N*-glycolyl-neuraminic acids. Structure (9) was assigned to the principal disialoganglioside.

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
3
↑
2
α-Neu*N*Gl*p*

(9)

The presence of a ganglioside containing L-fucose in ox brain is further evidence of the relation between gangliosides in neural and extraneural tissues.

Lipid intermediates are formed during the biosynthesis of glycoproteins in microsomes.[51] Column chromatography was used to separate dolichyl D-[^{14}C]mannosyl phosphate from a D-[^{14}C]manno-oligosaccharide which was also bound to lipid; after removal of the lipid, the oligosaccharide fraction appeared to be heterogeneous in its reaction with concanavalin A.

Gangliosides in the membranes of bovine erythrocytes and the fat globules of cow's milk were not hydrolysed by neuraminidase, although prior treatment with trypsin rendered these membrane-associated glycolipids susceptible to attack by neuraminidase.[52] The results indicate that the carbohydrate residues of the gangliosides of bovine erythrocytes and milk-fat globules are located principally on the environmental face of the membrane, where they are shielded by membrane proteins from attack by neuraminidase. The determinant of bovine blood-group J activity has been transferred from a serum protein on to the erythrocyte membrane by incubation *in vitro*.[53] The transferred J activity was detected only in the lipid fraction of the erythrocytes, implying that the J determinant, which is probably a carbohydrate moiety, is detached from a serum glycoprotein and transferred to a lipidic acceptor (probably a glycosphingolipid) at the erythrocyte membrane.

Gangliosides inhibited the binding of [^{125}I]thyrotrophin to receptors on the plasma membranes of bovine thyroid and guinea-pig retro-orbital tissues and to human adipocyte membranes.[54] The inhibition is markedly influenced by the number and location of sialic acid residues in the ganglioside molecule, the efficiency for inhibition being $G_{D_{1b}} > G_{T_1} > G_{M_1} = G_{M_3} > G_{D_{1a}}$. The possibility that a ganglioside, or a ganglioside-like structure, is a component of the thyrotrophin receptor is suggested by the observation that the amounts of gangliosides more complex than *N*-acetylneuraminyl-D-galactosyl-D-glucosyl-

[51] P. Zatta, D. Zakim, and D. A. Vessey, *Biochim. Biophys. Acta*, 1976, **441**, 103.
[52] J. M. Tomich, I. H. Mather, and T. W. Keenan, *Biochim. Biophys. Acta*, 1976, **433**, 357.
[53] F. Krotlinger, O. W. Thiele, and C. Ohl, *European J. Biochem.*, 1976, **67**, 495.
[54] B. R. Mullin, P. H. Fishman, G. Lee, S. M. Aloj, F. D. Ledley, R. J. Winand, L. D. Kohn, and R. O. Brady, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 842.

ceramide present in thyroid membranes are higher than those found in extra-neural tissues. The finding that the B component of cholera toxin – which also interacts with gangliosides – and the β-subunit of thyrotrophin have a common peptide sequence suggests that the modes of action of thyrotrophin and cholera toxin on membranes are similar.

A fucoganglioside isolated from boar testis has been purified by chromatography on silicic acid and a cellulose anion-exchanger.[55] A combination of chemical and enzymic methods indicated the structure (10) for the fucoganglioside, which contains mainly saturated, long-chain fatty acids.

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

(10)

The incorporation of D-[^{14}C]galactose into the individual gangliosides of sheep peripheral lymphocytes stimulated with concanavalin A was appreciably enhanced over that of control cells.[56] A marked enhancement of incorporation into trihexosylceramide and an alteration in the pattern of incorporation into gangliosides were noted in the mitogen-treated cells. The results suggest that significant alterations in the compositions and metabolism of glycolipids in lymphocytes may occur during certain of the complex stages of differentiation.

The presence of CMP-*N*-acetylneuraminyl-D-galactosyl-2-acetamido-2-deoxy-D-glucosyl-[*N*-acetylneuraminyl]-D-glucosylceramide:sialyltransferase activity has been demonstrated in rabbits during neurohypophysis.[57] The product of the reaction was identified as the ganglioside $G_{D_{1a}}$.

Membrane-bound 2-acetamido-2-deoxy-α- and -β-D-galactosyltransferases from guinea-pig microsomes were able to catalyse the formation of the non-reducing, terminal residues of Forssman hapten and globoside, respectively.[58] The enzymes can be readily differentiated, since the α-transferase is inhibited, and the β-transferase is stimulated, by UDP; UDP-D-glucose can suppress both α-transferase and pyrophosphatase activities.

Changes in cerebrosides, sulphatides, and monogalactosyldiglycerides during the development of rat cerebellum have been investigated.[59] Retinyl phosphate stimulated the incorporation of D-[^{14}C]mannose from GDP-D-[^{14}C]mannose into two principal D-mannosyl-lipids, which were identified as dolichyl D-mannosyl phosphate and D-mannosyl retinyl phosphate.[60] The principal neutral glycolipids of rat sublingual and submaxillary glands have been identified as mono-, di-, tetra-, and penta-hexosylceramides, but there is no evidence for the presence of fucolipids in these tissues.[61]

[55] A. Suzuki, T. Ishizuka, and T. Yamakawa, *J. Biochem.* (*Japan*), 1976, **78**, 947.
[56] R. Narasmhan, J. B. Hay, M. F. Greaves, and R. K. Murray, *Biochim. Biophys. Acta*, 1976, **431**, 578.
[57] J. T. R. Clarke and M. R. Mulcahey, *Biochim. Biophys. Acta*, 1976, **441**, 146.
[58] T. Ishibashi, T. Atsuta, and A. Makita, *Biochim. Biophys. Acta*, 1976, **429**, 759.
[59] S. Vrbaški and D. Kostić, *J. Neurochem.*, 1976, **27**, 983.
[60] G. C. Rosso, L. de Luca, C. D. Warren, and G. Wolf, *J. Lipid Res.*, 1975, **16**, 235.
[61] A. Slomiany, C. Annese, and B. L. Slomiany, *Biochim. Biophys. Acta*, 1976, **441**, 316.

UDP-D-galactose:G_{M_2}-galactosyltransferase has been isolated from the Golgi apparatus of rat livers.[62] Newly synthesized G_{M_1} (the product of the enzymic reaction) was incorporated into, or became closely associated with, the membranes of the Golgi apparatus. The synthesis of gangliosides in rat liver *in vivo* was impaired following the administration of 2-amino-2-deoxy-D-galactose; the amounts of UDP-D-galactose and UDP-D-glucose in the liver were reduced, resulting in severe hepatocellular damage.[63] Comparison of the glycosphingolipids isolated from the livers of rats treated in succession with 2-amino-2-deoxy-D-galactose and D-[1^{14}C]galactose with those obtained from control livers showed that the labelling of D-glucosylceramide was not altered and that only slight changes occurred in the level of G_{M_3}. The highly malignant rat hepatoma 27 was found to contain more lipid-bound sialic acid than normal liver, whereas the level of lipid-bound sialic acid was reduced in regenerating liver.[64] In comparison with the liver, the hepatoma contained more disialogangliosides but no trisialogangliosides, which are abundant in the liver. The principal disialoganglioside in the hepatoma cells was identified as $G_{D_{1b}}$ [D-galactosyl-2-acetamido-2-deoxy-D-galactosyl-(*N*-acetylneuraminyl-*N*-acetylneuraminyl)-D-glucosylceramide], which is known to be a precursor of trisialogangliosides in other tissues. These findings may be explained by a reduced activity of the glycosyltransferases – most probably the loss of a sialyltransferase activity – in the hepatoma that results in failure to complete the carbohydrate chain and in the accumulation of $G_{D_{1b}}$, which has no analogue in the liver.

Hyperplastic nodules induced in rat liver by the carcinogen *N*-(2-fluorenyl)-acetamide contained less tri- and tetra-sialogangliosides, but more disialoganglioside, than control livers and the surrounding tissues.[65] These differences represent at least one of the ways in which hyperplastic nodules deviate towards the malignant phenotype, in keeping with the view that hyperplastic nodules are a pre-malignant form of hyperplasia. The main ganglioside present in rat intestinal mucosa is the haematoside G_{M_3}, which contains *N*-glycolylneuraminic acid as the principal sialic acid.[66] The haematoside content and the level of CMP-sialic acid:lactosylceramide sialyltransferase activity of crypt cells are significantly lower than those of villus cells, demonstrating that marked differences in the contents and biosynthesis of gangliosides occur in contiguous populations of cells at varying stages of differentiation. Measurements of trihexosylceramide, ceramide, and UDP-D-glucose:hydroxy-fatty acid-ceramide D-glucosyltransferase and UDP-D-galactose:lactosylceramide D-galactosyltransferase activities also demonstrated that there are significant differences in the distribution and biosynthesis of individual glycolipids in crypt and villus cells.[67]

The amounts of haematosides, monosialogangliosides, and disialogangliosides extracted from the blood sera of Morris hepatoma-bearing rats are higher than those extracted from the sera of normal rats, whereas the content of trisialo-

[62] F. E. Wilkinson, D. J. Morré, and T. W. Keenan, *J. Lipid Res.*, 1976, **17**, 146.
[63] E. Rupprecht, C. Hans, G. Leonard, and K. Decker, *Biochim. Biophys. Acta*, 1976, **450**, 45.
[64] E. V. Dyatlovitskaya, A. M. Novikov, N. P. Gorkova, and L. D. Bergelson, *European J. Biochem.*, 1976, **63**, 357.
[65] W. D. Merritt, T. W. Keenan, and D. J. Morré, *Cancer Biochem. Biophys.*, 1976, **1**, 179.
[66] R. M. Glickman and J. F. Bouhours, *Biochim. Biophys. Acta*, 1976, **424**, 17.
[67] J. F. Bouhours and R. M. Glickman, *Biochim. Biophys. Acta*, 1976, **441**, 123.

gangliosides is substantially lower.[68] The changes in the pattern of gangliosides in the blood sera from hepatoma-bearing rats reflect alterations occurring in the tumour. Preliminary evidence has indicated the presence of two novel fucolipids in normal rat cells and in rat cells transformed with murine sarcoma virus.[69]

The development of gangliosides in mouse brain has been studied from birth, and chromatographic evidence suggested the presence of a new ganglioside.[70] There appear to be no abnormalities in the brain gangliosides of shambling mutant mice.[71]

A sialyltransferase from mouse-brain microsomes was able to catalyse the synthesis of sialylgalactosylceramide (G_7) from D-galactosylcerebroside and CMP-*N*-acetylneuraminic acid.[72] This microsomal preparation synthesized haematoside when lactosylceramide was used as the acceptor. Strains of established mouse neuroblastoma cells growing *in vivo* contained detectable amounts of the ganglioside $G_{T_{1a}}$, whereas they did not when growing in tissue culture.[73] This result suggests that the synthesis of $G_{T_{1a}}$ is suppressed by the conditions used for culturing the tissue, rather than by the loss of genotypic expression. Cell-free enzyme preparations from the tissue-cultured cells were able to catalyse the synthesis of G_{M_3} from lactosylceramide and CMP-*N*-acetylneuraminic acid.[74] The enzyme preparation also contained a 2-acetamido-2-deoxy-D-galactosyltransferase (UDP-D-Gal*N*Ac:G_{M_3}-2-acetamido-2-deoxy-D-galactosyltransferase) that catalyses the conversion of G_{M_3} into G_{M_2}. The results support the view that the first sialic acid residue of the brain gangliosides is introduced into lactosylceramide.

The patterns of long-chain, neutral glycolipids and gangliosides in established L-cell-derived murine fibroblast lines and their low- and high-tumourigenic hybrids have been studied.[75] High-tumourigenic lines were characterized by the absence of, or by a decrease in, long-chain, neutral glycolipids, or complex gangliosides (G_{D_1} or G_T), and/or haematosides. Low-tumourigenic hybrids derived from the fusion of low- and high-tumourigenic cells were characterized by the presence of additional long-chain, neutral glycolipids, or complex gangliosides, or both. A comparison between the membrane glycolipids and glycopeptides from mouse cells transformed by a murine sarcoma virus and those from normal cells showed that the virus-transformed cells contain less mono- and di-sialogangliosides (G_{M_1} and $G_{D_{1a}}$) and a lower activity of the specific D-galactosyltransferase required for the synthesis of these gangliosides.[76] Changes were also detected in the sialoglycopeptides.

The presence of vitamin A appears to be necessary for the biosynthesis of D-mannose-containing glycoproteins and glycolipids in hamster liver, but it

[68] V. P. Skipski, N. Katopodis, J. S. Prendergast, and C. C. Stock, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 1122.
[69] J. Skelly, M. Gacto, M. R. Steiner, and S. Steiner, *Biochem. Biophys. Res. Comm.*, 1976, **68**, 442.
[70] N. Baumann, S. Pollet, and M. L. Harpin, *Compt. rend.*, 1976, **283**, *D*, 1113.
[71] T. N. Seyfried, E. J. Weber, and W. L. Daniel, *J. Neurochem.*, 1976, **27**, 295.
[72] R. K. Yu and S. H. Lee, *J. Biol. Chem.*, 1976, **251**, 198.
[73] G. Dawson and A. C. Stoolmiller, *J. Neurochem.*, 1976, **26**, 225.
[74] S. F. Kemp and A. C. Stoolmiller, *J. Neurochem.*, 1976, **27**, 723.
[75] K. Itaya, S.-I. Hakomori, and G. Klein, *Proc. Nat. Acad. Sci. U.S.A.*, 1976, **73**, 1568.
[76] P. H. Fishman, R. O. Brady, and S. A. Aaronson, *Biochemistry*, 1976, **15**, 201.

was not established whether this results from the involvement of retinol or a metabolite in the glycosyl-transfer reactions.[77] Evidence has accumulated that the principal role of phospholipids in the activation of UDP-D-galactose: α-D-galactosyltransferase is to provide a suitable environment to enable the enzyme to act on the substrate (lactosylceramide).[78] The properties of enzymes catalysing the transfer of D-galactose from UDP-D-galactose to exogenous ceramide mono- and di-hexosides derived from the Syrian hamster cell line NIL 2 have been studied.[79] Kinetic analyses showed that the enzymes are susceptible to inhibition and to activation by a variety of reagents; for example, the enzymes are very sensitive to inhibition by their own substrates. The activities of two D-galactosyltransferases in NIL 2 hamster cells, which catalyse the formation of di- and tri-glycosylceramides, were found to increase markedly as the culture density increased, and the highest transferase activities were contained in the endoplasmic reticulum.[80] Virus transformation reduced the activity of the transferase catalysing the synthesis of triglycosylceramide, whereas that of the transferase catalysing the synthesis of diglycosylceramides was slightly increased. The transformed cells do not appear to produce a dialysable, soluble inhibitor of the transferase activities.

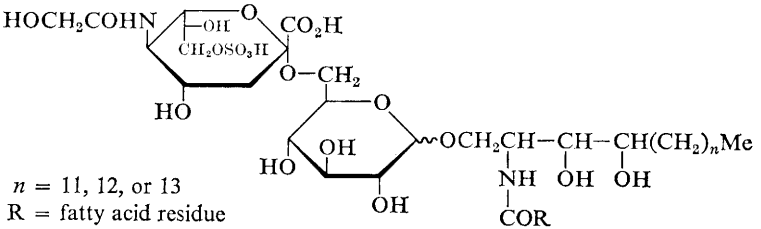
The sialic acid content of the gangliosides in the outer segments of frog retinal rods has been compared with those in other neural and muscular structures of a number of species.[81]

Intact liver cells obtained from chicken embryos exhibit D-mannosyltransferase activity, which is localized at the cell surface.[82] A D-mannosyltransferase in the cells of eight-day-old embryos catalysed the formation of several isoprenoid lipids containing D-mannose, one of which served as the substrate in the highly specific, rate-limiting second step involving the transfer of D-mannose to glycoprotein.[83] Subtle differences have been detected in the ceramide D-galactosyltransferase and cerebroside sulphotransferase activities of chicken-brain cellular fractions and those in cultured cells of neuronal and glial origin.[84]

A comparative study of the glycolipids in the testes of a number of birds and fish indicated that none contain sulphogalactosylmonoalkylmonoacylglycerol, which is the principal glycolipid in the testes and sperm of a number of animals; sulphogalactosylceramide is the principal glycolipid found in mature fowl, duck, and skate.[85]

A sulphated sialosphingolipid has been isolated from the gonads of the sea urchin *Echinocardium cordatum*.[86] Methylation analysis, periodate oxidation, and hydrolysis with neuraminidase established its structure as the sialic acid 8-sulphate-(2 → 6)-D-glucopyranosyl-(1 → 1)-ceramide (11).

[77] L. M. de Luca, C. S. Silverman-Jones, and R. M. Barr, *Biochim. Biophys. Acta*, 1975, **409**, 342.
[78] F. A. McEvoy, D. E. Ellis, and S. Shall, *Biochim. Biophys. Acta*, 1976, **450**, 418.
[79] K. A. Chandrabose and I. A. Macpherson, *Biochim. Biophys. Acta*, 1976, **429**, 95.
[80] K. A. Chandrabose, J. M. Graham, and I. A. Macpherson, *Biochim. Biophys. Acta*, 1976, **429**, 112.
[81] H. H. Hess, P. Stoffyn, and K. Sprinkle, *J. Neurochem.*, 1976, **26**, 621.
[82] D. Arnold, E. Hommel, and H. J. Risse, *Mol. Cell. Biochem.*, 1975, **10**, 81.
[83] D. Arnold, E. Hommel, and H. J. Risse, *Mol. Cell. Biochem.*, 1976, **11**, 137.
[84] L. L. Sarliéve, N. M. Neskovic, L. Freysz, P. Mandel, and G. Rebel, *Life Sci.*, 1976, **18**, 251.
[85] M. Levine, J. Bain, R. Narashimhan, B. Palmer, A. J. Yates, and R. K. Murray, *Biochim. Biophys. Acta*, 1976, **441**, 134.
[86] N. K. Kochetkov, G. P. Smirnova, and N. V. Chekareva, *Biochim. Biophys. Acta*, 1976, **424**, 274.



(11)

The phospholipid and glycolipid contents of the brains, and several other characteristics, of thirty-two species of fish belonging either to the elasmobranches or to the teleosts have been reported.[87] Three gangliosides isolated from the starfish *Asterina pectinifera* have sialic acid residues present in the interior of the carbohydrate chains.[88] Lactosylceramide and water-soluble materials containing arabinose, galactose, and sialic acids were liberated during attempts to remove the sialic acid residues with acid.

Lipophosphonoglycan, a major component of the plasma membrane of *Acanthamoeba castellanii*, has been shown to contain inositol (8%) and C_{25}- and C_{24}-phytosphingosines (13%), in addition to neutral sugars (26%), aminosugars (3%), aminophosphonates (10%), acid-labile phosphate (3%), and long-chain fatty acids.[89] Electrophoresis on dodecyl sulphate–polyacrylamide gel separated the lipophosphonoglycan into two principal components that differed mainly in their sugar constituents.

Plant and Microbial Glycolipids

Mono- and di-D-galactosyldiglycerides from numerous plant species have been separated from one another on a preparative scale.[90] D-Galactose is the principal sugar present, although D-glucose occurs as a minor component of the glycolipids in a number of the plants.

A partial separation of two particulate lipid:D-glucosyltransferases present in etiolated pea (*Pisum sativum*) epicotyls has been achieved.[91] The mitochondrial fraction contained most of the neutral lipid-glycosylating activity that catalyses the formation of products similar to stearyl D-glucosides, whereas a microsomal fraction contained most of the glycosylating activity for lipids of the polyprenol phosphate type. Some of these components appear to be the final receptors for the sugar residues of polysaccharides and glycoproteins. The metabolic turnover, lability to acid, and chromatographic properties of glucolipids obtained on feeding D-[^{14}C]glucose to embryos of *Zea mays* and *P. sativum* have been investigated.[92]

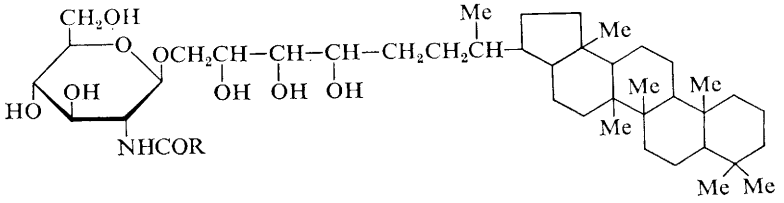
The incorporation into glycolipids of the sugars from GDP-D-mannose and UDP-2-acetamido-2-deoxy-D-glucose catalysed by a crude membrane fraction

[87] E. M. Kreps, N. F. Avrova, M. A. Chebotareva, E. V. Chirkovskaya, V. I. Krasilnikova, E. E. Kruglova, M. V. Levitina, E. L. Obukhova, L. F. Pomazanskaya, N. I. Pravdina, and S. A. Zabelinskii, *Comp. Biochem. Physiol.*, 1975, **52B**, 283.
[88] M. Sugita and T. Hori, *J. Biochem.* (*Japan*), 1976, **80**, 637.
[89] D. G. Dearborn, S. Smith, and E. D. Korn, *J. Biol. Chem.*, 1976, **251**, 2976.
[90] G. R. Jamieson and E. H. Reid, *Phytochemistry*, 1976, **15**, 135.
[91] R. P. Lezica, P. A. Romero, and M. A. Dankert, *Plant Physiol.*, 1976, **58**, 675.
[92] Y. Morohashi and R. S. Bandurski, *Plant Physiol.*, 1976, **57**, 846.

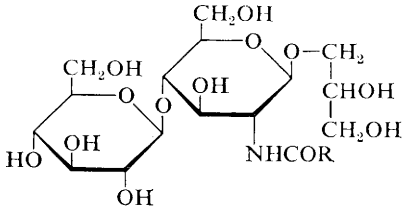
from *Phaseolus aureus* hypocotyls has been studied.[93] Amino-sugar-containing lipids obtained consisted of a mixture of P^1-2-acetamido-2-deoxy-D-glucosyl and P^1-di-*N*-acetylchitobiosyl P^2-dolichyl pyrophosphates. The disaccharyl-lipid can accept D-mannose from GDP-D-mannose, forming a lipid-bound D-mannosyl-di-*N*-acetylchitobiose.

Evidence has been obtained that the synthesis of polymers containing sialic acid in *Escherichia coli* requires membrane-bound undecaprenyl phosphate as an intermediate carrier of the sialic acid residues.[94] Cells of *E. coli* have been shown to contain a novel class of glycolipid consisting of D-gluco-oligosaccharides substituted with glyceryl phosphate units (derived from membrane lipids) and succinic acid in ester linkage.[95] Further studies have shown that the glyceryl phosphate is probably linked to O-6 of D-glucopyranosyl residues in the oligosaccharides.[96]

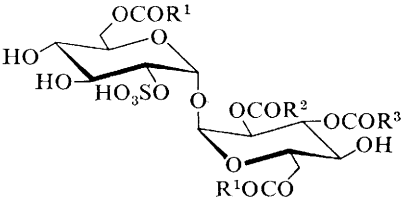
A novel glycolipid (12) isolated from the extreme thermoacidophile *Bacillus acidocaldarius* is a 2-acylamino-2-deoxy-β-D-glucopyranosyl derivative of 1,2,3,4-tetrahydroxypentane linked to hopane (a pentacyclic triterpene) at C-29.[97] Three other glycolipids possess a common structure (13) having the hydroxy-groups of their glycerol residues unsubstituted, or monoacylated, or diacylated.



(12) R = acyl



(13) R = acyl



R^1 = CHMe[CH_2CH]$_7$CHOH$C_{15}H_{31}$; R^2 = $C_{15}H_{31}$; R^3 = CHMe[CH_2CH]$_6C_{16}H_{33}$

(14)

[93] L. Lehle, F. Fartaczek, W. Tanner, and H. Kauss, *Arch. Biochem. Biophys.*, 1976, **175**, 419.
[94] F. A. Troy, I. K. Vijay, and N. Tesche, *J. Biol. Chem.*, 1975, **250**, 156.
[95] L. M. G. van Golde, H. Schulman, and E. P. Kennedy, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 1368.
[96] E. P. Kennedy, M. K. Rumley, H. Schulman, and L. M. G. van Golde, *J. Biol. Chem.*, 1976, **251**, 4208.
[97] T. A. Langworthy, W. R. Mayberry, and P. F. Smith, *Biochim. Biophys. Acta*, 1976, **431**, 550.

During conditions of rapid growth of *Bifidobacterium bifidum*, a precursor–product relationship exists between phosphatidylglycerol and glycerophosphoryl-galactosyldiglyceride.[98] Preferential conversion of phosphatidylglycerol into diphosphatidylglycerol occurred when cell-wall synthesis was inhibited with penicillin. A particulate fraction from *B. bifidum* catalysed the synthesis of phosphogalactolipids and diphosphatidylglycerol from phosphatidylglycerol.[99] The phosphogalactolipids result from the transfer of *sn*-glycerol 1-phosphate from phosphatidylglycerol to the (acylated) monogalactosyldiacylglycerols.

The general structural features of five families of polyacylated αα-trehalose 2-sulphates elaborated by *Mycobacterium tuberculosis* have been described.[100] Biological acylation of the trehalose core does not appear to be very specific, but yields a family of sulpholipids; the principal member has the structure (14).

D-Glucose and myristic, palmitic, and stearic acids, as well as D-(−)-glyceric acid, are components of a glycolipid isolated from *Nocardia caviae*.[101]

Wheat-germ agglutinin and concanavalin A have been used in studies of the binding characteristics of mycoplasma cells and membranes.[102]

A pentaglycosyldiacylglycerol isolated from *Acholeplasma modicum* is composed of residues of D-galactose, D-glucose, and a heptose (2 : 2 : 1).[103] A structure (15)

D-Gal*p*-(1 → 2)-α-D-Gal*p*-(1 → 3)-α-D-*glycero*-D-*manno*-Hep*p*-(1 → 3)-
β-D-Glc*p*-(1 → 2)-α-D-Glc*p*-diacylglycerol

(15)

was assigned to the glycolipid on the basis of the results of partial hydrolysis with acid, periodate oxidation, and oxidation with chromium trioxide.

Lysates of the protoplasts of *Saccharomyces cerevisiae* incorporated D-mannose from either GDP-D-mannose or P^2-dolichyl P^1-D-mannosyl pyrophosphate – probably at the polysomal level – into yeast cell-wall glycoproteins.[104] The acetamido-sugar from UDP-2-acetamido-2-deoxy-D-[^{14}C]glucose was also incorporated into endogenous glycolipids and a polymer fraction by a membrane fraction of *S. cerevisiae*; two of the three glycolipids were identified as P^1-2-acetamido-2-deoxy-D-glucosyl and P^1-di-*N*-acetylchitobiosyl P^2-dolichyl pyrophosphates.[105] The membrane preparation also contained a D-mannosyl-transferase that catalyses the transfer of two D-mannosyl residues to P^1-di-*N*-acetylchitobiosyl P^2-dolichyl pyrophosphate to form a lipid-linked tetrasaccharide, which is probably an intermediate in the glycosylation of the yeast mannan-protein. A dolichyl pyrophosphate derivative (a glycosylated endogenous acceptor) containing two residues of 2-acetamido-2-deoxy-D-glucose and, probably, twelve residues of D-mannose and four residues of D-glucose is synthesized by rat-liver microsomes.[106] Incubation of the acceptor with a particulate fraction from yeast resulted in transfer of the oligosaccharide chain to endogenous protein in a manner akin to that known to occur in liver microsomes.

[98] F. W. van Schak and J. H. Veerkamp, *F.E.B.S. Letters*, 1976, **67**, 13.
[99] J. H. Veerkamp, *Biochim. Biophys. Acta*, 1976, **441**, 403.
[100] M. B. Goren, O. Brokl, P. Roller, H. M. Fales, and B. C. Das, *Biochemistry*, 1976, **15**, 2728.
[101] M. T. Pommier and G. Michel, *Biochim. Biophys. Acta*, 1976, **441**, 327.
[102] I. Kahane and J. G. Tully, *J. Bacteriol.*, 1976, **128**, 1.
[103] W. R. Mayberry, T. A. Langworthy, and P. F. Smith, *Biochim. Biophys. Acta*, 1976, **441**, 115.
[104] G. Larriba, M. V. Elorza, J. R. Villanueva, and R. Sentandreu, *F.E.B.S. Letters*, 1976, **71**, 316.
[105] L. Lehle and W. Tanner, *Biochim. Biophys. Acta*, 1975, **399**, 364.
[106] A. J. Parodi, *F.E.B.S. Letters*, 1976, **71**, 283.

8

Chemical Synthesis and Modification of Oligosaccharides, Polysaccharides, Glycoproteins, Enzymes, and Glycolipids

BY J. F. KENNEDY

Synthesis of Polysaccharides, Oligosaccharides, Glycoproteins, Glycopeptides, Enzymes, and Glycolipids

A series of papers dealing with recent applications of solid-phase synthesis in chemistry and biochemistry includes articles on the solid-phase synthesis of oligosaccharides.[1]

Polysaccharides.—The glycosylation of t-butyl ethers under Helferich conditions has been investigated as an approach to the synthesis of polysaccharides; for example, glycosylation of 2,2′,3,3′,4,4′-hexa-*O*-acetyl-6′-*O*-t-butylgentiobiosyl bromide, which cannot undergo intramolecular glycosylation, gave a D-glucan containing both α- and β-(1 → 6)-linkages.[2]

Phosphorus pentaoxide in DMSO acted on methyl α-D-glucopyranoside, sucrose, and αα-trehalose to afford non-dialysable, phosphorylated D-glucans containing carbohydrate (46—59%) and phosphorus (11—14%), which gave reducing-sugar values of 5—31%.[3] Polysucrose (mol. wt. *ca.* 9500) was depolymerized by alkali, yielding sugar phosphates and oligosaccharides. Oxidation with periodate and methylation analysis demonstrated the presence of branch-points in the D-glucans which bind concanavalin A. The D-glucans are considered to be formed from non-reducing mono- and oligo-saccharides by dehydration, transglycosylation, and phosphorylation.

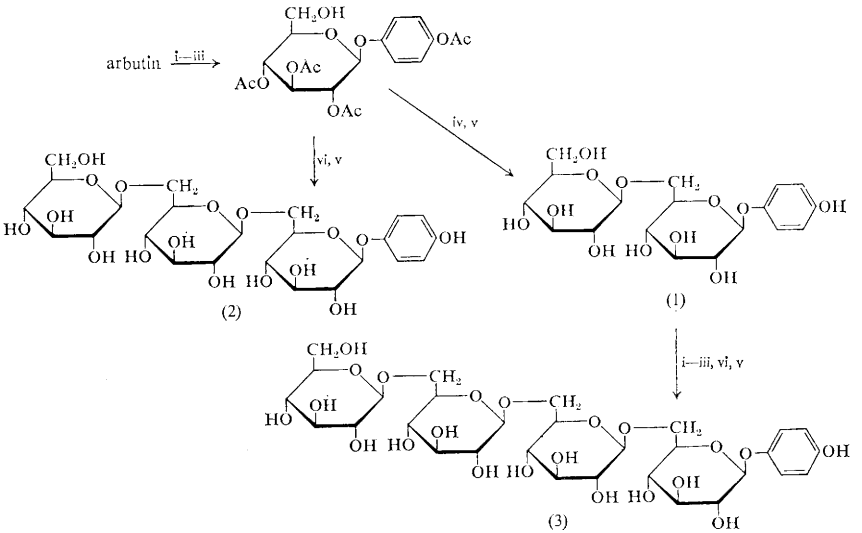
Xylanases from *Aspergillus niger* catalysed a reaction that yielded D-xylans from D-xylo-tetraose or -pentaose.[4]

Oligosaccharides and Glycosides thereof.—Various steps in the synthesis of benzyl 2-acetamido-4,6-di-*O*-acetyl-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-α-D-glucopyranoside using a functionalized 'popcorn' polystyrene have been examined.[5]

A route used to synthesize oligosaccharides containing α-linked 2-amino-2-deoxy-D-glucopyranosyl residues has been described (see Vol. 9, p. 14).[6]

[1] 'Solid-Phase Synthesis', ed. E. C. Blossey and D. C. Neckers, Dowden, Hutchinson, and Ross, 1975.
[2] N. K. Kochetkov and E. M. Klimov, *Carbohydrate Res.*, 1975, **44**, 138.
[3] S. Hirano, T. Nishio, and T. Ito, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1963.
[4] S. Takenishi and Y. Tsujisaka, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2315.
[5] G. Excoffier, D. Gagnaire, J.-P. Utille, and M. Vignon, *Tetrahedron*, 1975, **31**, 549.
[6] H. Paulsen and W. Stenzel, *Angew. Chem. Internat. Edn.*, 1975, **14**, 558.

The phenolic glycosides (1)—(3) of gentio-oligosaccharides have been synthesized from arbutin (Scheme 1),[7] and *O*-α-D-glucopyranosyl-(1 → 4)-*O*-β-D-glucopyranosyl-(1 → 6)-D-glucose has been prepared by way of condensation of acetobromomaltose with benzyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside in a Koenigs–Knorr reaction.[8] Derivatives of the anomeric benzyl L-idopyranosides



Reagents: i, TrCl–py; ii, Ac_2O–py; iii, 80% AcOH; iv, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide–Ag_2O; v, MeONa; vi, 2,2′,3,3′,4,4′,6′-hepta-*O*-acetylgentiobiosyl bromide–Ag_2O

Scheme 1

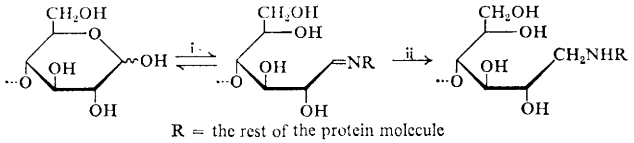
and L-idopyranosiduronates have been synthesized from D-glucose as models for conformational studies of L-iduronic acid residues in heparin.[9]

The abilities of α-amylases to synthesize malto-oligosaccharides from α-D-glucopyranosyl fluoride have been reviewed; related syntheses from α-maltosyl fluoride were also discussed.[10]

Glycoproteins.—Pseudoglycoproteins have been synthesized by treating proteins with carbohydrates (see, *e.g.*, Tables 1—5) and by coupling oligosaccharides (*e.g.* lactose) to proteins and derivatized gels (see Scheme 2).[11]

Glycopeptides.—*O*-[2-Acetamido-3- and -4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosyl]-*N*-benzyloxycarbonyl-L-serine methylamides have been synthesized by the reactions shown in Scheme 3.[12]

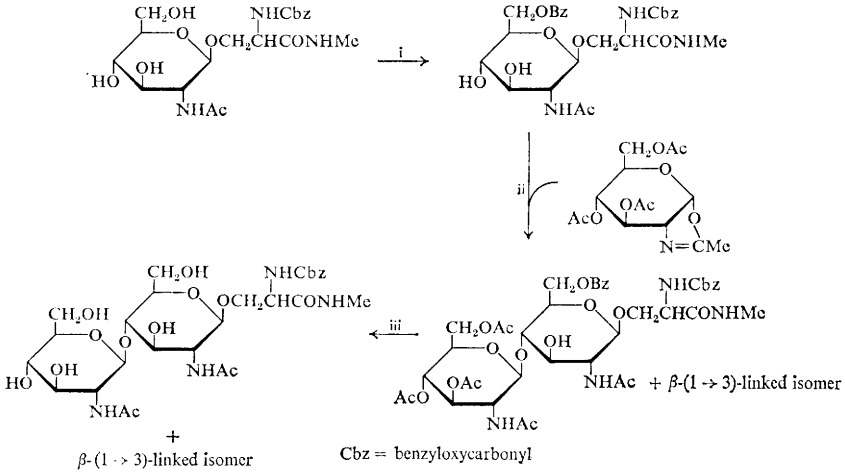
[7] K. Takiura, M. Yamamoto, Y. Miyaji, H. Takai, S. Honda, and H. Yuki, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2451.
[8] P. Nánási, A. Lipták, and L. Jánossy, *Acta Chim. Acad. Sci. Hung.*, 1976, **88**, 155.
[9] J. Kiss and P. C. Wyss, *Tetrahedron*, 1976, **32**, 1399.
[10] E. J. Hehre, G. Okada, and D. S. Genghof, in 'Carbohydrates in Solution', ed. R. F. Gould, *Adv. Chem. Ser.* No. 117, The American Chemical Society, Washington, 1973, p. 309.
[11] G. R. Gray, *Arch. Biochem. Biophys.*, 1974, **163**, 426.
[12] N. K. Kochetkov, V. A. Derevitskaya, and O. S. Novikova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 179.



R = the rest of the protein molecule

Reagents: i, RNH_2-phosphate buffer; ii, $NaBH_3CN$

Scheme 2



Reagents: i, BzCl–py; ii, TsOH–PhCl; iii, $MeNH_2$

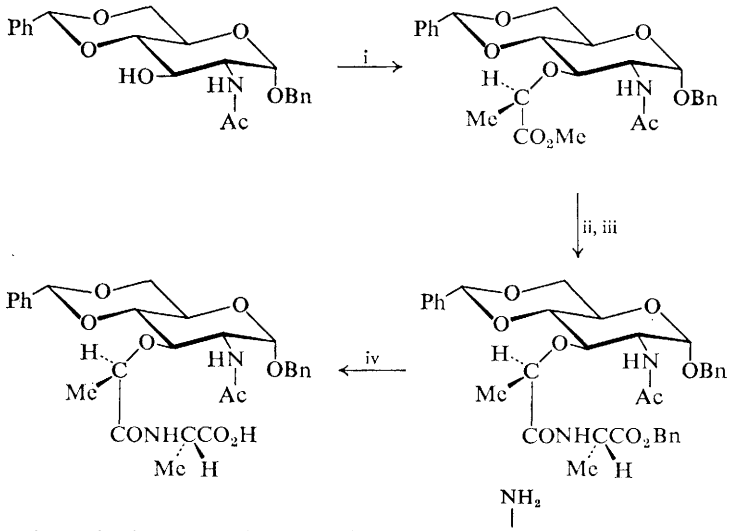
Scheme 3

2-Acetamido-2-deoxy-3-*O*-(D-propion-2-yl-L-alanine)- and -(D-propion-2-yl-L-alanyl-D-*iso*-glutamine)-D-glucopyranose have been obtained *via* condensation of benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranoside with an L-alanyl derivative and a dipeptide, respectively, followed by debenzylidenation and hydrogenolysis.[13] Only the glycodipeptide is adjuvant active, and is the smallest adjuvant-active structure known to date.

An efficient synthesis of (benzyl 4,6-*O*-benzylidene-*N*-acetylmuramyl)-L-alanine is shown in Scheme 4; this compound is a key intermediate in the synthesis of mucopeptides.[14] The synthesis of *N*-acetylmuramyl-L-alanyl-D-*iso*-glutamyl-LL-diaminopimelyl-D-alanine, a glycopeptide constituting part of the structure of the mureines of two bacteria, has been reported.[15]

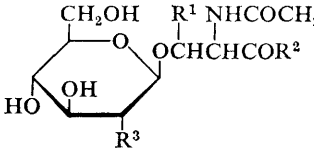
Modified Koenigs–Knorr reactions have been used in the synthesis of the glycopeptides (4)—(7) from hydroxyamino-acids,[16] and the synthesis of the bivalent lactosyl haptens (8)—(10) and the interactions of the haptens with anti-lactosyl antibodies have been reported.[17]

[13] C. Merser and P. Sinay, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 1316.
[14] A. Arendt, A. Kolodziejczyk, and T. Sokolowska, *Roczniki Chem.*, 1974, **48**, 1707.
[15] A. Arendt, A. Kolodziejczyk, and T. Sokolowska, *Roczniki Chem.*, 1974, **48**, 1921.
[16] K. Wakabayashi and W. Pigman, *Carbohydrate Res.*, 1974, **35**, 3.
[17] P. V. Gopalakrishnan and F. Karush, *Immunochemistry*, 1974, **11**, 279.



Reagents: i, $MeCHClCO_2Me$–Na/K alloy; ii, HO^-; iii, $MeCHCO_2Bn$–*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; iv, hydrolysis

Scheme 4



(4) $R^1 = H$; $R^2 = NH_2$; $R^3 = OH$
(5) $R^1 = Me$; $R^2 = OMe$; $R^3 = OH$
(6) $R^1 = Me$; $R^2 = NH_2$; $R^3 = OH$
(7) $R^1 = H$; $R^2 = NH_2$; $R^3 = NHCOCF_3$

NN'-bis(α-*N*Ac,ε-*N*-Q-Lys)-4-phenylenediamine
(8)

NN'-bis(α-*N*Ac,ε-*N*-Q-Lys-Pro-Pro)-4-phenylenediamine
(9)

NN'-bis(α-*N*Ac,ε-*N*-Q-Lys-$(Ala)_4$)-4-phenylenediamine
(10)

Q = β-lactosyloxy·$C_6H_4N{:}NC_6H_4NHCOCH_2SCH_2CO$·

Enzymes.—The possibility of synthesizing an artificial enzyme has been discussed.[18]

Condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-β-D-glucopyranosylamine with suitably protected derivatives of L-alanine, L-alanyl-L-threonine, L-alanyl-L-threonyl-L-leucine, and L-alanyl-L-threonyl-L-leucyl-L-alanine afforded glycopeptides corresponding to the sequences 18—19, 18—20, 18—21, and 18—22 of deoxyribonuclease A.[19]

[18] 'Advances in Polymer Science,' ed. Z. A. Rogovin, Israel Program for Scientific Translations Ltd. and John Wiley and Sons, Ltd., London, 1974.
[19] H. G. Garg and R. W. Jeanloz, *J. Org. Chem.*, 1976, **41**, 2480.

L-Lys → L-Val → L-Phe → Gly → L-Arg
(11)

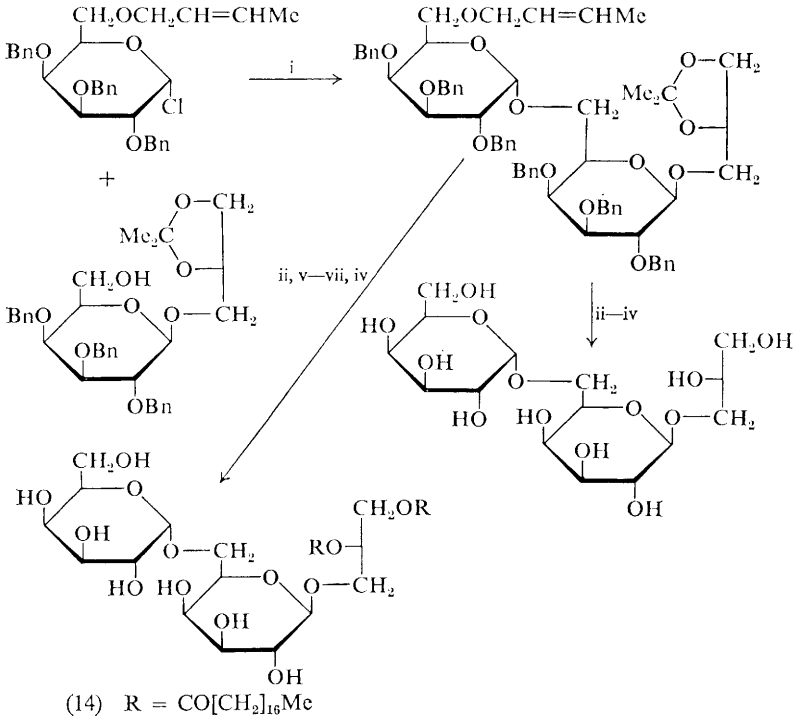
L-Thr → L-Pro → Gly → L-Ser → L-Arg
(12)

L-Lys → L-Val → L-Tyr → Gly → L-Arg
(13)

Syntheses of tryptic peptides corresponding to the sequences 1—5 (11) and 69—73 (12) of hen egg-white lysozyme and to the sequence 1—5 (13) of turkey egg-white lysozyme have been described.[20] The anti-histaminic activity of (11)—(13) was examined.

Glycolipids and Gangliosides.—Approaches to the synthesis of D-galactosylglycerol, D-galactosyldiglycerides, and plant galactolipids have been reviewed.[21]

A new approach to the synthesis of cord factor (the toxic glycolipid of *Mycobacterium tuberculosis*) is based on the use of ester derivatives of αα-trehalose



Reagents: i, $Et_4\overset{+}{N}\overset{-}{Cl}$–$Et_3N$–$CH_2Cl_2$; ii, Bu^tOK–DMSO; iii, HCl–MeOH; iv, H_2–Pd–C; v, BnCl–NaH–PhH; vi, HCl–dioxan; vii, $Me[CH_2]_{16}COCl$–py

Scheme 5

[20] K. Suzuki, N. Endo, R. Tani, and H. Kikuchi, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2462.
[21] H. C. van Hummel, *Fortschr. Chem. org. Naturstoffe*, 1975, **32**, 267.

and α-branched β-hydroxy-fatty acids.[22] Another synthesis of cord factor (6,6′-di-*O*-mycoloyl-αα-trehalose) and a synthesis of an analogue, 6,6′-di-*O*-palmitoyl-αα-trehalose, have been reported.[23] 3-*O*-(6-*O*-α-D-Galactopyranosyl-β-D-galactopyranosyl)-1,2-di-*O*-stearoyl-L-glycerol (14) has been synthesized by way of condensation of 2,3,4-tri-*O*-benzyl-6-*O*-(but-2-enyl)-α-D-galactopyranosyl chloride and 3-*O*-(2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl)-1,2-*O*-isopropylidene-L-glycerol (Scheme 5).[24]

Phytosphingosine prepared from the yeast *Hansenula ciferrii* has been used as the starting material in a synthesis of D-galactosylsphingolipids; glycosylation of *N*-dichloroacetylated and 3,4-di-*O*-benzoylated phytosphingosine was accomplished with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide in the presence of mercuric cyanide, whereafter removal of the protecting groups with alkali gave 1-*O*-β-D-galactopyranosylphytosphingosine, which was *N*-acylated using the 4-nitrophenyl esters of octadecanoic and D-2-hydroxyoctadecanoic acids to give cerebrosides.[25]

Modification of Polysaccharides and Oligosaccharides, and Uses of Modified Polysaccharides and Oligosaccharides

Introduction.—The gaseous and other products formed on γ-radiolysis of D-glucose and D-glucans have been examined.[26]

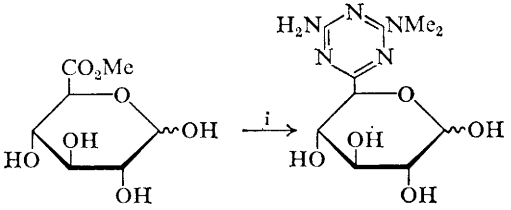
Recent advances in the preparation of matrices for affinity chromatography by modification of polysaccharides, *etc.*, have been reviewed, with particular reference to the chemistry of the matrices, spacer arms, molecular probes, physical parameters, and new applications.[27] A theoretical approach to affinity chromatography on modified polysaccharide and other matrices has been developed by considering how the interaction between the polysaccharide derivative and the substrate depends on such factors as substrate loading and the concentration of the washing agent, *etc.*[28]

The usefulness of attaching reactive residues to insoluble polymer supports, including polysaccharides, has been dealt with in a series of papers on solid-phase synthesis.[1]

The cleavage of polysaccharides by hydrolysis with acid, by oxidation with periodate or chromium trioxide, by base-catalysed β-elimination, by deamination, and by Weermann and Lossen rearrangements has been ably reviewed.[29] A new approach to selective cleavage of the glycosidic bond to the reducing-end residue of oligosaccharides has involved base-catalysed β-elimination on a model 5-deoxy-5-nitromaltitol derivative.[30]

[22] J. F. Tocanne, *Carbohydrate Res.*, 1975, **44**, 301.
[23] R. Toubiana, B. C. Das, J. Defaye, B. Mompon, and M. J. Toubiana, *Carbohydrate Res.*, 1975, **44**, 308.
[24] P. A. Gent and R. Gigg, *J.C.S. Perkin I*, 1975, 1521.
[25] I. Pascher, *Chem. and Phys. Lipids*, 1974, **12**, 303.
[26] S. I. Goldin, A. A. Ivko, N. T. Bondarenko, S. V. Markevich, and V. A. Sharpatyi, *Doklady Akad. Nauk S.S.S.R.*, 1976, **228**, 389.
[27] H. Guilford, *Ann. Reports (B)*, 1974, **71**, 56.
[28] P. C. Wankat, *Analyt. Chem.*, 1974, **46**, 1400.
[29] B. Lindberg, J. Lönngren, and S. Svensson, *Adv. Carbohydrate Chem. Biochem.*, 1975, **31**, 185.
[30] N. Kashimura, K. Yoshida, and K. Onodera, *Carbohydrate Res.*, 1975, **40**, 375.

Polysaccharides can be fully benzylated using benzyl chloride in DMSO under nitrogen in the presence of methylsulphinyl methanide.[31] Only one treatment is usually necessary. Standard methodologies for dyeing polysaccharides with Methylene Blue, *etc.*,[32] and for the specific cleavage of polysaccharides containing uronic acid residues [33] have been described. The carboxy-group of a hexuronic acid can be incorporated into a *s*-triazinyl ring (Scheme 6) and the procedure has been extended to polyuronates.[34]



Reagent: i, *NN*-dimethylbiguanidinium hydrochloride–MeOH–THF

Scheme 6

Enhancement of the intensity of the fluorescence of dansylated protamine in the presence of sulphated carbohydrates has been used in assays for mono-, oligo-, and poly-saccharide sulphates and glycosaminoglycans.[35]

The interactions of polysaccharides with homogeneous myeloma immunoglobulins have been reviewed.[36]

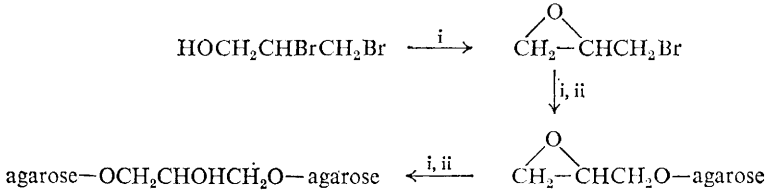
Agaroses.—Neoagaro-oligosaccharides and reduced forms thereof have been used as substrates for neoagarases.[37]

Aldehydo-groups have been introduced into agarose by hydrolysis (pH 3) of the product obtained from the reaction of agarose cyclic imidocarbonate with 4-aminobutyraldehyde diethyl acetal.[38] The d.s. of the original agarose derivative could be determined by measuring the amount of ethanol released on acid hydrolysis. Periodate-oxidized agarose has been used to immobilize chymotrypsin, with retention of enzymic activity.[39]

Macroporous agarose activated with 1,4-bis-(2,3-epoxypropyloxy)butane reacted with cyclohexa-amylose to give an affinity matrix used in the purification of β-amylase.[40]

A benzylated, cross-linked agarose gel has been prepared.[41] Gels variously cross-linked with 2,3-dibromopropan-1-ol (Scheme 7) have been used in the chromatography of enzymes, including polysaccharide hydrolases. In some instances, the enzyme was denatured on elution from the gel, although the

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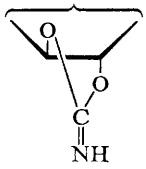


Reagents: i, NaOH; ii, agarose

Scheme 7

adsorbed enzyme was highly active. Since the gels are amphophilic, they may find use in electrophoresis.

Cyclic imidocarbonate derivatives of agarose (15) (Vol. 7, p. 511) continue to be used extensively for the immobilization of biologically active macromolecules



(15)

and for affinity chromatography. 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) by such methods are summarized in Tables 1, 2, and 3, 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.

Table 1 *Use of agarose cyclic imidocarbonates for the preparation of active immobilized enzymes*

Enzyme coupled to agarose cyclic imidocarbonate	*E.C. No.*	*Ref.*
Aminopeptidase (cytosol)	3.4.11.1	42
Aspartate aminotransferase	2.6.1.1	43 [a]
Chymotrypsin	3.4.21.1	39
Dextranase	3.2.1.11	44
Diol dehydrase (B_{12}-dependent)	4.2.1.28	45
D-Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	46
Laccase	1.10.3.2	47

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Table 1 (*cont.*)

Enzyme coupled to agarose cyclic imidocarbonate	*E.C. No.*	*Ref.*
Lactate dehydrogenase	1.1.1.27	48
Malate dehydrogenase	1.1.1.37	43 [b]
α-D-Mannosidase	3.2.1.24	49
Pepsin A	3.4.23.1	50
Ribonuclease	3.1.4.22/33	51
Subtilisin	3.4.21.14	39
Trypsin	3.4.21.4	48, 52
Tryptophanase	4.1.99.1	53, 54

[a] Coupled together with malate dehydrogenase. Coupled together with aspartic aminotransferase.

Table 2 *Use of agarose cyclic imidocarbonate for the preparation of immunoadsorbents*

Immunologically active compound coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
Adenovirus hexon antigens	—	Investigations of the antigenic determinants of adenovirus capsids	55
Anti-arginase antibody	—	Purification of arginase by immunoadsorption	56
Anti-(*Bacillus subtilis* α-amylase) antibody	—	Removal of cross-reacting materials from modified *B. subtilis* α-amylase by immunoadsorption	57 [b]
Anti-enterotoxin antibody	— (16)[a]	Purification of the enterotoxin from *Clostridium perfringens* by immunoadsorption	58
Anti-(human C3PA) antibody	—	Purification of human C3 proactivator by immunoadsorption	59
Anti-(human α-foetoprotein) antibody	—	Radioimmunoassay of human α-foetoprotein	60
Anti-(D-glucose oxidase) antibody	—	Purification of D-glucose oxidase by immunoadsorption	61

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Table 2 (*cont.*)

Immunologically active compound coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
Anti-(β-D-glucuronidase) antibody	—	Characterization of porcine antibodies (immunoglobulins IgG) to rabbit β-D-glucuronidase	62
Anti-(human immunoglobulin IgG) antibody	—	Enzyme-linked immunoassay of human immunoglobulin IgG	63
Anti-(human serum albumin) antibody	— (16) [a], (17) [a]	Immunoadsorption of human serum albumin modified for affinity electrophoresis (see p. 452)	64
Anti-(P-450$_{CAM}$) antibody	—	Purification of the P-450 haemeproteins of *Rhizobium japonicum* by immunoadsorption	65
Anti-(porcine insulin) antibody	—	Combination of insulin chains on an immobilized anti-insulin antibody	66
Angiotensin	(18) [a, c]	Purification of rabbit immunoglobulin anti-(angiotensin II) antibodies by immunoadsorption	67
Escherichia coli antigens Ferritin	— —	Isolation of rabbit immunoglobulin anti-milk antibodies	68
Fetuin	—	Fractionation of normal and of immune sera by immunoadsorption	69
D-Glucose oxidase	—	Purification of anti-(D-glucose oxidase) antibodies by immunoadsorption	61
Human Clq protein	—	Isolation of Clq-binding immune complexes	70
Human immunoglobulin IgG	—	Isolation of Clq-binding immune complexes	70
	—	Microfluorometric immunoassays using solid-phase antigens by an inhibition process	71
Human immunoglobulin IgM	—	Microfluorometric immunoassays with solid-phase antigens	71

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Table 2 (*cont.*)

Immunologically active compound coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
Insulin	— (17) [a]	Isolation of guinea-pig immunoglobulin anti-insulin antibodies by immuno-adsorption	72
Mouse immunoglobulin anti-(*Bacillus subtilis* α-amylase) antibody	—	Removal of cross-reacting materials from modified *B. subtilis* α-amylase by immunoadsorption	57 [b]
Mouse immunoglobulin IgM paraprotein	—	Microfluorometric immuno-assays with solid-phase antigens by an inhibition process	71
Mouse nerve-growth factor	—	Isolation of sheep anti-(nerve-growth factor) antibodies	73
Ovalbumin	—	Microfluorometric immuno-assay with solid-phase antigens by a competition process	71
Peptides from the cell walls of *Bacillus megaterium*	—	Pseudoimmunoadsorption in studies of the immunological properties of bacterial cell walls	74
Porcine immunoglobulin IgG anti-(rabbit β-D-glucuronidase) antibody	—	Characterization of porcine antibodies (immuno-globulins IgG) to rabbit β-D-glucuronidase	62
Rabbit immunoglobulin anti-arginase antibody	—	Purification of arginase by immunoadsorption	56
Rabbit immunoglobulin Ig anti-(D-glucose oxidase) antibody	—	Purification of D-glucose oxidase by immuno-adsorption	61
Rabbit immunoglobulin Ig anti-(human α-foetoprotein) antibody	—	Radioimmunoassay of human α-foetoprotein	60
Rabbit immunoglobulin IgG	—	Investigation of the reversible binding of T-cell factor on immobilized immuno-globulins	75
Rabbit immunoglobulin IgG anti-human C3PA antibody	—	Purification of human C3 proactivator by immuno-adsorption	59
Rabbit immunoglobulin IgG anti-(human immuno-globulin IgG) antibody	—	Enzyme-linked immunoassay of human immuno-globulin IgG	63

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Table 2 (*cont.*)

Immunologically active compound coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
Rabbit immunoglobulin IgG anti-(P-450$_{CAM}$) antibody	—	Purification of the P-450 haemeproteins of *Rhizobium japonicum* by immunoadsorption	65
Rabbit immunoglobulin IgG $F(ab')_2$ fragment	—	Investigation of the reversible binding of T-cell factor on immobilized immunoglobulins	75
Rabbit immunoglobulin IgM	—		
Rabbit immunoglobulin anti-enterotoxin antibody	— (16) [a]	Purification of the enterotoxin from *Clostridium perfringens* by immunoadsorption	58

[a] See text p. 432 and Scheme 9. [b] Derivative not certain. [c] Protein = albumin.

Table 3 *Use of agarose cyclic imidocarbonates for the preparation of affinity chromatography materials*, etc.

Ligand or affinant coupled to agarose cyclic imidocarbonate [a]	*Use of product*	*Ref.*
2-Acetamido-*N*-(6-aminohexanoyl)-2-deoxy-β-D-glucopyranosylamine	Purification of β-D-2-acetamido-2-deoxy-hexosidases A and B by affinity chromatography	76
2-Acetamido-1-(4-L-aspartamido)-1,2-dideoxy-β-D-glucose	Isolation of wheat-germ agglutinin by affinity chromatography	77 [b]
α_1-Acid glycoprotein	Isolation of neuraminidase by affinity chromatography	78
Amines (primary)	Affinity chromatography of lipase using hydrophobic ligands	79
1-Aminoalkanes	Hydrophobic affinity chromatography of chloroamphenicol acetyltransferases	80
4-Aminobenzoic acid	Purification of phenoloxidase by affinity chromatography	81
2-Amino-2-deoxy-D-glucose	Large-scale purification of glucokinase	83
6-Aminohexanoic acid	Hydrophobic affinity chromatography of D-galactosyltransferase	84
	Large-scale purification of glucokinase	83
2-Acetamido-*N*-(6-aminohexanoyl)-2-deoxy-β-D-glucopyranosylamine	Isolation of β-D-2-acetamido-2-deoxy-hexosidase by affinity chromatography	85
N^6-(6-Aminohex-1-yl)adenosine 2′,5′-diphosphate	Purification of D-glucose 6-phosphate dehydrogenase by affinity chromatography	86

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Table 3 (*cont.*)

Ligand or affinant coupled to agarose cyclic imidocarbonate [a]	*Use of product*	*Ref.*
N-(6-Aminohexan-1-yl)amino-2-deoxy-D-glucose	Large-scale purification of glucokinase	83
N-(6-Aminohexanoyl)-L-fucopyranosylamine	Removal of contaminating α-L-fucosidase from preparations of glycoside hydrolases by affinity chromatography	87
	Purification of α-L-fucosidase by affinity chromatography; studies of the behaviour of isoenzyme forms on the matrix	82
(6-Aminohexanoyl)-D-tryptophan methyl ester	Investigations of specific and non-specific binding of chymotrypsin during affinity chromatography	88
N^6-(6-Aminohex-1-yl)adenosine 2′,5′-diphosphate	Separation of the isoenzymes of alcohol dehydrogenase and isolation of the enzyme by affinity chromatography	89
N^6-(6-Aminohex-1-yl)adenosine 5′-phosphate	Affinity chromatography and purification of lactate dehydrogenase; comparisons of a variety of coupling routes to the matrix	90 [c]
	Application as an immobilized enzyme cofactor and as an affinity support for the purification of dehydrogenases and kinases	91
	Purification of the steroid-active isoenzyme of alcohol dehydrogenase by affinity chromatography	92
P^3-(6-Aminohex-1-yl)deoxyguanosine triphosphate	Isolation of ribonucleotide reductase by affinity chromatography	93
N-[*N*-(6-Aminohexyl)glycyl]-ansanilic acid	Investigations of the effects of different spacer arms on the affinity chromatography of alkaline phosphatase	94
3-Aminophenobarbital	Purification of P-450 haemeproteins of *Rhizobium japonicum* by affinity chromatography	65
4-Aminophenyl 1-thio-β-D-galactopyranoside	Purification of bacterial glycoside hydrolases by affinity chromatography	95
α-Amylase-inhibitor proteins from wheat flour	Purification of α-amylases by affinity chromatography	96
Bovine serum albumin (sulphydryl blocked)	Measurement of ligand-binding by proteins or peptides	97
Caprylyl hydrazide	Study of lyotropic salt effects in hydrophobic chromatography	98

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Table 3 (*cont.*)

Ligand or affinant coupled to agarose cyclic imidocarbonate [a]	*Use of product*	*Ref.*
Castor-bean lectin	Comparison of the binding capacities of two forms of α-L-iduronidase	99
Concanavalin A	Affinity chromatography of serum proteins	100
	Affinity chromatography of low-mol.-wt. human folate-binding protein	101
	Comparison of the binding capacities of two forms of α-L-iduronidase	99
	Crossed immunoaffinoelectrophoresis of glycoprotein enzymes	102
	Fractionation of carcinoembryonic antigen and investigation of the heterogeneity of the antigen by affinity chromatography	103
	Investigations of the binding of human-fibroblast interferon to concanavalin A; affinity-chromatography characteristics of immobilized concanavalin A	104
	Investigation of the glycoproteinaceous nature of αα-trehalases	105
	Isolation of peroxidase by affinity chromatography	106
	Matrix for the immobilization of laccase	47
	Purification of β-D-galactosidase and β-D-glucuronidase isoenzymes from normal human liver by affinity chromatography	107
	Purification of β-D-2-acetamido-2-deoxy-hexosidases A and B by affinity chromatography	76
	Purification of arylsulphatase A by affinity chromatography	108
	Purification of glycoside hydrolases by affinity chromatography	109
	Purification of hyaluronidase by affinity chromatography	110
	Purification of α-D-mannosidase by affinity chromatography	87

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Table 3 (*cont.*)

Ligand or affinant coupled to agarose cyclic imidocarbonate [a]	*Use of product*	*Ref.*
	Removal of polysaccharide contaminants from DNA preparations by affinity chromatography	111
	Separation of membrane glycoproteins from rat-brain synoptic vesicles by affinity chromatography	112
	Studies by a buoyant density method of the interactions of the carbohydrate-binding sites on concanavalin A with adipocyte receptors	113, 114
	Studies of the binding of glycophorin A to immobilized lectins	115
	Studies of the binding of glycoproteins in microsomal and Golgi membranes to lectins	116
	Studies of the interactions of *Mucor* sp. acid proteases with concanavalin A	117
Crotalaria juncea lectin	Affinity chromatography of serum proteins	100
Crotalaria sp. lectin	Studies of the binding of glycoproteins in microsomal and Golgi membranes to lectins	116
Deacetylcolchicine	Purification of tubulin by affinity chromatography	118
1,6-Diaminohexane	Hydrophobic affinity chromatography in the fractionation of enzymes from halophilic bacteria	119
α,ω-Diaminohexanes	Hydrophobic affinity chromatography of D-galactosyltransferase	84
Fetuin	Purification of neuraminidase to a protease-free preparation	120
Fungal cell-wall glycoproteins	Isolation of glycoside hydrolases by affinity chromatography	121
α-D-Galactopyranosyl-binding lectin (from *Bandeiraea simplicifolia*)	Studies of the interactions of *Bandeiraea simplicifolia* lectin with model carbohydrate–protein conjugates and polysaccharides by affinity chromatography	122

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Table 3 (*cont.*)

Ligand or affinant coupled to agarose cyclic imidocarbonate [a]	*Use of product*	*Ref.*
[U-^{3}H]Gibberellic acid GA_3 4-aminobutyramide	Comparison of methods for the preparation of affinity adsorbents for gibberellin	123
Glycoprotein from the cell wall of *Saccharomyces cerevisiae*	Isolation of an *exo*-β-D-glucanase from a mixture of D-glucanases and D-glucosidases by affinity chromatography	121
2-(*N*-Glycylglycyl)amido-2-deoxy-D-glucose	Large-scale purification of glucokinase	83
Glycylglycyl-L-arginine	Affinity chromatography of trypsin and related enzymes	124
Heparin(cetyl pyridinium salt)	Separation of two forms of α-L-iduronidase by affinity chromatography	99
Hexyl 6-amino-6-deoxy-αβ-L-fucopyranoside	Isolation of *Ulex europeus* haemagglutinin by affinity chromatography	125
Insulin	Investigation of the interaction of immobilized insulin with bovine serum albumin	126
Isodeacetylcolchicine	Purification of tubulin by affinity chromatography	118
α-Lactalbumin	Purification of D-galactosyltransferase from skim milk	84
L-Leucine L-Leucyl amide	Hydrophobic affinity chromatography of D-galactosyltransferase	84
[D-Leucine^6]octapeptide [d]	Purification of renin by affinity chromatography	127
[8-Lysine]vasopressin	Isolation of anti-([8-lysine]vasopressin) antibodies by affinity chromatography	128
Micrococcus lysodeikticus cell-wall lysozyme lysate	Purification of lysozymes and other bacteriolytic enzymes by affinity chromatography	129
	Purification of lysozyme by affinity chromatography	130
Norleucine	Purification of D-galactosyltransferase from skim milk	84
Ovomucoid	Isolation of trypsin by affinity chromatography	131
Pepsin	Purification of a pepsin inhibitor from *Ascaris lumbricoides* by affinity chromatography	50
Pepsin inhibitor (from *Ascaris lumbricoides*)	Purification of pepsin by affinity chromatography	50

[123] H. D. Knöfel, P. Müller, R. Kramell, and G. Sembdner, *F.E.B.S. Letters*, 1975, **60**, 39.
[124] T. Kumazaki, K. Kasai, and S. Ishii, *J. Biochem.* (*Japan*), 1976, **79**, 749.
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[130] T. Yoshimoto and D. Tsuru, *J. Biochem.* (*Japan*), 1974, **76**, 887.
[131] E. B. Gilliam and G. B. Kitto, *Comp. Biochem. Physiol.*, 1976, **54**, 21.

Table 3 (*cont.*)

Ligand or affinant coupled to agarose cyclic imidocarbonate [a]	*Use of product*	*Ref.*
L-Phenylalanine	Purification of phenoloxidase by affinity chromatography	81
Phenylamine	Affinity chromatography of lipase using hydrophobic ligands	79
4-Phenylbutylamine	Studies of the interaction of α-D-glucosidase with a hydrophobic matrix	132
Phenylethylamine, Phenylmethylamine, Phenylpropylamine	Affinity chromatography of lipase using hydrophobic ligands	79
Polyadenylate	Purification of mammalian proteins having affinity for polynucleotides by affinity chromatography	133
Poly(adenylic acid)	Purification of RNA-complexing proteins by affinity chromatography	134
Poly(uridylic acid)	Purification of reticulocyte ribosomes by affinity chromatography	135
Pteridine (reduced)	Purification of phenylalanine 4-monooxygenase by affinity chromatography	136 [e]
Pyran copolymer	Affinity chromatography and isolation of viral DNA polymerases	137
Red kidney-bean agglutinin, *Ricinis communis* agglutinin	Studies of the binding of glycoprotein A to immobilized lectins	115
	Purification and separation of polysaccharides containing terminal, non-reducing D-galactosyl residues by affinity chromatography	138
Salmine (tryptic digest)	Isolation of *Streptomyces griseus* trypsin from pronase	139
Soybean trypsin inhibitor	Analytical affinity chromatography used in studies of the kinetics of trypsinogen activation	140
Trypsin	Purification of isoinhibitors of trypsin from cows' colostrum by affinity chromatography	52
Trypsin inhibitor (from lima bean), (from soybean)	Isolation of trypsin by affinity chromatography	131
	Separation of trypsins and chymotrypsins by affinity chromatography	141
	Separation of α- and β-anhydrotrypsins by affinity chromatography	142
L-Tryptophan, L-Trypsin	Hydrophobic affinity chromatography of D-galactosyltransferase	84

[132] R. E. Huber and R. D. Mathison, *Canad. J. Biochem.*, 1976, **54**, 153.
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[140] V. Kasche, *Arch. Biochem. Biophys.*, 1976, **173**, 269.
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[142] B. Y. Yung and C. G. Trowbridge, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 927.

Ligand or affinant coupled to agarose cyclic imidocarbonate [a]	*Use of product*	*Ref.*
UDP-hexanolamine	Purification of D-galactosyltransferase from skim milk	84
L-Valine	Hydrophobic affinity chromatography of D-galactosyltransferase	84
Wheat-germ agglutinin	Isolation of glycophorin A by affinity chromatography	115
	Studies of the binding of glycoproteins in microsomal and Golgi membranes to lectins	116

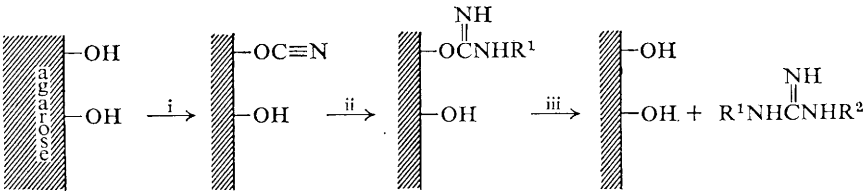
[a] In some cases the same product may be formed by a multistage reaction incorporating a bridge, as outlined in Table 4 and Scheme 9. [b] Using epichlorohydrin-cross-linked, desulphated agarose. [c] Using Ultrogel® (an agarose–polyacrylamide copolymer). [d] His → Pro → Phe → His → Leu → D-Leu → Val → Tyr. [e] An agarose cyclic imidocarbonate type of matrix is assumed.

The repeated use of derivatives of agarose cyclic imidocarbonate in affinity chromatography has been discussed from the viewpoints of the nature of the binding of the protein to the affinant and the methods for regenerating 'used' columns, *etc.*[83] Agarose cyclic imidocarbonate has been used in automated immunoadsorption and affinity chromatographies.[68]

The number of alkylamino side-chains introduced into agarose *via* the cyclic imidocarbonate derivative has been determined by spectroscopic and elemental analyses or by the use of labelled derivatives,[143] and the amount of an enzyme covalently attached to agarose, *via* the cyclic imidocarbonate derivative, has been measured by five independent methods, which were assessed for use in routine analyses.[42]

An affinant for neuraminidase, which was formed by reaction of α_1-acid glycoprotein with agarose cyclic imidocarbonate, did not display non-specific adsorption that is characteristic of the analogous oxamic acid derivatives.[78] Concanavalin A immobilized on agarose cyclic imidocarbonate effected different bindings, depending on whether or not ethylene glycol was present.[104]

Problems have arisen in using agarose cyclic imidocarbonate for immobilization purposes, since material with high insulin-specific activity was released from an insulin-agarose derivative (an *N*-substituted isourea) in the presence of bovine serum albumin, presumably by the type of reaction shown in Scheme 8.[126]



Reagents: i, CNBr; ii, R^1NH_2 (R^1 = the rest of the insulin molecule); iii, R^2NH_2 (R^2 = the rest of the bovine serum albumin molecule)

Scheme 8

[143] M. Sharma and W. R. Slaunwhite, *Analyt. Biochem.*, 1975, **68**, 79.

Table 4 *Use of agarose cyclic imidocarbonate for the preparation of affinity-chromatography materials* via *linkage extension*

Ligand or affinant coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
N-Acetyl-L-phenylalanine	(17)	Purification of phenoloxidase by affinity chromatography	81
Adenosine 5′-phosphate	(17)	Isolation of lactate dehydrogenase from muscle extracts by affinity chromatography	144 [b]
Adenosine 5′-phosphate (periodate-oxidized)	(17)	Purification of ribonuclease by affinity chromatography	145 [c]
Adenosine triphosphate (periodate-oxidized)	(19)	Investigation of the binding of ATP to the progesterone receptor; affinity chromatography of the progesterone–ATP complex	146
1-Alkylamines Amines (primary)	(16)	Affinity chromatography of lipase on hydrophobic matrices	79
4-Aminobenzoic acid	(20)	Purification of phenoloxidase by affinity chromatography	81
4-Aminochloroamphenicol	(16)	Separation of chloroamphenicol transferases by affinity chromatography	145
2-Amino-2-deoxy-D-glucose	(16)	Isolation of wheat-germ agglutinin from wheat-germ lipase	116
3-Aminophenobarbital	(16)	Purification of the P-450 haemeproteins of *Rhizobium japonicum* by affinity chromatography	65
4-Aminophenyl 2-acetamido-2-deoxy-1-thio-β-D-glucopyranoside	(20)	Purification of β-D-2-acetamido-2-deoxyhexosidase from other glycoside hydrolases, *etc.*, by affinity chromatography	148
4-Aminophenyl 1-thio-β-D-galactopyranoside	(16)	Purification of β-D-galactosidase by affinity chromatography	149 [d]
Butylamines	(16)	Affinity chromatography of lipase on hydrophobic matrices	79
Chloroamphenicol	(16)	Separation of chloroamphenicol transferases by affinity chromatography	147
4-Chlorobenzylamine	(16)	Affinity chromatography of thrombins	150
4-Chloromercuribenzoate	(17A)	Purification and separation of β-D-galactosidases	151

Cytidine diphosphate *sn*-1,2-diacylglycerol (periodate-oxidized)	(19)	Separation of membrane-bound enzymes involved in phosphatidylglycerol synthesis by affinity chromatography	152
Deoxycholic acid	(17)	Affinity chromatography of lipase on hydrophobic matrices	79
Deoxycorticosterone hemisuccinate	(17)	Purification of progesterone-binding globulin by affinity chromatography	153
4-(4-Diazophenylazo)phenylarsonic acid	(21), (22)	Investigations of the effects of different spacer arms in the affinity chromatography of alkaline phosphatase; isolation of alkaline phosphatase	94
N-(4-Diazophenyl)oxamic acid	(23)	Purification of bacterial glycoside hydrolases by affinity chromatography	95
	(24) [c]	Purification of neuraminidase by affinity chromatography; separation of two forms of neuraminidase	154
Di-*O*-oleoylglycerol	(16)	Isolation of triacylglycerol lipase by affinity chromatography	155
2-Ethylhexylamine	(16)	Affinity chromatography of lipase on hydrophobic matrices	79
Folic acid	(17)	Purification of low-mol.-wt. human folate-binding protein by affinity chromatography	101
[U-^{3}H]Gibberellic acid GA_3 4-amino-butyramide	(25)	Comparison of the methods used to prepare affinity adsorbents for gibberillin	123
[U-^{3}H]Gibberellic acid GA_3 anhydride	(17)		

[144] M. J. Harvey and P. D. G. Dean, *Science Tools*, 1976, **23**, 36.
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[147] Y. Zaidenzaig and W. V. Shaw, *F.E.B.S. Letters*, 1976, **62**, 266.
[148] E. Bamberg, F. Dorner, and W. Stöckl, *Experientia*, 1975, **31**, 516.
[149] P. Dunnill and M. D. Lilly, *Biotechnol. and Bioeng.*, 1974, **16**, 987.
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[151] M. Sato and I. Yamashina, *J. Biochem. (Japan)*, 1974, **76**, 1155.
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[155] A. Verine, H. Giudicelli, and J. Boyer, *Biochim. Biophys. Acta*, 1974, **369**, 125.

Table 4 (*cont.*)

Ligand of affinant coupled to agarose cyclic imidocarbonate	*Agarose intermediate* [a]	*Use of product*	*Ref.*
UDP-D-Glucuronic acid	(17)	Affinity chromatography of collagen D-glucosyl-transferase	156
D-Mannopyranosylamine	(16)	Purification of α-D-mannosidase by affinity chromatography	87
Nicotinamide adenine dinucleotide	(16)	Isolation of diphtheria toxin by affinity chromatography	157
Nicotinamide adenine dinucleotide (reduced)	*e*	Purification of nitrate reductase by affinity chromatography	158
Nicotinamide adenine dinucleotide phosphate	(17)	Purification of D-glucose-6-phosphate dehydrogenase by affinity chromatography	86
	(19)	Purification of D-glucose-6-phosphate dehydrogenase by affinity chromatography; studies of the interactions of nicotinamide adenine dinucleotide phosphate and the reduced form thereof with the enzyme	159
19-Nortestosterone 17-hemisuccinate	(17A)	Purification of progesterone-binding globulin by affinity chromatography	153
t-Octylamine	(16)	Affinity chromatography of lipase on hydrophobic matrices	79
Oestradiol derivative (E_2-7α-$(CH_2)_{10}CO_2^-$)	(17A)	Investigations of the parameters affecting the purification of calf-uterus oestrogen 'receptor' by affinity chromatography	160

Pepstatin (treated with *N*-hydroxysuccinimide)	(17)	Isolation of catlepsins D by affinity chromatography	161
2-L-Phenylalanyl-DL-leucine	(26)	Affinity chromatography of proteases from *Bacillus subtilis* and thermolysin	162
6-Phospho-*N*-[(1*H*)-1,2,4-triazol-3-yl]-D-glucopyranosylamine	(27)	Purification of *myo*-inositol-1-phosphate synthase by affinity chromatography	163
Potassium oxalate	(17)	Isolation of phenylmaleimide-modified lactate dehydrogenases	164
t-Ribonucleic acid (periodate-oxidized)	(19)	Isolation of aminoacyl-tRNA synthases by affinity chromatography	165
Rose Bengal	(17)	Investigations of the photo-oxidation of proteins	166[f]
Starch cyclic imidocarbonate	(17)	Purification of phosphorylases by affinity chromatography	167

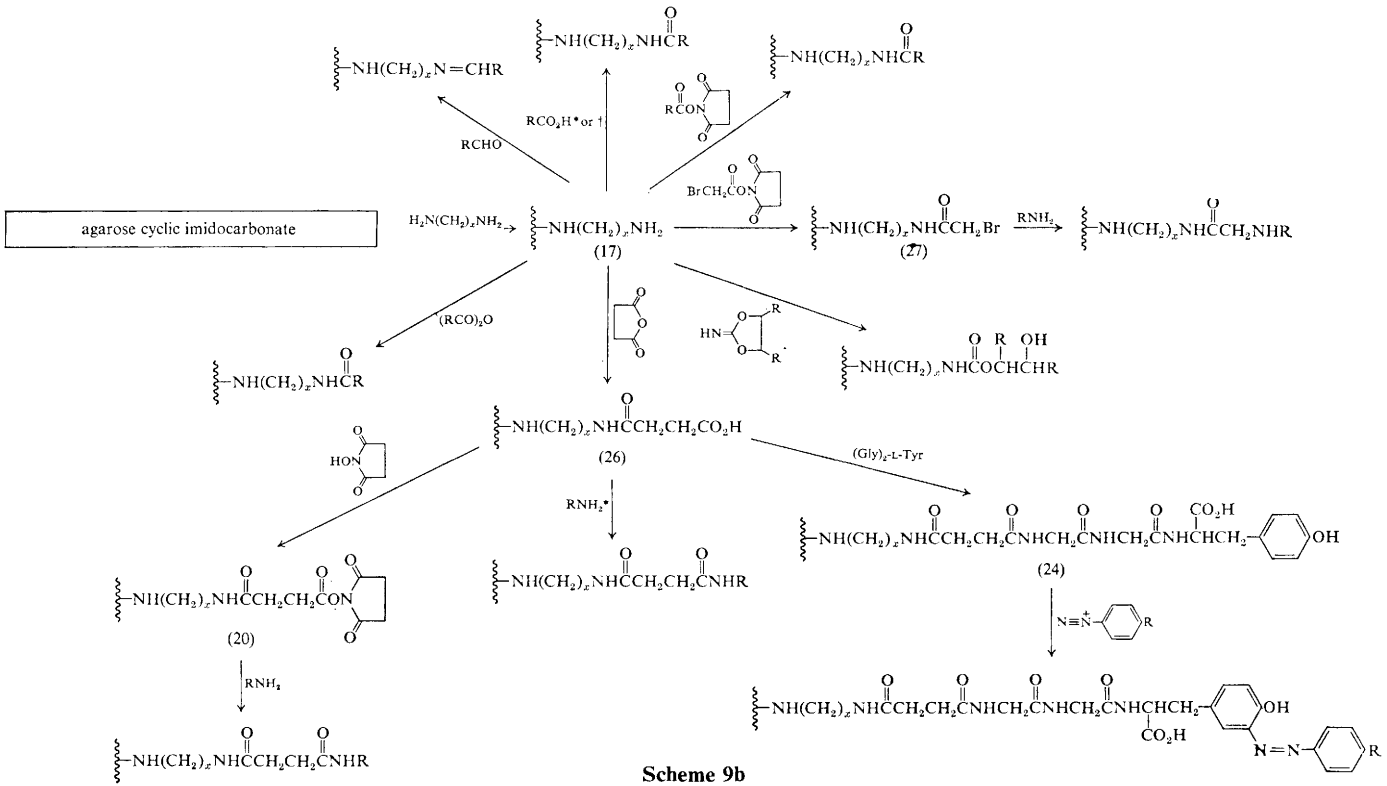
[a] Multistage reactions can sometimes be avoided by prior attachment of a bridge to the affinant molecule. [b] Using polyacrylamide-agarose as the initial matrix former. [c] The coupled product was reduced with sodium borohydride. [d] Derivative not certain. [e] Not stated. [f] Presumably a non-covalent derivative.

156 H. Anttinen and K. I. Kivirikko, *Biochim. Biophys. Acta*, 1976, **429**, 750.
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158 Y. M. Heimer, S. Krashin, and E. Riklis, *F.E.B.S. Letters*, 1976, **62**, 30.
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161 O. V. Kazakova and V. N. Orekhovich, *Biochemistry* (*U.S.S.R.*), 1975, **40**, 826.
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163 E. A. Funkhouser and F. A. Loewus, *Plant Physiol.*, 1975, **56**, 786.
164 W. E. Trommer and G. Becker, *Biochim. Biophys. Acta*, 1976, **422**, 1.
165 C. M. Joyce and J. R. Knowles, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 1278.
166 N. M. C. Kaye and P. D. J. Weitzman, *F.E.B.S. Letters*, 1976, **62**, 334.
167 N. K. Matheson and R. H. Richardson, *Phytochemistry*, 1976, **15**, 887.

agarose cyclic imidocarbonate
see Scheme 9b
(Gly)$_x$Tyr
(21)
(22) x = 2
(23) x = 1
(16)
(17)
(18)
(19)
(25)
RCHO
ROH*
RNH$_2$
RNH$_2$*
RCO$_2$H*
H$_2$N(CH$_2$)$_2$NH$_2$
NH$_2$(CH$_2$)$_x$CO$_2$H
H$_2$N-protein

Scheme 9a

R = rest of affinant, ligand, *etc.*; * = reaction involving carbodi-imide. In subsequent references to this Scheme, the suffix A denotes the use of $H_2N(CH_2)_3NH(CH_2)_3NH_2$ in place of $H_2N(CH_2)_xNH_2$ for the production of (17), *etc.*



Scheme 9b

R = rest of affinant, ligand, *etc.*; * = reaction involving carbodi-imide; † = reaction involving isobutylchloroformate. In subsequent references to this Scheme, the suffix A denotes the use of $H_2N(CH_2)_3NH(CH_2)_3NH_2$ in place of $H_2N(CH_2)_xNH_2$ for the production of (17), *etc.*

Reaction of cyanogen bromide-treated agarose with ammonium hydroxide (to form the ammonia derivative) and then with *N*-α-acetylornithine or poly-(L-ornithine) yielded arginine after hydrolysis with acid, indicating that the ammonia derivative is an *O*-substituted isourea. Reaction of cyanogen bromide-treated agarose in turn with *N*-α-acetylornithine and ammonium hydroxide released *N*-α-acetyl-arginine and -citrulline, indicating that they are derived from base-catalysed hydrolysis of a substituted isourea.

Direct attachment of an affinity ligand or enzyme to agarose cyclic imidocarbonate has often proved to be unrewarding, since the approach to the ligand is sterically impeded. This difficulty can be overcome by inserting a covalent bridge between the matrix and the ligand, and bridging is also used when functional groups on the intended ligand do not react with those on the matrix, since a new type of functionality can be introduced. Modifications used recently to provide bridges in the preparation of affinity-chromatography matrices, *etc.*, from agarose cyclic imidocarbonate are shown in Schemes 9a and 9b and Table 4. When bridging has been used to immobilize antibodies and antigens, details are given in Table 2. Other derivatives of agarose cyclic imidocarbonate used for miscellaneous purposes are listed in Table 5.

Table 5 *Use of agarose cyclic imidocarbonate for the preparation of miscellaneous derivatives*

Species coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
N-α-Acetylornithine	Investigations of the structures formed on reaction of cyclic imidocarbonates with amines	126
4-Aminobutyraldehyde diethyl acetal	Intermediate for introducing an aldehydic function into agarose (see p. 432)	38
6-Aminohexanoic acid	Control in the separation of chloro-amphenicol acetyltransferases by affinity chromatography	147
	Controls in the interactions of haeme-proteins with affinity-chromatography matrices	65
Ammonia	Investigations of the structures formed on reaction of cyclic imidocarbonates with amines	126
Diamines	Determination of alkylamino side-chains coupled to agarose beads	123
1,6-Diaminohexane	Controls in the interactions of haeme-proteins with affinity-chromatography matrices	65
Flavodoxin	Studies of the properties of immobilized flavodoxin and of the replacement of ferredoxin as an electron-carrier	168
Glutathione (reduced form)	Investigation of the hydrolysis of 4-nitrophenyl acetate by immobilized glutathione	169

[168] S. G. Mayhew and M. J. J. Strating, *European J. Biochem.*, 1975, **59**, 539.
[169] T. Murachi and K. Okumura, *J. Polymer Sci., Polymer Letters*, 1976, **14**, 361.

Table 5 (*cont.*)

Species coupled to agarose cyclic imidocarbonate	*Use of product*	*Ref.*
n-Hexylamine	Control in the affinity chromatography of alcohol dehydrogenase and in demonstrating the absence of interactions with an n-hexyl spacer arm	89
Myosin	Preparation of an immobilized form of myosin and characterization of the adenosine triphosphatase activity generated	170
Ovalbumin	Control in the development of micro-fluorometric immunoassays with solid-phase antigens	71
Peptides (from the cell walls *Bacillus megaterium*)	Stimulation of immunity to hormones in mice as a property of bacterial cell walls	74
Poly(L-ornithine)	Investigations of the products formed on reaction of cyclic imidocarbonates with amines	126
Reduced 5-carboxymethylated trypsinogen	Study of how the refolding of trypsinogen depends on the endogenous disulphide bridges	171
Rose Bengal [a]	Control of the immobilization of Rose Bengal	166 [b]
2,4,6-Trinitrobenzene-sulphonic acid [c]	Determination of alkylamino side-chains coupled to agarose beads	143
Trypsin inhibitor (from soybean)	Investigations of the effect of the heterogeneity of immobilized soybean trypsin inhibitor on the separation of pancreatic proteases	141

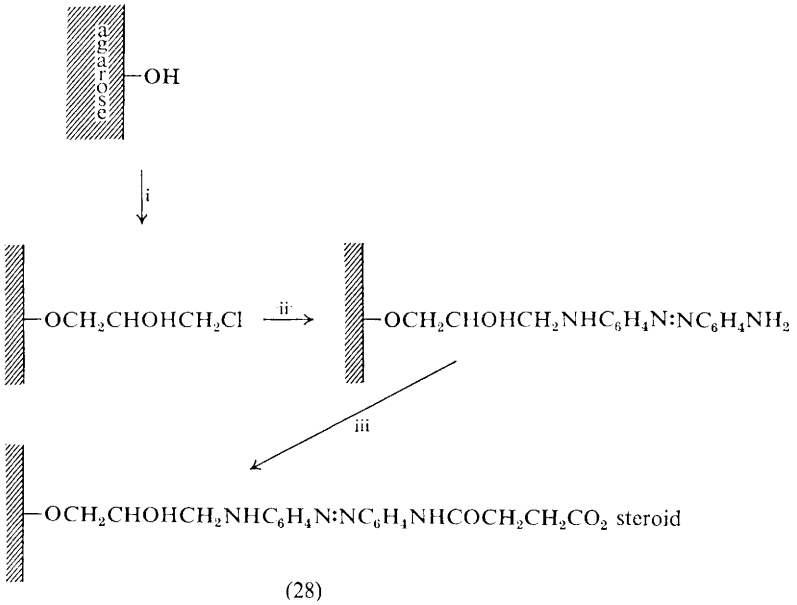
[a] See (16), Scheme 9. [b] No reaction detected. [c] Agarose derivative not certain.

Side-chains containing amino-groups linked to agarose cyclic imidocarbonate are too labile to be used in the isolation of steroid-orientated globulins from plasma.[172] An alternative method for coupling steroids to agarose is shown in Scheme 10. The derivative (28) was used to isolate a steroid-binding globulin from human plasma.

Divinylsulphonylagarose has been activated and then coupled to human chorionic gonadotrophin, ovine luteinizing hormone, and rat prolactin to give immunoadsorbents that are suitable for isolating the corresponding antibodies.[173]

Agarose has been cross-linked and activated by reaction with epichlorohydrin.[174] The epoxide groups of this agarose derivative reacted with 1,6-diaminohexane to give a derivative to which *myo*-inositol 2-phosphate could be coupled. The resulting phosphated derivative of agarose was used in the affinity chromatography of enzymes that act on phosphorylated derivatives of *myo*-inositol. Epichlorohydrin-treated agarose also reacted with choline to give an affinity matrix suitable for the joint purification of choline and ethanolamine kinases.[80]

[170] E. Elgart, T. Gusovsky, and M. D. Rosenberg, *Biochim. Biophys. Acta*, 1975, **410**, 178.
[171] A. Light and N. K. Sinha, *Biochem. Biophys. Res. Comm.*, 1976, **68**, 1180.
[172] W. Rosner and R. N. Smith, *Biochemistry*, 1975, **14**, 4813.
[173] M. R. Sairam and J. Porath, *Biochem. Biophys. Res. Comm.*, 1976, **69**, 190.
[174] O. Scheiner and M. Breitenbach, *Monatsh.*, 1976, **107**, 581.



(28)

Reagents: i, epichlorohydrin; ii, 4,4′-azodianiline; iii, androstanediol succinate

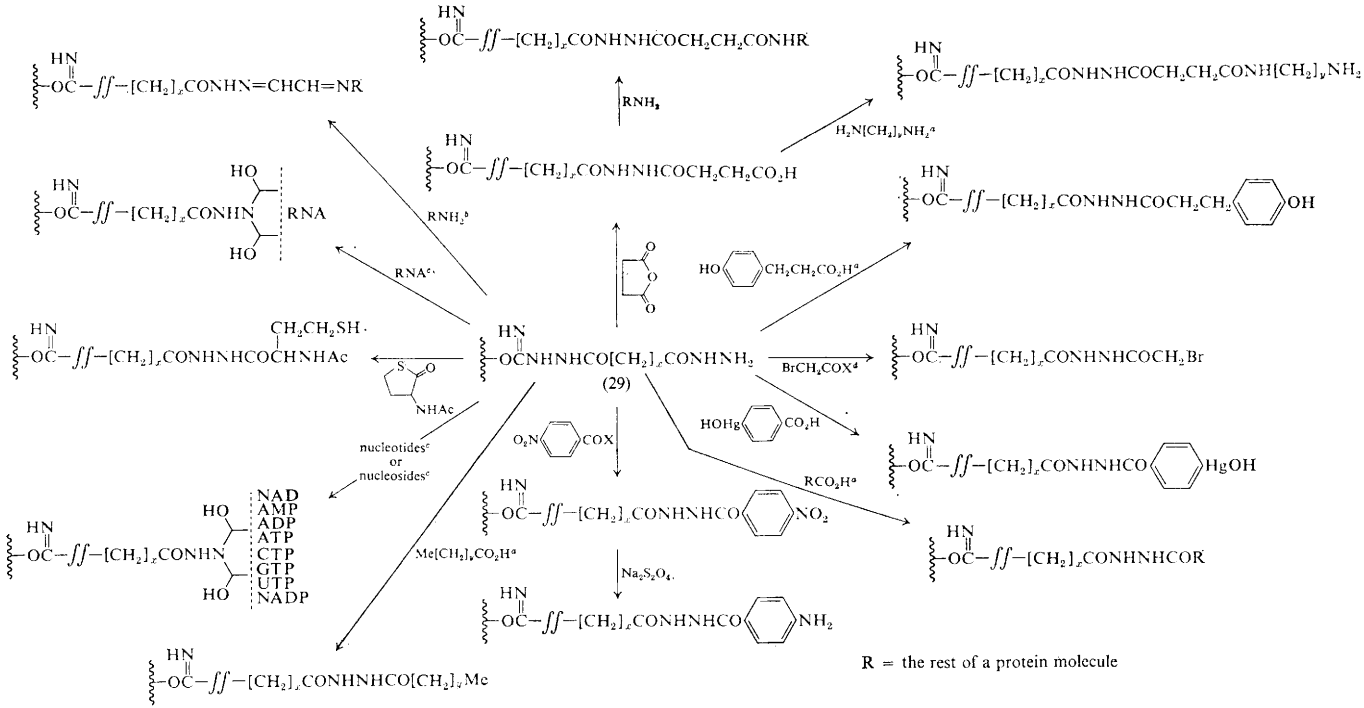
Scheme 10

Agarose has been used as a matrix for the immobilization of glucoamylase.[175] Hydrazidoalkylagarose (29) has proved useful in the purification of biologically active molecules by affinity chromatography.[176] Various functional groups can be attached to the derivative (29) by means of extended, hydrocarbon spacer arms (Scheme 11). The leakage of functional groups attached to univalent spacer arms was overcome by coupling either poly(glutamic acid hydrazide) or linear poly(acrylic hydrazide) to agarose. Multipoint attachment of these polymers to agarose [*e.g.* (30)] confers greater stability.

A hydroxypropyl-phloroglucinol derivative has been prepared by cross-linking phloroglucinol to agarose with epichlorohydrin.[64] Proteins could be coupled to this derivative after it had been treated with cyanogen bromide. An immunoadsorbent based on anti-(human serum albumin) antibody so produced (see also Table 2) was used to investigate the migration of human serum albumin through the adsorbent in an electric field and the absorption and elution of this antigen. The effects of different spacer arms and of steric hindrance in affinity electrophoresis and in affinity chromatography were discussed.

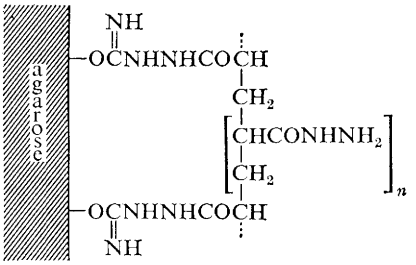
An agarose derivative containing 2-hydroxy-3-thiolpropyl ether groups has been treated with 2,2′-bipyridyl disulphide to give a product (31) that was used in the isolation of urease by a chromatographic procedure involving disulphide-linkage interchange.[177]

[175] J. Kučera and J. Hanus, *Coll. Czech. Chem. Comm.*, 1975, **40**, 2536.
[176] M. Wilchek and T. Miron, *Mol. Cell. Biochem.*, 1974, **4**, 181.
[177] P. Eigtved, D. Steen Jensen, A. Kjaer, and E. Wieczorkowska, *Acta Chem. Scand.* (*B*), 1976, **30**, 180.

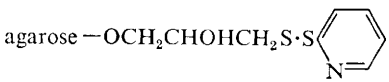


[a] Carbodi-imide-assisted reaction; [b] glutaraldehyde-assisted reaction; [c] oxidized with periodatebefore coupling; [d] or using *N*-bromoacetylsuccinimide

Scheme 11



(30)



(31)

Cross-linking of desulphated agarose with epichlorohydrin gave a derivative that exhibited improved properties for the preparation of affinity-chromatography matrices.[77]

The carbohydrate moiety of an agarose–polyacrylamide copolymer (Ultrogel®) could be activated by treatment with either periodate or cyanogen bromide, whereas the polyacrylamide moiety could be activated by treatment with either acylazide or glutaraldehyde.[90] N^6-(6-Aminohexyl)-AMP was coupled to the products to yield affinity matrices that bound lactate dehydrogenase. Polyacrylamide–agarose has been recommended for the preparation of affinity-chromatography matrices *via* the cyclic imidocarbonate derivative of the polysaccharide moiety.[144] The advantages of this composite matrix include a variable pore-size and the presence of two distinct functional groups that can be independently activated. Spacer arms can be incorporated in the usual way.

Poly(adenylic acid), poly(guanylic acid), and poly(inosinic acid) reversibly associate with agarose gels at high salt concentrations at pH 6—10.[178] The association of poly(guanylic acid) and poly(inosinic acid) with agarose gels decreased progressively with deprotonation of the purine residue, and both polymers interacted only weakly with the gels at pH >10, regardless of the salt concentration; the interaction was also temperature dependent. The association of poly(adenylic acid) with agarose gels was less sensitive to deprotonation and temperature. The immobilization of several polynucleotides was prevented by urea at salt concentrations where saturation of agarose normally occurred.

Rose Bengal did not bind to macroporous agarose or the agarose derivative (16) either on mixing or in the presence of a carbodi-imide.[166] The strong binding that occurs between Rose Bengal and the agarose derivative (17) is therefore ascribed to an ionic reaction between anionic groups on the dye and amino-groups on the matrix.

Alginic Acids.—Alginic acid cyclic imidocarbonate reacted with lysozyme to give an active immobilized form of the enzyme that is soluble at pH >4 and insoluble at pH <3.[179]

Treatment of alginic acid methyl ester with *NN*-dimethylbiguanidine afforded a *s*-triazinyl derivative having C-6 of the hexuronic acid residues incorporated into

[178] S. L. Petrović, M. B. Novaković, and J. S. Petrović, *Biopolymers*, 1975, **14**, 1905.
[179] M. Charles, R. W. Coughlin, and F. X. Hasselberger, *Biotechnol. and Bioeng.*, 1974, **16**, 1553.

the heterocyclic ring.[34] Triazinylated monosaccharides were released on hydrolysis of the derivatized polysaccharide with acid.

Amyloses.—Reduced malto-oligosaccharides with a ^{14}C-label at their reducing end have been used in mapping the specificity site of an α-amylase system.[180]

Acetoglycosyl bromide derivatives of higher malto-oligosaccharides have been prepared from cyclohepta-amylose, maltohexaose, and maltoheptaose.[181] The products were treated either with 2,3-di-*O*-phenylcarbamoyl-6-*O*-tritylamylose in the presence of silver perchlorate or with 2,3-di-*O*-phenylcarbamoylamylose in the presence of mercuric cyanide and bromide. After removal of the protecting groups, the branched molecules were characterized by their iodine-binding properties, susceptibilities to β-amylase, and priming activities in phosphorolytic synthesis. The distance between the branch points of the backbone chain was 25—55 and 100—150 residues, respectively, in the products from these reactions.

The type of spherulite and the extent of aggregation of fibrils in the crystal growth of amylose acetate and propionate depended on the solvent and the concentration of the polymer used for crystallization.[182]

Glass-transition temperatures for amylose acetate and propionate have been measured as a function of the chain-length using differential scanning calorimetry and torsional braid techniques.[183] Critical chain-lengths could be predicted if the D-glucopyranosyl ring is regarded as a rigid unit in the amylose chain. The dependence of the glass-transition temperature on the reciprocal of the molecular weight was not predicted adequately by Gibbs–Di Marzio theory over the complete range of molecular weights, and branching of the polysaccharide chain was shown to lower the glass-transition temperature slightly.

Fibrillar crystals of amylose acetate have been grown under various conditions using a film-formation method.[184] The type of fibrillar aggregation depended on the solvent, the concentration of polymer, and the temperature of crystallization. It was proposed that the fibrils are fundamental units of most polymer crystallization processes and that the growth of fibrous crystals occurs as often as lamellar growth of polymer crystals. A common feature of the fibres is the twisting and branching habit, which can be compared with the morphology of fibres of natural polymers. The adsorption of amylose triacetate onto porous adsorbents has been investigated.[185]

The activity of β-amylase towards dialdehydo(periodate-oxidized)-amylose distinguishes it from other D-glucanases.[186] The benzylation of amylose [31] and molecular parameters for dilute solutions of carboxymethylamylose (sodium salt) [187] have been reported.

Ion-exclusion chromatography, gel-permeation chromatography, and enzymolysis have been used to assess the purity and amylopectin contents of amyloses used in assays of α-amylase.[188]

[180] J. A. Thoma and J. D. Allen, *Carbohydrate Res.*, 1976, **48**, 105.
[181] B. Pfannemüller, G. Richter, and E. Husemann, *Carbohydrate Res.*, 1976, **47**, 63.
[182] R. M. Gohil and R. D. Patel, *European Polymer J.*, 1976, **12**, 477.
[183] J. M. G. Cowie and S. A. E. Henshall, *European Polymer J.*, 1976, **12**, 215.
[184] R. M. Gohil and R. D. Patel, *Stärke*, 1976, **28**, 45.
[185] P. R. M. Nair, K. C. Patel, and R. D. Patel, *Stärke*, 1976, **28**, 267.
[186] J. J. Marshall, *F.E.B.S. Letters*, 1974, **46**, 1.
[187] P. K. Manna and P. K. Choudhury, *J. Macromol. Sci. Chem.*, 1974, **A8**, 909.
[188] J. F. Kennedy, *Stärke*, 1976, **28**, 196.

The 4-nitro- and 2,4-dinitro-benzoyl derivatives of amylose have been prepared [189] and the solution properties of amylose sulphate have been investigated.[190]

D-Glucopyranosyl side-chains have been introduced into amylose by condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with either 2,3-di-*O*-phenylcarbamoyl-6-*O*-tritylamylose or the detritylated derivative, although degradation of the main chain occurred in the latter instance.[191] The distance between the branch points on the backbone chain was 2—4 residues. α-(1 → 6)-Linkages were formed preferentially in the former reaction and β-(1 → 6)-linkages in the latter reaction.

Graft copolymers of polystyrene and amylose containing different proportions of polystyrene have been prepared in a reaction initiated by Fe^{2+}–hydrogen peroxide.[192] The thermal stability of amylose is enhanced by the graft. Thermal degradation of the copolymers is independent of the degree of grafting and occurs in two stages.

The crystal and molecular structures of the complex of amylose with DMSO have been elucidated by stereochemical analysis, potential-energy calculations, and *X*-ray diffraction.[193] The complex crystallizes in a pseudotetragonal unit cell, with two antiparallel chains in each unit cell. The amylose chain is present as a left-handed helix, with three turns per crystallographic repeat, and one DMSO molecule is located inside the helix for every three D-glucosyl residues, and four DMSO and eight water molecules are located in large interstices between the chains; the interaction of these molecules with the helix results in pseudo-tetragonal chain-packing.

Celluloses.—Papers presented at a symposium on 'Cellulose as a Chemical and Energy Source' have emphasized the industrial applications of cellulose and also include information on various modifications and derivatives of cellulose.[194] Aspects of cellulose chemistry have been reviewed.[195]

Ethers and water-soluble derivatives of cellulose have been assayed spectrophotometrically following reaction of D-glucose or substituted D-glucose derivatives released on hydrolysis with 4-hydroxybenzoylhydrazine.[196] Hydrolysis of the polysaccharides was achieved more readily with trifluoroacetic acid than with sulphuric acid.

Diffusion coefficients for cellodextrin alditols (d.p. 1—5) were found to be 22% less in 0.32M sodium hydroxide than in water.[197] The alditols had slightly lower rates of diffusion and higher coefficients of friction and Einstein–Stokes hydrodynamic radii in water than the corresponding cellodextrins.

The free radicals produced by various types of cellulose on u.v.-irradiation at 77 K have been examined by e.s.r. spectroscopy.[198] The effects of crystallinity

[189] C. Simionescu and S. Dumitriu, *Cellulose Chem. Technol.*, 1972, **6**, 663.
[190] K. S. Patel, C. K. Patel, K. C. Patel, and R. D. Patel, *Stärke*, 1975, **27**, 265.
[191] B. Pfannemüller, G. C. Richter, and E. Husemann, *Carbohydrate Res.*, 1975, **43**, 151.
[192] R. M. Gohil, C. K. Patel, K. C. Patel, and R. D. Patel, *Stärke*, 1976, **28**, 95.
[193] W. T. Winter and A. Sarko, *Biopolymers*, 1974, **13**, 1461.
[194] 'Cellulose as a Chemical and Energy Source' (Biotechnol. and Bioeng. Symposium, No. 5, 1975), ed. C. R. Wilke, John Wiley and Sons Ltd., London, 1976.
[195] F. Shafizadeh, *Pure Appl. Chem.*, 1973, **35**, 195.
[196] D. W. Leedy, *Carbohydrate Res.*, 1976, **47**, 337.
[197] J. E. Tostevin and H. A. Swenson, *J. Polymer Sci., Polymer Phys.*, 1976, **14**, 2047.
[198] N.-S. Hon, *J. Polymer Sci., Polymer Chem.*, 1976, **14**, 2513.

and the amount of sensitizer (Fe^{3+}) adsorbed indicated that photochemical reactions in cellulose occur only in amorphous regions. E.s.r.-spectroscopic measurements showed that the largest concentration of radicals is formed when cellulose is irradiated in the absence of oxygen and that cellulose can be protected from photodegradation by lignin.[199] The reactions of hydrogen atoms and other radicals formed on photo-irradiation of cellulose were investigated,[200] and mechanisms were suggested to account for the formation of radicals and products.[201]

The effects of photosensitizers on the formation of free radicals on u.v.-irradiation of cellulose at 77 K have been investigated.[202] Samples of cellulose treated with benzoyl peroxide, hydrogen peroxide, benzophenone, riboflavin, Cr^{2+} ions, or Ni^{2+} ions exhibited single-line e.s.r. spectra, whereas those treated with Pb^{2+} or Fe^{3+} ions exhibited three- and five-line e.s.r. spectra, respectively. Studies of the decay of radicals in photo-irradiated cellulose at low temperatures indicated that at least six types of radical are formed.[203] Changes in the radicals with the reaction conditions were carefully examined.

A comment on a model for the degradation of cotton cellulose corrects errors in a previous paper (R. J. Elema, *J. Polymer Sci., Part C, Polymer Symposia*, 1973, **42**, 1545).[204] The availability of hydroxy-groups in completely mercerized cotton cellulose and in hydrocelluloses has been assessed by reaction with *NN*-diethylaziridinium chloride.[205]

Mercerization of fibrous *Valonia* cellulose has been achieved with aqueous ethanolic sodium hydroxide and the course of mercerization was monitored by electron microscopy.[206] The production of single-cell proteins from cellulosic wastes has been described.[207]

Ion-exchange derivatives of cellulose are more efficient than carbon black in removing the colour from textile effluents and are well suited for the purification of pretreated or rinsing waters.[208] They are also able to remove surfactants from effluents, breaking up emulsions of water with solvents.

Acetyl, benzoyl, lauryl, 4-phenylbutyryl, phenoxyacetyl, and other esters of cellulose have been prepared.[209] Investigation of these esters as hydrophobic materials for immobilizing enzymes showed that the phenoxyacetyl derivative is the most versatile. A procedure for acetylating bagasse cellulose has been reported.[210]

Changes in the structure of cellulose on extensive acetylation and butyrylation have been discussed.[211] Crystallization of these derivatives occurred on

[199] N.-S. Hon, *J. Polymer Sci., Polymer Chem.*, 1975, **13**, 2641.
[200] N.-S. Hon, *J. Polymer Sci., Polymer Letters*, 1976, **14**, 225.
[201] N.-S. Hon, *J. Polymer Sci., Polymer Chem.*, 1976, **14**, 2497.
[202] N.-S. Hon, *J. Polymer Sci., Polymer Chem.*, 1975, **13**, 1933.
[203] N.-S. Hon, *J. Polymer Sci., Polymer Chem.*, 1975, **13**, 2653.
[204] R. J. Elema, *J. Polymer Sci., Polymer Letters*, 1975, **13**, 533.
[205] S. P. Rowland and E. J. Roberts, *J. Polymer Sci., Polymer Chem.*, 1974, **12**, 2099.
[206] H. D. Chanzy and E. J. Roche, *J. Polymer Sci., Polymer Phys.*, 1975, **13**, 1859.
[207] W. D. Bellamy, *Biotechnol. and Bioeng.*, 1974, **16**, 869.
[208] A. Gangneux, D. Wattiez, and E. Marechal, *European Polymer J.*, 1976, **12**, 551.
[209] L. G. Butler, *Arch. Biochem. Biophys.*, 1975, **171**, 645.
[210] Y. A. Kostrov, *Cellulose Chem. Technol.*, 1974, **8**, 125.
[211] N. I. Klenkova, O. M. Kulakova, N. D. Tsimara, L. A. Volkova, E. P. Shirokov, and Y. L. Pogosov, *Cellulose Chem. Technol.*, 1974, **8**, 449.

heating. The fibrillar crystal-growth habit of cellulose acetate has been compared with that of amylose acetate.[184] The adsorption of water or piezoelectricity deformed the crystal lattice of cellulose triacetate.[212]

Data on the electro-osmosis of water through a membrane of cellulose acetate have been analysed in terms of non-equilibrium thermodynamics.[213] The linear phenomenological equations were found to be valid for the system. The efficiencies of energy conversion for both electro-osmosis (electrical energy into mechanical work) and streaming potential (mechanical work into electrical energy) were calculated.

The kinetics of acetolysis of cellulose acetate in mixtures of chloroform and acetic anhydride containing either perchloric acid or sulphuric acid have been examined.[214] The rate of change of the molecular weight of cellulose acetate was followed by viscometry, and the dependences of the first-order rate constant on the concentrations of catalyst, acetic anhydride, and cellulose acetate and on the molecular weight and temperature were determined. A mechanism was proposed for the acetolysis.

A modified form of cellulose acetate containing some periodate-oxidized D-glucopyranosyl residues has been prepared.[215] Fibres of cellulose acetate have been used to immobilize enzymes.[216] Selectively oxidized cellulose (dialdehydocellulose) has been treated with hydroxylamine, bisulphite, and acetic acid.[215] Dialdehydocellulose provides a matrix suitable for coupling with trypsin, giving an active immobilized enzyme.[217] Reduction of the unchanged aldehydo-groups on the matrix stabilized the enzyme.

Diaminocellulose, a cationic polymer, is best prepared by conversion of dialdehydocellulose into the dioxime and then reduction with sodium borohydride.[218] Treatment of diaminocellulose with methyl iodide gave a derivative containing secondary amino-groups.

Aminoethylcellulose has been used for the immobilization of enzymes, which retain their activity. Coupling with carboxypeptidase C [219] and chymotrypsin [39] was mediated by glutaraldehyde and that of other enzymes by diethyl adipimidate.[220]

Cellulose either substituted with quaternary ammonium groups or grafted with polyacrylic acid has been used for ion-exchange chromatography, particularly in the purification of textile effluents.[221] Graft copolymers of acrylic acid and a quaternary ammonium derivative of cellulose (obtained by condensation of cellulose with epoxypropyltriethylammonium chloride) were prepared; quaternary ammonium derivatives of cellulose can also be obtained in one step by heating cellulose with epichlorohydrin and an amine. The

[212] S. Sasaki and E. Fukada, *J. Polymer Sci., Polymer Phys.*, 1976, **14**, 565.
[213] R. C. Srivastava and A. K. Jain, *J. Polymer Sci., Polymer Phys.*, 1975, **13**, 1603.
[214] M. A. Bhatti and P. Howard, *Makromol. Chem.*, 1976, **177**, 101.
[215] S. Nadzhimutdinov, A. Sarymsakov, and K. U. Usmanov, *Cellulose Chem. Technol.*, 1975, **9**, 617.
[216] W. Marconi, F. Morisi, and R. Mosti, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1323.
[217] E. van Leemputten and M. Horisberger, *Biotechnol. and Bioeng.*, 1974, **16**, 997.
[218] T. Nishiuchi and S. Segawa, *Nippon Kagaku Kaishi*, 1974, **8**, 2435.
[219] H. Ueki, S. Nagai, S. Shoji, T. Funakoshi, and Y. Kubota, *J. Biochem. (Japan)*, 1974, **76**, 191.
[220] J. Campbell and W. E. Hornby, *Biochim. Biophys. Acta*, 1975, **403**, 79.
[221] A. Gangneux, D. Wattiez, and E. Marechal, *European Polymer J.*, 1976, **12**, 535.

structural and ion-exchange properties of the cellulose derivatives were studied and it was shown that the exchange equilibrium is reached after 2 h.[222] The grafted cellulose derivative is a mid-strength cation-exchanger (pK_a = 5.7) and the quaternary ammonium derivative a strong anion-exchanger (pK_a = 12.9).

Cotton linters have been subjected to acid hydrolysis for various times and the products converted into samples of cellulose tricarbanilate (weight-average mol. wt. 0.21—1.1 × 10^6).[223] Measurements of the intrinsic viscosities of dilute solutions of the derivatives in *p*-dioxan and butanone showed that the environment is well displaced from the normal θ-state and is closer to that at the lower critical solution temperature (234 °C in *p*-dioxan).

Glass-transition temperatures have been measured for cellulose tricarbanilate as a function of the chain-length; the critical chain-length can be predicted if the D-glucopyranosyl residues are regarded as flexible.[183] The data for cellulose tricarbanilate were compared with those for derivatives of amylose.

Cellulose *trans*-2,3-carbonate reacted with rabbit ala3/b4 immunoglobulin IgG to give an immunoadsorbent that was used in isolating sheep anti-b4 antibodies.[224] Coupling of the antibodies with cellulose *trans*-2,3-carbonate gave an immunoadsorbent that is suitable for the separation of rabbit allotypic b4 immunoglobulin [b4 (κ) IgG] from b4-negative immunoglobulin [b4-negative (κ) IgG].

The d.s. of macroporous cellulose *trans*-2,3-carbonate obtained when cellulose reacts with ethyl chloroformate can be controlled by the addition of small quantities of water.[225] This procedure produced a matrix that is suitable for the covalent binding of chymotrypsin; the insolubilized enzyme is appreciably active towards high-molecular-weight substrates. The amount of chymotrypsin covalently bound to macroporous cellulose *trans*-2,3-carbonate and the caseinolytic activity of the immobilized enzyme are substantially improved by swelling the matrix in DMSO before the enzyme is coupled.[226] The porosity of the insoluble support is increased by swelling, so that macromolecules can diffuse into it. The physical properties of cellulose *trans*-2,3-carbonate are irreversibly destroyed unless it is properly stored.

A highly-substituted carboxymethylcellulose gel with a high degree of hydration has been prepared by precipitation from a solution of the sodium salt, treatment with formaldehyde, and freezing.[175] The gel was converted into an azide derivative, which was used to immobilize glucoamylase. A method for determining the d.s. of carboxymethylcelluloses has been improved.[227] Carboxymethylcellulose has been used in studies of the action of rennin on κ-casein.[228]

Microcrystalline cellulose reacted with thionyl chloride in DMF to yield chlorodeoxycellulose, which was then converted into deoxyhydrazinocellulose

[222] A. Gangneux, D. Wattiez, and E. Marechal, *European Polymer J.*, 1976, **12**, 543.
[223] J. T. Guthrie, M. B. Huglin, R. W. Richards, V. I. Shah, and A. H. Simpson, *European Polymer J.*, 1975, **11**, 527.
[224] J. F. Kennedy, P. A. Keep, and D. Catty, *Biochem. Soc. Trans.*, 1976, **4**, 135.
[225] J. F. Kennedy, in 'Insolubilised Enzymes', ed. M. Salmona, C. Saronio, and S. Garattini, Raven Press, New York, 1974, p. 29.
[226] J. F. Kennedy and A. Rosevear, *J.C.S. Perkin I*, 1974, 757.
[227] J. G. Zadow, *Svensk Papperstidn.*, 1975, **78**, 376.
[228] M. Miyoshi, C.-H. Yoon, F. Ibuki, and M. Kanamori, *Agric. and Biol. Chem. (Japan)*, 1976, **40**, 347.

on treatment with hydrazine hydrate.[229] Deoxyhydrazinocellulose complexed metal ions in aqueous solution.

The e.s.r. spectra of cupriethylenediamine dihydroxide and cupriammonium hydroxide complexes of cellulose, hydrocellulose, and other carbohydrates have been measured at ambient and low temperatures.[230] It appears that complexation is chemical rather than physical, with the covalent character of the copper ligand being comparatively weaker in the complexes. A strong bond is formed between the copper atom and the vicinal 2,3-diol group of D-glucopyranosyl residues.

Deoxythiocyanatocelluloses have been prepared in high yield by treating chlorodeoxycellulose with potassium thiocyanate in DMF.[231] The chlorodeoxy- and deoxythiocyanato-celluloses exhibited moderate antibacterial activities. 6-Deoxycellulose has been prepared from 2,3-di-*O*-acetyl-6-*O*-toluene-*p*-sulphonyl-cellulose.[232]

Celluloses dyed with Remazol Brilliant Blue R, *etc.*, have been used as substrates in determining the solubilizing activities of cellulase complexes.[233]

Tris and epichlorohydrin reacted with cellulose to give a weakly cationic adsorbent (ECTHAM-cellulose), which was used in the fractionation of chromatins.[234]

The effects of salts, solvents, and substituents on the reactions of cellulose with epoxy-compounds have been reinvestigated and the results compared with previously published data.[235]

DEAE-cellulose has been used to prepare active immobilized derivatives of chymotrypsin,[236] lactate dehydrogenase, alcohol dehydrogenase, penicillin amidase, and glucoamylase,[237, 238] and water-soluble conjugates with gluco-amylase.[238] The adsorption is presumed to be of an ionic nature.

Hydroxypropylmethylcellulose (a surface-active agent) is superior to agarose for determining chloride, bromide, and iodide ions.[239]

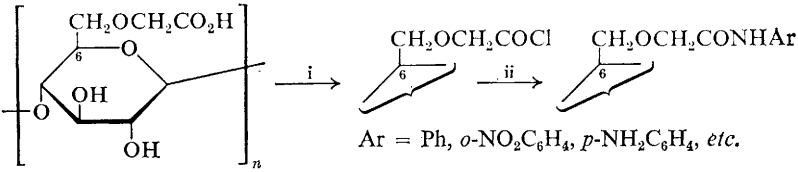
An active immobilized form of the enzyme was obtained when β-D-glucosidase reacted with cellulose cyclic imidocarbonate.[240]

Studies on the degradation of cellulose nitrites in DMF have been reported.[241]

Syntheses of 2,4-dinitro-, 3,5-dinitro-, and 4-nitro-benzoylcelluloses and of arylcarboxymethylcellulose amides (Scheme 12) have been reported.[189] Macro-molecular antibiotics [*e.g.* (32)] were obtained when semisynthetic penicillin derivatives were coupled with cellulose derivatives of this type.

A cellulose ion-exchanger containing salicylic acid has been prepared by the reactions outlined in Scheme 13.[242] The material is stable in acid and in alkali.

[229] S. Machida and Y. Sueyoshi, *Angew. makromol. Chem.*, 1976, **49**, 171.
[230] M. Z. Elsabee, S. M. Mattar, and G. M. Habashy, *J. Polymer Sci., Polymer Chem.*, 1976, **14**, 1773.
[231] T. L. Vigo, G. F. Danna, and C. M. Welch, *Carbohydrate Res.*, 1975, **44**, 45.
[232] A. I. Usov, R. G. Krylova, and I. Kostelian, *Bull. Acad. Sci. U.S.S.R.*, 1975, **24**, 2657.
[233] M. Leisola and M. Linko, *Analyt. Biochem.*, 1976, **70**, 592.
[234] R. T. Simpson, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 552.
[235] Y. Tanaka, Y. Shimura, and H. Shiozaki, *Makromol. Chem.*, 1976, **177**, 1301.
[236] D. L. Regan, M. D. Lilly, and P. Dunnill, *Biotechnol. and Bioeng.*, 1974, **16**, 1081.
[237] E. N. Oreshkin, L. A. Nakhapetyan, and L. M. Vainer, *Priklad. Biokhim. i Mikrobiol.*, 1974, **10**, 856.
[238] B. Solomon and Y. Levin, *Biotechnol. and Bioeng.*, 1974, **16**, 1161.
[239] M. Kapel, J. C. Fry, and D. R. Shelton, *Analyst*, 1975, **100**, 570.
[240] V. R. Srinivasan and M. W. Bumm, *Biotechnol. and Bioeng.*, 1974, **16**, 1413.
[241] D. Mislovičová and M. Pašteka, *Cellulose Chem. Technol.*, 1974, **8**, 481.
[242] P. Burba and K. H. Lieser, *Z. analyt. Chem.*, 1976, **279**, 17.

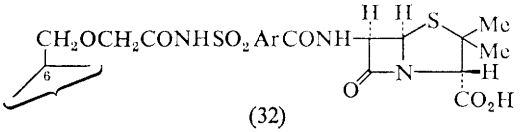


Reagents: i, $[Me_2\overset{+}{N}{:}CHCl]Cl^-$; ii, $ArNH_2$

Scheme 12

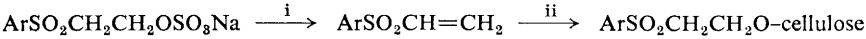
Exchange equilibria with Fe^{3+}, UO_2^{2+}, Cu^{2+}, and Co^{2+} ions were reached fairly rapidly, and Th^{4+} and Fe^{3+} ions were exchanged preferentially.

Studies of the molecular conformation of cellulose sulphate in gels indicated that there are no double helices present.[243] Stereoviews of the cellulose sulphate molecule are shown in Figure 1.



(32)

An enzymic synthesis of cDNA has been carried out on thymidine polynucleotide–cellulose, which is formed when cellulose reacts with thymidine 5′-phosphate in the presence of a carbodi-imide.[244] The product DNA was used in assays of mRNA.



Ar = salicylic acid

Reagents: i, NaOH; ii, cellulose–NaOH–DMSO

Scheme 13

The dynamic mechanical properties of cellophanes (from cellulose xanthate) containing various amounts of water have been measured at 100—520 K and 10^{-2}—10^2 Hz.[245] Four mechanical transitions were identified.

Cellulose has been cross-linked using epichlorohydrin.[246] The cross-linked cellulose reacted with *NN*-diethyl-2,3-epoxypropylamine to give 3-(*NN*-diethylamino)-2-hydroxypropylcellulose, which was used in the fractionation of polysaccharides and other polymers.[247] Reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with either 6-*O*-tritylcellulose 2,3-diphenylcarbanilate or the corresponding detritylated derivative followed the same course as that described for the corresponding amylose derivative (see p. 456), yielding a modified cellulose with 5—8 D-glucosyl residues between the branch points.[191]

[243] S. Arnott, D. W. L. Hukins, R. L. Whistler, and C. W. Baker, *Carbohydrate Res.*, 1974, **35**, 259.
[244] S. Levy and H. Aviv, *Biochemistry*, 1976, **15**, 1844.
[245] S. A. Bradley and S. H. Carr, *J. Polymer Sci., Polymer Phys.*, 1976, **14**, 111.
[246] L. Kuniak, *Cellulose Chem. Technol.*, 1974, **8**, 255.
[247] M. Antal and R. Toman, *J. Chromatog.*, 1976, **123**, 434.

Colloidal solutions of a graft copolymer of wood-pulp cellulose and poly-(acrylate-acrylamide) have been obtained by high-speed shearing of a slurry of fibres of the cellulose derivative.[248] The colloidal solutions exhibited high viscosity at low solute concentrations and are pseudoplastic and thixotropic.

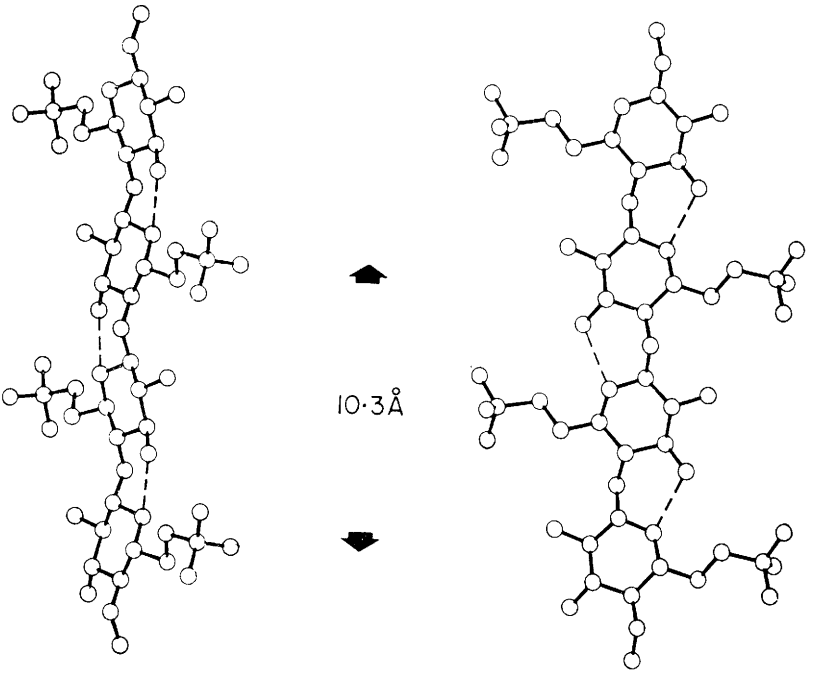


Figure 1 *Two views of cellulose sulphate; the broken lines represent hydrogen bonds between* O-3 *and* O-5 *on adjacent residues*

Graft copolymers of cellulose and poly(itaconic acid) have been prepared for the first time.[249]

The role of solvent alcohol in the photosensitized copolymerization or graft polymerization of styrene with cellulose has been investigated.[250] Photo-induced grafting of poly(styrene-*alt*-acrylonitrile) to cellulose yielded a product containing hetero- and homo-polymer chains.[251] The effects of the matrix and concentration on the rate of reaction and the products have been investigated in persulphate-initiated grafting of poly(styrene-*alt*-acrylonitrile) onto cellulose.[252]

The graft polymerization of vinyl monomers onto cellulose has been initiated using a system containing sodium bisulphite and soda-lime glass.[253] Both the

[248] P. Le Poutre and A. A. Robertson, *Tappi*, 1974, **57**, 87.
[249] K. Dimov, D. Dimitrov, E. Terlemezian, and M. Semkova, *Cellulose Chem. Technol.*, 1975, **9**, 575.
[250] N. P. Davis, J. L. Garnett, and R. Urquhart, *J. Polymer Sci., Polymer Letters*, 1976, **14**, 537.
[251] N. G. Gaylord and T. Tomono, *J. Polymer Sci., Polymer Letters*, 1975, **13**, 697.
[252] N. G. Gaylord and T. Tomono, *J. Polymer Sci., Polymer Letters*, 1975, **13**, 689.
[253] O. Y. Mansour and A. A. B. Moustafa, *J. Polymer Sci., Polymer Chem.*, 1975, **13**, 2795.

crude and true grafting yields increased to a limit with increased ratios of methyl methacrylate to cellulose, but decreased when the rate of addition of sodium bisulphite radicals exceeded that of polymerization. The mechanism of initiation is assumed to involve the trapping of bisulphite radicals within the glass to form a so-called 'sulphur-impregnated solid', which is paramagnetic and acts as a source of free radicals in inducing polymerization and graft copolymerization.[254]

An extensive study has been made of the graft polymerization of vinyl monomers (*e.g.* methyl methacrylate, sodium vinyl sulphonate, 4-vinylpyridine, acrylamide, and acrylic acid) onto dissolving pulp and groundwood initiated by acetic acid and hydrogen peroxide.[255] It is possible to predict accurately the extent of grafting and the properties of the graft copolymers. The effects of solvents on the radiation-induced grafting of vinylpyridines onto cellulose have been examined.[256]

Chitin-coated cellulose (produced by treating cellulose with alkali-chitin) has been used in the purification of lysozyme [257] and a derivative of lysozyme [258] by affinity chromatography. DNA derivatives of cellulose have been used in the chromatography of phosphorylated, nucleolar nonhistone [925] and in the purification of a glucocorticoid receptor from rat liver.[260]

A method for trapping enzymes (*e.g.* β-D-galactosidase and D-glucose isomerase) within cellulose fibres is based on addition of the enzyme to a solution of cellulose and *N*-ethylpyridinium chloride in DMF and then precipitation of the cellulose derivative with water.[261] The product could be fixed with glutaraldehyde.

Oligo(deoxythymidylic acid) adsorbed onto cellulose has been used as an affinity-chromatography matrix for isolating mRNA; RNAs containing poly-(adenylic acid) are retained on the matrix.[262]

The decomposition of benzoyl peroxide on cellulose has been studied.[263]

Chitins.—4-Methylumbelliferyl [*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-$(1 \rightarrow 4)]_{1 \text{ or } 2}$-2-acetamido-2-deoxy-β-D-glucopyranosides have been obtained in good yield by reaction of the peracetylated glycosyl chlorides with sodium 4-methylumbelliferone in DMF.[264] The trisaccharide glycoside is a good substrate for lysozyme.

The 1,5-lactone obtained by oxidation of tetra-*N*-acetylchitotetraose has been used to probe the active site of lysozyme. [265]

Chitosans.—Acylated chitosan gels have been prepared by treatment of chitosan with acetic, propionic, and butyric anhydrides.[266] The molecular aggregation of

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[255] H. Hatakeyama and B. Rånby, *Cellulose Chem. Technol.*, 1975, **9**, 583.
[256] J. L. Garnett and E. C. Martin, *J. Polymer Sci., Polymer Letters*, 1976, **14**, 35.
[257] T. C. Fletcher and A. White, *Comp. Biochem. Physiol.*, 1976, **55B**, 207.
[258] T. Imoto, M. Fujimoto, and K. Yagishita, *J. Biochem.* (*Japan*), 1974, **76**, 745.
[259] J. Bearden, jun., and T. Chandra, *Mol. Cell. Biochem.*, 1976, **10**, 3.
[260] H. J. Eisen and W. Glinsmann, *Biochem. Biophys. Res. Comm.*, 1976, **70**, 367.
[261] Y. Y. Linko, L. Pohjola, R. Viskari, and M. Linko, *F.E.B.S. Letters*, 1976, **62**, 77.
[262] R. E. Pemberton, P. Liberti, and C. Baglioni, *Analyt. Biochem.*, 1975, **66**, 18.
[263] A. B. Baskina, G. M. Korneva, V. I. Ivanov, V. P. Melnikov, L. V. Karmilova, and N. S. Enikolopian, *Doklady Akad. Nauk S.S.S.R.*, 1976, **228**, 348.
[264] F. M. Delmotte, J. P. D. J. Privat, and M. L. P. Monsigny, *Carbohydrate Res.*, 1975, **40**, 353.
[265] L. O. Ford, L. N. Johnson, P. A. Machin, D. C. Phillips, and R. Tjian, *J. Mol. Biol.*, 1974, **88**, 349.
[266] S. Hirano and Y. Ohe, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 1337.

the partially *N*- and *O*-acylated gels is similar to that of cellulose gels. Conditions for deacylation of the gels were also investigated.

Glycolchitosan has been used as a substrate in studies of the cell-lytic activities of species of *Rhizopus* and *Bacillus*.[267]

Chitosan reacted with heparin to give a polyelectrolyte that is soluble in water–hydrochloric acid–methanol, or water–potassium bromide–acetone, or formic acid.[268]

Cycloamyloses.—The preparation of acetylated glycosyl bromide derivatives of higher malto-oligosaccharides has been studied using cyclohepta-amylose, and the products were coupled to derivatives of amylose.[181]

Cyclohexa-amylose reacted with an epoxy derivative of agarose to yield an affinity-chromatography matrix that is suitable for use in the purification of β-amylase.[40]

Partial methylation of cyclohexa-amylose gave dodeca-*O*-methylcyclohexa-amylose in which all the primary and C-2 hydroxy-groups are etherified.[269] *O*-Alkylated polymers obtained by treating cyclohexa-amylose with epichlorohydrin under basic conditions catalysed the chlorination of anisole with hypochlorous acid, giving 4-chloroanisole with high (99%) regioselectivity. Dodeca-*O*-methylcyclohexa-amylose afforded greater selectivity than cyclohexa-amylose, and various features of the chlorination were discussed.

Heptakis-(2-*O*-methyl)cyclohepta-amylose has been prepared by methylation of heptakis-(6-bromo-6-deoxy)cyclohepta-amylose and regeneration of the primary hydroxy-groups in the resulting heptakis-(6-bromo-6-deoxy-2-*O*-methyl)-cyclohepta-amylose by benzoate exchange and debenzoylation.[270] The *O*-methyl groups in tetradeca-*O*-methylcyclohepta-amylose are located at O-2 and O-6 of the hexose residues.

Determination of the crystal and molecular structures of the cyclohexa-amylose (α-cyclodextrin, α-CD)–methanol complex has shown that cyclohexa-amylose assumes an unstrained 'relaxed' structure, which is stabilized by a ring of hydrogen bonds between O-2 and O-3 of adjacent D-glucopyranosyl residues, on inclusion of methanol.[271] The results support the general mechanism (Figure 2) proposed for the formation of inclusion complexes.

The molecular motions in inclusion complexes formed by cyclohexa-amylose with 4-methylcinnamate, 3-methylcinnamate, and 4-t-butylphenate anions have been studied by ^{2}H and ^{13}C nuclear relaxation methods.[272] The results showed that the time taken for the substrate to reorientate increases by a factor of *ca.* 4 on inclusion, whereas the increase in tumbling motion depends on the substrate. It was pointed out that, in general, a molecular complex should be described not only by its thermodynamic stability and formation and dissociation kinetics, but also by the *dynamic rigidity*, defined by the coupling between the molecular motions of the two (or more) entities of which it is composed.

[267] Y. Tsujisaka, Y. Tominaga, and M. Iwai, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 145.
[268] Y. Kibuchi, *Makromol. Chem.*, 1974, **175**, 2209.
[269] R. Breslow, H. Kohn, and B. Siegel, *Tetrahedron Letters*, 1976, 1645.
[270] K. Takeo and T. Kuge, *Stärke*, 1976, **28**, 226.
[271] B. Hingerty and W. Saenger, *J. Amer. Chem. Soc.*, 1976, **98**, 3357.
[272] J. P. Behr and J. M. Lehn, *J. Amer. Chem. Soc.*, 1976, **98**, 1743.

The *X*-ray structure of a cyclohexa-amylose–kryptin complex has been reported and a mechanism proposed for the inclusion process.[273] Cycloamyloses have been shown to form complexes with nitroxyl radicals; the complexes were examined by e.s.r. spectroscopy.[274]

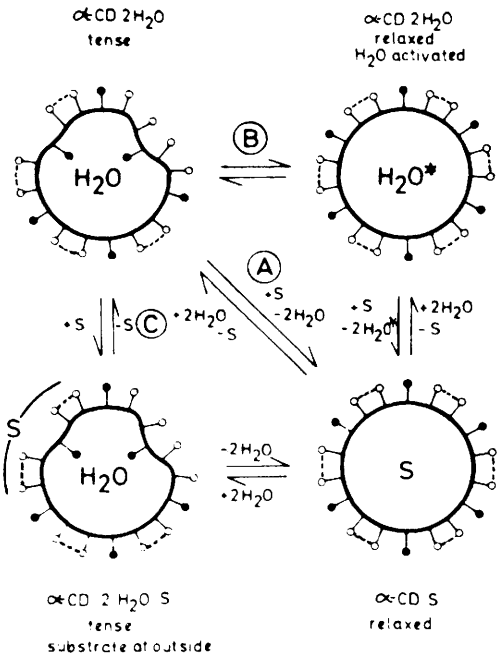


Figure 2 *Schematic representation of the α-cyclodextrin (CD)-substrate inclusion process. The 'empty' α-CD molecule in the upper left-hand corner corresponds to the α-CD,$(H_2O)_2$ complex found in the crystalline state. The molecule has a collapsed, distorted conformation with only four* O-2···O-3 *hydrogen bonds formed and corresponds to a high-energy, 'tense' state. Upon inclusion of a substrate molecule,* via *routes* A, B, *or* C, *it goes into a low-energy, 'relaxed' state with an unstrained conformation and all six* O-2···O-3 *hydrogen bonds (indicated by broken lines) formed.* H_2O^* *represents 'activated water' in unstrained, relaxed α-CD*

Cyclohepta-amylose is able to bind non-polar substrates (*e.g.* ferrocene, fluorobenzene, anisole, pyridine, toluene, and 4-t-butylcyclohexanol) in polar, non-aqueous media (*e.g.* DMSO and DMF).[275] Significant rate increases were found for cycloamylose-promoted processes (*e.g.* the deacylation of 3-t-butylphenyl acetate) in non-aqueous and in mixed (*e.g.* 60% DMSO) solvents.

C.d., u.v., and ^1H n.m.r. spectroscopy have been used to investigate the inclusion of *N*-substituted phenothiazines (tranquillizing drugs) by cyclohepta-amylose in aqueous solution.[276] Formation constants were calculated for the 1:1 complexes obtained. The spectral changes produced on complexation

[273] S. Wolfrom and N. Mathias, *Chem. Ber.*, 1976, **109**, 503.
[274] J. Martinie, J. Michon, and A. Rassat, *J. Amer. Chem. Soc.*, 1975, **97**, 1818.
[275] M. Siegel and R. Breslow, *J. Amer. Chem. Soc.*, 1975, **97**, 6869.
[276] M. Otagiri, K. Uekama, and K. Ikeda, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 188.

indicated that the aromatic ring of the phenothiazine is included in the cavity, whereas the *N*-substituents interact with groups outside the cavity.

The interactions of cyclohexa- and cyclohepta-amyloses with non-steroidal anti-inflammatory drugs, particularly the effects of inclusion on the solubility and stability of the drugs in water, have been studied.[277] The formation of inclusion complexes with cyclohepta-amylose increased the solubility of a number of the drugs in water and also accelerated the degradation of agar–propazone in water. These inclusion complexes can also be obtained in powdered form by freeze-drying and coprecipitation methods.[278]

The fluorescence of 6-*p*-toluidinylnaphthalene 2-sulphonate was appreciably enhanced in solutions of cyclohexa-, cyclohepta-, and cyclo-octa-amyloses.[279] The increased fluorescence was used to study the kinetics of inhibition by maltose and cyclohexa-amylose of the hydrolysis of cyclohepta-amylose by α-amylase. The binding of cyclohepta-amylose to α-amylase has been studied.[280]

Dextrans.—End-labelled isomalto-oligosaccharides have been used in studies of the action pattern of a dextranase.[281]

Periodate-oxidized dextrans have been coupled to the ragweed-pollen allergen (antigen E) and then reduced with sodium borohydride to stabilize the linkages.[282] The ratio of dextran to antigen E in two of the products (mol. wt. 1.0×10^5 and 1.40×10^5) was in the range 2—5 : 1. The antigenic and allergenic activities of the coupled antigen are *ca.* 7 times less than those of the free antigen, but the activities were restored on treatment of the coupled antigen with dextranase. The coupled antigen stimulated the formation of anti-dextran antibodies in rabbits. Periodate-oxidized dextran coupled readily with trypsin to give a soluble immobilized derivative of the enzyme.[283]

Perbenzylated dextran has been prepared.[31]

In an attempt to clarify the effects of ionic and hydrophobic substituents on the polyelectrolytic behaviour of polysaccharides, potentiometric titrations and activity measurements of the counter-ions have been conducted on carboxymethyldextrans containing either substituted carboxy-groups or benzyl groups.[284]

'Dextran glass' (10^4 M_r dextran) attached to an alkylamine-bonded phase of glass has been used for the hydrophobic immobilization of enzymes.[209]

A Blue Dextran–agar complex and tritiated dextran have been used in a spectrophotometric assay of dextranase activity and in studies of the action patterns of dextranases, respectively.[285]

Dextran cyclic imidocarbonate reacted with nicotinamide adenine dinucleotide$^+$–N^6-[*N*-(6-aminohexyl)acetamide] to yield a water-soluble immobilized derivative of the coenzyme.[286]

[277] Y. Hamada, N. Nambu, and T. Nagai, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1205.
[278] M. Kurozumi, N. Nambu, and T. Nagai, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 3062.
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[280] S. Móra, I. Simon, and P. Elödi, *Mol. Cell. Biochem.*, 1974, **4**, 205.
[281] G. J. Walker and M. D. Dewar, *Carbohydrate Res.*, 1975, **39**, 303.
[282] T. P. King, L. Kochoumian, K. Ishizaka, L. M. Lichtenstein, and P. S. Norman, *Arch. Biochem. Biophys.*, 1975, **169**, 464.
[283] R. L. Foster, *Experientia*, 1975, **31**, 772.
[284] K. Gekko and H. Noguchi, *Biopolymers*, 1975, **14**, 2555.
[285] R. H. Staat and C. F. Schachtele, *Infection and Immunity*, 1974, **9**, 467.
[286] P. O. Larsson and K. Mosbach, *F.E.B.S. Letters*, 1974, **46**, 119.

The reactions of dextran, Sephadex, and methyl 4,6-*O*-benzylidene-α-D-glucopyranoside with cyanogen bromide have been carefully examined, and cyclic imidocarbonate, carbamate, and cyclic carbonate groups were found in the products.[287] An acyclic imidocarbonate structure could also be formed between two D-glucosyl residues in different dextran chains. Coupling between an activated carbohydrate and an amino-compound (*e.g.* a protein) involves the formation of isourea groups. The simultaneous release of ammonia, derived in part from the hydrolysis of carbamate groups but mostly from other groups in the activated carbohydrate, is independent of the coupling reaction.

Nicotinamide adenine dinucleotide^{+}–N^{6}-[*N*-(6-aminohexyl)acetamide] has been coupled to dextran cyclic imidocarbonate with a view to incorporating the immobilized coenzyme, as a cofactor, into a multi-enzyme electrode and model enzyme reactors.[288]

An improved procedure for the synthesis of stearoyldextrans of various d.p. relies on formation of the 2,4-benzeneboronate, which is then treated with stearoyl chloride in pyridine.[289] The chemical and immunological properties of these esters, particularly the detection of plaque-forming cells specific for the α-(1 → 6)-determinant, were examined.

Dextran sulphate has been shown to trigger off another pathway of complement activation.[290] The effects of the molecular weight and the degree of sulphation on this activity were studied; above a critical molecular weight (5×10^3), only the degree of sulphation is responsible for complement activation. Clusters of several D-glucosyl residues, each carrying one or two sulphate groups, are thought to be essential for activating the alternative pathway.

Acetylated forms of Sephadex (dextran cross-linked with epichlorohydrin) have been used in the isolation of various proteolipids,[291] and Sephadex has been etherified by reaction with alkaline sodium borohydride and 1-allyloxy-2,3-epoxypropane.[292] Treatment of the etherified Sephadex with mercuric acetate gave a polymer containing covalently-bound, monofunctional mercury, which was used in the separation of nucleotides.

A Sephadex cyclic imidocarbonate derivative reacted with colicins E1, E2, and E3 to give products that were used to investigate the reactivities of colicins with cells.[293]

A spacer arm, 3,3′-iminobispropylamine, has been attached to Sephadex LH-20 (a lipophilic gel).[294] A phenyltrimethylammonium group was then attached to the free end of the spacer arm and the product was used to isolate a cholinergic proteolipid.

Sephadexes have been used as affinity matrices in the purification of an *exo*-amylase from *Pseudomonas stutzeri*,[295] mammalian glucoamylase ('glucoamylase–

[287] K. Broström, S. Ekman, L. Kågedal, and S. Akerström, *Acta Chem. Scand.* (*B*), 1974, **28**, 102.
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[290] R. Burger, U. Hadding, H. U. Schorlemmer, V. Brade, and D. Bitter-Suermann, *Immunology*, 1975, **29**, 549.
[291] J. Monreal, *Biochim. Biophys. Acta*, 1976, **427**, 15.
[292] D. W. Gruenwedel, M. G. Heskett, and J. E. Lammert, *Biochim. Biophys. Acta*, 1975, **402**, 7.
[293] C. Lau and F. M. Richards, *Biochemistry*, 1976, **15**, 666.
[294] H. Saraceno and E. de Robertis, *Biochem. Biophys. Res. Comm.*, 1976, **69**, 555.
[295] H. Dellweg, M. John, and J. Schmidt, *European J. Appl. Microbiol.*, 1975, **1**, 191.

maltase complex'),[296] porcine-kidney α-L-fucosidase,[297] and human-liver α-D-glucosidases.[298]

An investigation of the interaction of lysozyme with Sephadex showed that there are only a few binding sites on the polysaccharide matrix (*ca.* 1 in 10^5 D-glucosyl residues).[299]

Glycogens.—The complete hydrolysis of glycogen with acid [300] and the complete benzylation of glycogen [31] have been reported.

Glycosaminoglycans and Proteoglycans.—Standard procedures for the depolymerization of glycosaminoglycans with nitrous acid, to give products containing anhydro-sugar residues,[301] and for dyeing glycosaminoglycans with Methylene Blue have been reported.[32]

The precipitation of glycosaminoglycans with scandium ions forms the basis of a spectrophotometric determination, the excess of scandium ions being determined with Xylenol Orange.[302]

Chondroitin 4-[^{35}S]sulphate, which was prepared using a rat chondrosarcoma, yielded a labelled heptasaccharide having a terminal, non-reducing 2-acetamido-2-deoxy-D-galactopyranosyl 4-sulphate residue on enzymic hydrolysis.[303]

Chondroitin 4-sulphate and cartilagenous proteoglycans form ionic complexes with lysozyme.[304] The complexes are solubilized by either salts or alkali, although a higher salt concentration is needed to solubilize the proteoglycan complexes. The distribution of charges on the complexes and the implications of the formation of ionic complexes in tissues were discussed.

Self-diffusion coefficients have been measured for Na^+, Ca^{2+}, and Sr^{2+} cations associated with a preparation of chondroitin 6-sulphate that also contained hyaluronic acid.[305] These studies have implications for the transport of cations in connective tissues.

Deaminative cleavage of heparin and reduction of the resulting disulphated disaccharide with sodium borotritide afforded 2,5-anhydro-4-*O*-(α-L-idopyranuronosyl 2-sulphate)-D-[1-^{3}H]mannitol 6-sulphate, which was used as the substrate in an assay for α-L-idopyranosyluronic acid 2-sulphate sulphatase.[306] Heparin has been labelled *in vivo* with ^{35}S-sulphate.[307]

Trimethylsilylated derivatives of heparin (d.s. 1 or 4) have been prepared.[308]

A method for converting the hexuronic acid residues of heparin into hexose residues has been described.[309] Thus, treatment of heparin with 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide transformed the carboxy-groups into

[296] S. Sivakami and A. N. Radhakrishnan, *Indian J. Biochem. Biophys.*, 1973, **10**, 283.
[297] G. Ya. Vidershain and A. A. Prokopenkov, *Biochemistry* (*U.S.S.R.*), 1975, **40**, 694.
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[304] R. A. Greenwald and C. E. Schwartz, *Biochim. Biophys. Acta*, 1974, **359**, 66.
[305] H. Magdelenat, P. Turq, and M. Chemla, *Biopolymers*, 1974, **13**, 1535.
[306] T. W. Lim, I. G. Leder, G. Bach, and E. F. Neufeld, *Carbohydrate Res.*, 1974, **37**, 103.
[307] S. Ögren and U. Lindahl, *J. Biol. Chem.*, 1975, **250**, 2690.
[308] R. E. Harmon and K. K. De, *J. Carbohydrates, Nucleosides, Nucleotides*, 1974, **1**, 477.
[309] J. E. Shively and H. E. Conrad, *Methods in Carbohydrate Chem.*, 1976, **7**, 149.

activated intermediates (lactones ?), which could be reduced with sodium borohydride.

Heparin (as the cetylpyridinium salt) has been immobilized by reaction of its residual peptide chain with agarose cyclic imidocarbonate; the immobilized mucopolysaccharide was used in separating two forms of α-L-iduronidase.[99] Heparin reacted with chitosan to give a novel polyelectrolyte complex.[268]

Hyaluronic acid can be labelled with fluorescein, with only slight degradation, to give a derivative of d.s. 0.001—0.05.[310]

The carboxy-groups of hyaluronic acid have been esterified using diazomethane and the esterified product was used to test the substrate specificities of hyaluronate lyases.[311]

Treatment of hyaluronic acid with hydrazine removed some of the *N*-acetyl groups, which were reconstituted on reacetylation with tritiated acetic anhydride; the tritiated mucopolysaccharide was used as a substrate in a semimicro assay for hyaluronidase.[312] Tritium-labelled hyaluronic acid, labelled by the tritium-recoil technique, has been treated with β-D-glucuronidase to yield tritiated oligosaccharides containing terminal, non-reducing 2-acetamido-2-deoxy-β-D-glucopyranosyl residues.[313]

Laminarins.—Laminarin has been fully benzylated by two cycles of treatment with benzyl chloride in DMSO–sodium hydride.[31]

Levans.—The kinetics of the acid hydrolysis of *Streptococcus salivarius* levan have been measured by continuous examination of the weight-average molecular weight using light-scattering techniques.[314] Two first-order reactions were observed: a rapid reaction in which linkages at branch points are cleaved and a slower reaction in which linkages in the main chain are cleaved. Thus, the hydrolysis of this levan proceeds by a mechanism that differs from the completely random hydrolysis of dextran, glycogen, and amylopectin.

Mannans.—A low-molecular-weight mannan has been fully benzylated by two cycles of treatment with benzyl chloride in DMSO–sodium hydride.[31]

Mannan from baker's yeast has been stearoylated and phosphorylated; gel filtration was used to separate the water-soluble component of the resulting stearoylated mannan into two fractions.[315]

Pachymans.—Acetylated pachyman [a β-(1 → 3)-D-glucan] has been cast and stretched to form an oriented film.[316] The *X*-ray diffraction pattern of the film showed that the polysaccharide chains are parallel, rather than antiparallel, and favour a right-handed helix.

Pectic Acids and Pectins.—Pectin has been converted into a *s*-triazinyl derivative having C-6 of the hexuronic acid residues incorporated into the heterocyclic ring.[34] The actions of polygalacturonase and acid on the derivative were investigated.

[310] A. N. De Belder and K. O. Wik, *Carbohydrate Res.*, 1975, **44**, 251.
[311] H. Greiling, H. W. Stuhlsatz, T. Eberhard, and A. Eberhard, *Connective Tissue Res.*, 1975, **3**, 135.
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[316] T. L. Bluhm and A. Sarko, *Biopolymers*, 1975, **14**, 2639.

Pullulans.—Conditions for the complete benzylation of pullulan with benzyl chloride in DMSO–sodium hydride in a single step have been reported.[31]

Starches.—Conditions have been reported for the complete hydrolysis of starch with acid so that no significant acid-catalysed reversion occurred.[300]

Periodate-oxidized starch has been incorporated into poly(vinyl alcohol) membranes; the properties, including the permeability, of the membranes were studied.[317]

A Blue Starch-agar plate,[318] Phadebas Blue starch,[319, 320] and Remazol Brilliant Blue starch [321] have been used in the detection of amylase activity.

A report on the preparation of 2-hydroxyethylated D-glucoses is of relevance to the preparation of the corresponding starch derivatives.[322]

Starch cyclic imidocarbonate reacted with a 6-aminohexyl derivative of agarose to give an affinity matrix suitable for use in the purification of phosphorylases.[167]

The uses of starch xanthide in the manufacture of paper [323] and rubber [324] have been discussed.

Starch cross-linked with epichlorohydrin reacted with *NN*-diethyl-2,3-epoxypropylamine to form an ion-exchange material that is suitable for the fractionation of polysaccharides and other biopolymers.[247] Starches cross-linked with either epichlorohydrin or phosphorus oxychloride adsorb α-amylase.[325]

Procedures for encapsulating pesticides in starch involve the oxidative cross-linking of starch xanthate in the presence of the pesticide.[326] Subsequent release of the pesticide can be slowed down by incorporating a small amount of latex with starch xanthate prior to cross-linking.

The reaction of a cross-linked starch with sodium 2-chloroethyl-3-chloropropyl- or 3-chloro-2-hydroxypropyl-sulphonate has been used in the preparation of ion-exchange matrices.[327] The reaction with 3-chloro-2-hydroxypropylsulphonate proceeded in high yield, even at low temperatures, and did not require an organic solvent.

Acrylonitrile has been grafted onto a gelatinized DEAE-starch to give stable, latex-like dispersions of the copolymer; the extent of grafting at ambient temperature is related to the nitrogen content of the starch derivative.[328] Dispersions of the copolymers having $\leqslant 50\%$ of grafted poly(acrylonitrile) afforded clear adhesive films on air- and heat-drying on glass.

[317] Y. Nozawa, F. Higashide, and T. Kanakoto, *J. Polymer Sci., Polymer Letters*, 1975, **13**, 433.
[318] T. Takeuchi, T. Matsushima, T. Sugimura, T. Kozu, T. Takeuchi, and T. Takemoto, *Clin. Chim. Acta*, 1974, **54**, 137.
[319] G. Skude, *Scand. J. Clin. Lab. Invest.*, 1975, **35**, 41.
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Starch has been converted into cold-soluble and cold-swelling derivatives by reaction with ethyl cyanide or by graft polymerization with acrylic esters and saponification.[329]

Graft copolymers of polychloroprene and a cationic starch imparted wet-strength properties to paper when applied as wet-end additives to pulp, and they also exhibited cold-contact adhesive properties without pressure being applied.[330] Poly(styrene-*alt*-acrylonitrile) has been grafted onto starch.[331]

β-Amylase adsorbed onto starch has been used as an immobilized form of the enzyme.[332] Lipoproteins interacted with starch and prevented the formation of starch–iodine complexes.[333]

Xylans.—Conditions for converting xylan into a fully benzylated derivative in one step have been reported.[31]

The hexuronic acid side-chains of a methylated xylan have been selectively eliminated by reaction with either sodium methoxide or sodium methylsulphinyl methide.[33]

Miscellaneous.—The properties of flavonoid glycosides of oligosaccharides have been briefly reviewed.[334] 5-Acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-α-nonulosonyl-(2 → 3)-[1-^{3}H]lactitol has been used as a substrate in an assay of neuraminidase.[335]

Stoicheiometric reduction of the hexuronic acid residues of the capsular polysaccharide from *Aerobacter aerogenes* has been described (see also p. 468).[309]

Barley β-D-glucan reacted with Reactone Red 2B to give a product that was used in an investigation of the substrate specificity of a barley β-D-glucanase.[336] The relative ease of hydrolysis of α-(1 → 6)-linkages in branched D-glucans has been investigated.[300]

β-(1 → 3)-Linked D-glucans with their reducing-end residue periodate-oxidized and reduced have been used as substrates in mechanistic studies of an *exo*-β-1,3-D-glucanase; the amount of glycerol released indicates the extent of initial attack on a chain, and the ratio of D-glucose to glycerol released indicates the extent of multiple attack.[337]

Gum arabic cross-linked with epichlorohydrin reversibly adsorbed an anti-(B haemagglutinin) from a *Streptomyces* species.[338]

Degradation of methylated leiocarpan A, the main polysaccharide in a gum exudate from *Anogeissus leiocarpus*, has been accomplished with DBU and acetic anhydride.[339]

A lysate of the cell walls of *Micrococcus lysodeikticus* reacted with agarose cyclic imidocarbonate to give an affinity matrix that is suitable for use in the purification of lysozyme and other bacteriolytic enzymes.[129]

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[333] A. Pastermack and U.-H. Stenman, *Clin. Chim. Acta*, 1975, **65**, 213.
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[338] Y. Fujita, K. Oishi, K. Suzuki, and K. Imahori, *Biochemistry*, 1975, **14**, 4465.
[339] G. O. Aspinall and A. S. Chaudhari, *Canad. J. Chem.*, 1975, **53**, 2189.

A procedure for replacing the labile acyl groups in the lipopolysaccharide from *Mycobacterium phlei* with radiolabelled *O*-methyl groups has been described.[340]

An oligosaccharide rich in 2-amino-2-deoxy-D-galactosyl residues from a *Neurospora* mutant has been *N*-acetylated using acetic anhydride.[341]

Reduction of the D-glucuronic acid residues of SIII pneumococcal polysaccharide yielded a substrate useful for distinguishing between various β-D-glucan hydrolases.[342]

A tritiated slime polysaccharide was obtained when seeds of *Zea mays* were grown in the presence of L-[^{3}H]fucose.[343]

Modification of Glycoproteins and Uses of Modified Glycoproteins

Introduction.—Procedures employing enzymes in the structural elucidation of glycoproteins have been reported.[344]

Albumins.—In a new technique called affinity electrophoresis, human serum albumin becomes bound in competitive fashion to two forms of immobilized anti-(human serum albumin) antibody.[64]

An 11-α-hydroxy-4-pregnene-3,20-dione hemisuccinyl derivative of bovine serum albumin has been prepared for use as an immunogen.[345]

Antibodies and Immunoglobulins.—The interactions of homogeneous myeloma immunoglobulins with polysaccharide antigens have been reviewed.[36]

Rabbit anti-(human immunoglobulin IgG) antibodies, horse anti-(rabbit immunoglobulin) antibodies, and ovalbumin, all labelled with fluorescein isothiocyanate, and goat anti-(rabbit immunoglobulin) antibodies labelled with tetramethylrhodamine isothiocyanate have been used with insolubilized antigens in new microfluorometric immunoassays.[71]

Reduced rabbit immunoglobulin IgG coupled to β-D-galactosidase has been used in an enzyme-linked immunoassay for human immunoglobulin IgG.[63]

Ceruloplasmins.—Desialylized ceruloplasmin has been shown to bind *Ricinis communis* lectin.[138]

Erythrocyte-membrane Glycoproteins.—Human erythrocyte-membrane glycoprotein has been modified to a tryptic glycopeptide, which was desialylized by mild acid treatment.[346] The non-reducing, terminal D-galactosyl residue of the glycopeptide was labelled by enzymic oxidation and reduction with sodium borotritide.

Fetuins.—Desialylized fetuin has been shown to bind *R. communis* lectin.[138] The terminal *N*-acetylneuraminic acid residues of fetuin have been converted into 5-acetamido-3,5-dideoxy-L-*arabino*-2-[7-^{3}H]heptulosonic acid residues by oxidation with periodate and reduction with potassium borotritide.[347] The modified fetuin is a substrate for neuraminidase.

The effect of phospholipase on the binding of desialylized, ^{125}I-labelled orosomucoid by rat-liver plasma membranes has been investigated.[348]

[340] G. R. Gray, *Methods in Carbohydrate Chem.*, 1976, **7**, 157.
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[343] K. Wright, D. H. Northcote, and R. M. Davey, *Carbohydrate Res.*, 1976, **47**, 141.
[344] Yu-Teh Li and Su-Chen Li, *Methods in Carbohydrate Chem.*, 1976, **7**, 221.
[345] F. Dray, J.-M. Andrieu, and F. Renaud, *Biochim. Biophys. Acta*, 1975, **403**, 131.

Table 6 *Modification of glycoproteins by coupling to insoluble matrices and other macromolecules*

Glycoprotein	*Macromolecule or matrix coupled*	*Use of product*	*Ref.*
α_1-Acid glycoprotein	Reaction with agarose cyclic imidocarbonate	Isolation of neuraminidase by affinity chromatography	78
Albumin		Spacer arm in the formation of affinity-chromatography material—other ligands were added with the aid of water-soluble carbodi-imides [a]	67
Albumin (bovine)	Glutaraldehyde-cross-linked with arylsulphatase on platinum	Enzyme electrode in a selective assay of sulphate ion	355
Anti-arginase antibody	Reaction with agarose cyclic imidocarbonate	Purification of arginase by immunoadsorption	56
Anti-(*Bacillus subtilis* α-amylase) antibody		Removal of cross-reacting materials from modified *B. subtilis* α-amylase by immunoadsorption	57 [b]
Anti-enterotoxin antibody	Reaction with (16) [c]	Purification of the enterotoxin from *Clostridium perfringens* by affinity chromatography	58
Anti-(D-glucose oxidase) antibody	Reaction with agarose cyclic imidocarbonate	Purification of D-glucose oxidase by immunoadsorption	61
Anti-(β-D-glucuronidase) antibody		Characterization of porcine antibodies (immunoglobulins IgG) to rabbit β-D-glucuronidase	62
Anti-(human C3PA) antibody		Purification of human C3 proactivator by immunoadsorption	59
Anti-(human α-foetoprotein) antibody		Radioimmunoassay of human α-foetoprotein	60
Anti-(human immunoglobulin IgG) antibody		Enzyme-linked immunoassay of human immunoglobulin IgG	63
Anti-(human serum albumin) antibody	Reaction with a cyanogen-bromide-activated phloroglucinol derivative of agarose [d] Reaction with agarose cyclic imidocarbonate Reaction with (17) [c] Reaction with (16) [c]	Immunoadsorption of human serum albumin modified for affinity electrophoresis	64
Anti-(rabbit immunoglobulin) antibody	Ethyl chloroformate-aided polymerization	Solid-phase reagent in an enzymic immunoassay of progesterone	345

Table 6 (*cont.*)

Glycoprotein	*Macromolecule or matrix coupled*	*Use of product*	*Ref.*
Castor-bean lectin	Reaction with agarose cyclic imidocarbonate	Comparison of the binding abilities of two forms of α-L-iduronidase	99
Concanavalin A	Binding to D-glucose oxidase	Studies on the interaction of concanavalin A with D-glucose oxidase and of the resulting enzymic activity	356
	Glutaraldehyde-cross-linked	Purification of carcinoembryonic antigen by affinity chromatography	357
	Glutaraldehyde-linked to nylon fibres	Isolation of immunogenic tumour cells by cell-affinity chromatography	358
	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of serum proteins	100
		Affinity chromatography of low-molecular-weight human folate-binding proteins	101
		Comparison of the binding capacities of two forms of α-L-iduronidase	99
		Crossed immunoaffinoelectrophoresis of glycoprotein enzymes	102
		Investigations of the binding of human-fibroblast interferon to concanavalin A and the affinity-chromatography characteristics of immobilized concanavalin A	104
		Investigation of αα-trehalases	105
		Isolation of peroxidase by affinity chromatography	106
		Matrix for the immobilization of laccase	47
		Purification of β-D-2-acetamido-2-deoxy-hexosidases A and B by affinity chromatography	76
		Purification of arylsulphatase A by affinity chromatography	108
		Purification of β-D-galactosidase and β-D-glucuronidase isoenzymes from normal human liver by affinity chromatography	327
		Purification of glycoside hydrolases by affinity chromatography	109
		Purification of hyaluronidase by affinity chromatography	110

		Purification of α-D-mannosidase by affinity chromatography	87
		Removal of polysaccharide contaminants from preparations of DNA by affinity chromatography	111
		Separation of membrane glycoproteins from rat-brain synaptic vesicles by affinity chromatography	112
		Studies of the interactions of the carbohydrate-binding sites on concanavalin A with adipocyte receptors	113, 114
		Studies of the binding of glycophorin A to immobilized lectins	115
		Studies of the binding of glycoproteins in microsomal and Golgi membranes to lectins	116
		Studies of the interactions of *Mucor* sp. acid proteases with concanavalin A	117
Crotalaria juncea lectin	Reaction with agarose cyclic imidocarbonate	Affinity chromatography of serum 'proteins'	100
Crotalaria lectin		Studies of the binding of glycoproteins in microsomal and Golgi membranes to lectins	116
Ferritin		Isolation of rabbit immunoglobulin anti-milk antibodies	68
Fetuin		Fractionation of normal and immune sera immunoglobulins that react with fetuin	69
		Preparation of a protease-free preparation of neuraminidase	120
	Reaction with bromoacetylcellulose	Preparation of a protease-free preparation of neuraminidase	120
Fungal cell-wall glycoproteins	Reaction with agarose cyclic imidocarbonate	Isolation of glycoside hydrolases by affinity chromatography	121
α-D-Galactopyranosyl-binding lectin from *Bandeiraea simplicifolia*		Studies of the interactions of *Bandeiraea simplicifolia* lectin with model carbohydrate–protein conjugates and polysaccharides by affinity chromatography	122
Glycoprotein from the cell walls of *Saccharomyces cerevisiae*		Isolation of an *exo*-β-D-glucanase from a mixture of D-glucanases and D-glucosidases by affinity chromatography	121
Human chorionic gonadotrophin	Reaction with activated divinylsulphonylagarose	Isolation of anti-(luteinizing hormone) antibody	173

Table 6 (*cont.*)

Glycoprotein	*Macromolecule or matrix coupled*	*Use of product*	*Ref.*
Human immunoglobulin IgG		Isolation of Clq-binding immune complexes	70
	Reaction with agarose cyclic imidocarbonate	Microfluorometric immunoassays with solid-phase antigens by an inhibition process	71
Human immunoglobulin IgM		Microfluorometric immunoassays with solid-phase antigens	71
Luteinizing hormone	Reaction with activated divinylsulphonylagarose	Isolation of anti-(luteinizing hormone) and anti-(luteinizing hormone β-subunit) antibodies	173
Mouse immunoglobulin anti-(*Bacillus subtilis* α-amylase) antibody		Removal of cross-reacting materials from modified *B. subtilis* α-amylase by immunoadsorption	57 [b]
Mouse immunoglobulin IgM paraprotein		Microfluorometric immunoassays with solid-phase antigens by an inhibition process	71
Ovalbumin	Reaction with agarose cyclic imidocarbonate	Microfluorometric immunoassays with solid-phase antigens by a competitive immunoadsorption process	71
Ovomucoid		Isolation of trypsin by affinity chromatography	131
Porcine immunoglobulin IgG anti-(rabbit β-D-glucuronidase) antibody		Characterization of porcine antibodies (immunoglobulins IgG) to rabbit β-D-glucuronidase	62
Prolactin	Reaction with activated divinylsulphonylagarose	Isolation of anti-prolactin antibody	173
Rabbit ala3/b4 immunoglobulin IgG	Reaction with cellulose *trans*-2,3-carbonate	Isolation of sheep anti-b4 antibody by immunoadsorption	224
Rabbit immunoglobulin anti-arginase antibody		Purification of arginase by immunoadsorption	56
Rabbit immunoglobulin Ig anti-(D-glucose oxidase) antibody		Purification of D-glucose oxidase by immunoadsorption	61
Rabbit immunoglobulin Ig anti-(human α-foetoprotein) antibody		Radioimmunoassay of human α-foetoprotein	60

Rabbit immunoglobulin IgG	Reaction with agarose cyclic imidocarbonate	Investigation of the reversible binding of T-cell factor on immobilized immunoglobulins	75
Rabbit immunoglobulin IgG anti-(human C3PA) antibody		Purification of human C3 proactivator by immunoadsorption	59
Rabbit immunoglobulin IgG anti-(human immunoglobulin IgG) antibody		Enzyme-linked immunoassay of human immunoglobulin IgG	63
Rabbit immunoglobulin IgG $F(ab)_2$ fragment		Investigations of the reversible binding of T-cell factor on immobilized immunoglobulins	75
Rabbit immunoglobulin IgM			
Rabbit immunoglobulins anti-enterotoxin	Reaction with (16) [c]	Purification of the enterotoxin from *Clostridium perfringens* by affinity chromatography	58
Red kidney-bean agglutinin	Reaction with agarose cyclic imidocarbonate	Studies of the binding of glycophorin A to immobilized lectins	115
Ricinis communis agglutinin		Purification and separation by affinity chromatography of polysaccharides containing terminal, non-reducing D-galactosyl residues	138
Sheep anti-b4 antibody	Reaction with cellulose *trans*-2,3-carbonate	Separation of rabbit allotypic b4 immunoglobulin (κ) IgG from b4-negative immunoglobulin (λ) IgG	224
Sheep immunoglobulin anti-(rabbit immunoglobulin) antibody	Ethyl chloroformate-aided polymerization	Solid-phase reagent in an enzymic immunoassay of progesterone	345
$[^{125}I]$Transferrin	Adsorption onto culture tubes of polypropylene	Investigations of the interactions of immobilized transferrin with reticulocytes and fibroblasts	359
Wheat-germ agglutinin	Reaction with agarose cyclic imidocarbonate	Isolation of glycophorin A by affinity chromatography	115
		Study of the binding of glycoproteins in microsomal and Golgi membranes to lectins	116

[a] See (18), Scheme 9a. [b] Derivative not adequately characterized. [c] See Schemes 9a and 9b. [d] See p. 452.

Ovalbumins.—Ovalbumin labelled with fluorescein isothiocyanate has been used with insolubilized antigens in microfluorometric immunoassays.[71] The chemical and spectroscopic properties of the product obtained when *N*-[4-(2-benzimidazolyl)phenyl]maleimide reacts with hen egg-white lysozyme have been examined.[349]

Phytohaemagglutinins.—Photoaffinity labelling of succinyl-concanavalin A with 4-azidophenyl α-D-mannopyranoside gave a derivative that showed weak, but definite, mitogenic activity against lymphocytes.[350] [^{3}H]Concanavalin A has been prepared.[351]

Transferrins.—A study of the metabolism of desialylized transferrins has shown that bovine, canine, and porcine asialotransferrins are not cleared from rabbit plasma *via* the Ashwell–Morell pathway (*cf.* human asialotransferrin).[352] The glycopeptides of human transferrin are heterogeneous, so that the removal of 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid residues exposed structures having different affinities for the hepatic asialoglycoprotein receptor.[353] Related work on the metabolism of desialylized transferrins has been reported.[354]

Immobilized Derivatives of Glycoproteins.—The modification of glycoproteins to give active immobilized derivatives continues to attract attention. These derivatives and their uses are listed in Table 6.[355–359]

Affinity chromatography on immobilized concanavalin A in the presence of sodium dodecyl sulphate has been used in separations of glycoproteins.[112]

Modification of Enzymes and Uses of Modified Enzymes

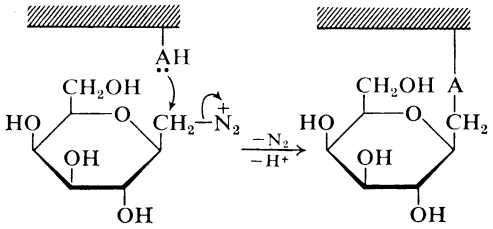
β-D-2-Acetamido-2-deoxyglucosidases.—Human placental β-D-2-acetamido-2-deoxyglucosidases A and B have been desialylized with neuraminidase, but desialylization did not transform the A form into the B form.[360]

β-D-Galactosidases.—β-D-Galactosidase derivatized with either reduced rabbit immunoglobulin IgG[63] or progesterone[345] has been used in enzyme-linked immunoassays of human immunoglobulin IgG and progesterone, respectively.

Mouse-liver β-D-galactosidase has been transformed into a denatured and reduced form.[361] Treatment of many forms of this enzyme with neuraminidase markedly reduced their electrophoretic mobilities.

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[358] J. J. Killion and G. M. Kollmorgen, *Nature*, 1976, **259**, 674.
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[361] S. Tomino and M. Meisler, *J. Biol. Chem.*, 1975, **250**, 7752.

2,6-Anhydro-1-deoxy-1-diazo-D-*glycero*-L-*manno*-heptitol blocked the active site of the β-D-galactosidase from *Escherichia coli* by the reaction shown in Scheme 14.[362] An immunoassay for anti-adenosine antibodies is based on *E. coli* β-D-galactosidase coupled to adenosine, the binding of the complex to the antibodies being measured with 2-nitrophenyl β-D-galactopyranoside as a substrate.[363]



Scheme 14

β-D-Glucuronidases.—Removal of the terminal neuraminic acid residues from rabbit-liver β-D-glucuronidase decreased the number of multiple forms, chiefly those of lowest p*I*, observed by isoelectric focusing.[364]

***endo*-β-D-2-Acetamido-2-deoxyglucanases.**—[^{3}H]Dansylated derivatives of (D-Glc*N*Ac*p*)$_{0 \text{ or } 2}$-(D-Man*p*)$_6$-(D-Glc*N*Ac*p*)$_2$-Asn were rapidly hydrolysed by an *endo*-β-D-2-acetamido-2-deoxyglucanase from *Streptomyces griseus*.[365]

α-Amylases.—Porcine pancreatic α-amylase has been *S*-carboxymethylated and the *S*-carboxymethylated derivative treated with trypsin.[366] Examination of the products suggested reasons for the heterogeneity of preparations of this enzyme. Although the ε-amino-group of the lysyl residue of porcine pancreatic α-amylase involved in binding Cl^- ions is blocked by reaction with 2,4-dinitrofluorobenzene, the enzyme derivative retained its substrate-binding ability and amylolytic activity.[367] α-Limit dextrins and maltose, which bind to the active site, protected the enzyme from dinitrophenylation at least as effectively as the Cl^- effector. A radioimmunoassay for porcine pancreatic amylase is based on the ^{125}I-derivative obtained by the lactoperoxidase method.[368]

Acetylation of a tyrosyl residue in *Bacillus subtilis* amylase led to partial loss of the enzymic activity towards a series of malto-oligosaccharides, indicating that a tyrosyl residue located near the catalytic site is involved in the productive binding of substrates.[369] The difference spectrum of a tryptophanyl residue in *Bacillus subtilis* α-amylase was significantly decreased following treatment with

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[369] M. Ohnishi, T. Suganuma, and K. Hiromi, *J. Biochem.* (*Japan*), 1974, **76**, 7.

N-bromosuccinimide.[370] Immune responses to a heavy conjugate of *Bacillus subtilis* α-amylase and 4-diazoquinoline 1-oxide have been examined.[57]

***exo*-β-D-1,3-Glucanases.**—An *exo*-β-D-1,3-glucanase from a Basidiomycete has been modified by photo-oxidation, or by treatment with hydrogen peroxide under acidic conditions or with diphenyldiazomethane, or by oxidation with *N*-bromosuccinimide.[371] Photo-oxidation rapidly inactivated the enzyme, owing to the decomposition of histidyl residues, whereas the results of oxidation with *N*-bromosuccinimide indicated that a tryptophanyl side-chain is involved in, but is not necessary for, catalytic activity.

Glucoamylases.—Although the molecular weight and the carbohydrate content of *Aspergillus awamori* glucoamylase were reduced on treatment with subtilisin, most of the enzymic activity was retained.[372] The modified enzyme was less stable and its ability to hydrolyse starch was diminished.

Reduction and carboxymethylation of *A. niger* glucoamylase resulted in complete loss of enzymic activity, whereas *O*-acetylation of three of the 13 tyrosyl residues resulted in loss of 20% of the enzymic activity.[373] The tryptophanyl residues of *Rhizopus niveus* glucoamylase have been modified by treatment with *N*-bromosuccinimide so that the subsite structure of the enzyme could be studied.[374]

Hyaluronate Lyases.—Hyaluronate lyase from group A Streptococci has had its histidyl and some other amino-acid residues modified by photo-oxidation in the presence of Methylene Blue.[311]

Lysozymes.—The properties of denatured forms of lysozyme and of α-lactalbumin have been compared.[375] Various reactions have been carried out on a peptide containing two disulphide linkages in order to locate the antigenic site of lysozyme,[376] and the effects of anhydrous hydrogen fluoride on hen egg-white lysozyme have been studied by u.v. and c.d. spectroscopy and *X*-ray crystallography.[377]

Modification of both tryptophanyl residues in a two-disulphide peptide of lysozyme by reaction with 2,3-dioxo-5-indolinesulphonic acid gave a derivative possessing a similar conformation to that of the unmodified peptide.[378] Immunological and o.r.d. and c.d. spectroscopic studies of the modified peptide yielded information on the structure of the antigenic site of the enzyme.

An enzymically inactive product obtained on treating hen egg-white lysozyme with iodine has been studied by *X*-ray diffraction methods.[379] The electron-density map showed that the carboxy-group of glutamic acid-35 had moved very close to tryptophan-108, probably by formation of an ester linkage.

[370] M. Ohnishi, H. Kegai, and K. Hiromi, *J. Biochem.* (*Japan*), 1975, **78**, 247.
[371] D. R. Peterson and S. Kirkwood, *Carbohydrate Res.*, 1975, **41**, 273.
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Tryptophan-62 or -108 (or both) of hen egg-white lysozyme has been specifically oxidized.[380] Lysozyme containing an oxindolylalanine residue at position 108 has been used to study the heats of dilution, saccharide-binding, and self association of hen egg-white lysozyme.[381] Lysozyme containing an oxindolylalanine residue at position 35 or 62 or 108 has also been prepared.[382]

The binding of 2-acetamido-2-deoxy-α- and -β-D-glucopyranoses to hen egg-white lysozyme with its tryptophan-62 residue oxidized has been investigated.[383] It appears that the α-anomer binds at the subsite in two mutually exclusive orientations, one of which corresponds to that adopted by the β-anomer. The oxidized enzyme has been purified by affinity chromatography on a chitin-coated cellulose, and its activities towards glycolchitin and bacterial cells were examined.[258] The activities of other derivatives of the enzyme were also examined.

Reduction of hen egg-white lysozyme with diborane and oxidation of the reduced disulphide linkages yielded a homogeneous derivative in which the carboxy-groups of aspartic acid-119 and the chain-end leucyl residue are reduced to the corresponding alcohols; peptide mapping showed that the disulphide linkages had reformed correctly.[384]

The enzymic activity of a reduced peptide (residues 1—27) from hen egg-white lysozyme was restored by oxidation with glutathione.[385] Studies on the mechanism of this process have provided information on the folding pathway of the reduced enzyme.[386]

Succinylation of hen egg-white lysozyme yielded at least six products (disc electrophoresis).[387] Homogeneous derivatives were isolated by ion-exchange chromatography and the positions of derivatization were established. One of the fractions exhibited slight (10%) enzymic activity.

Tritiated and ^{131}I-labelled hen egg-white lysozymes have been used to measure the intestinal adsorption, tissue distribution, and degradation of the enzyme.[388]

Oligo-1,6-D-glucosidase : Sucrose α-D-Glucohydrolases.—Reaction of an oligo-1,6-D-glucosidase : sucrose α-D-glucohydrolase (sucrase–isomaltase) complex from rabbit small intestines with [^{3}H]conduritol-B epoxide (an active-site-directed inhibitor) has permitted the amino-acid sequences around the essential carboxy-groups in the active site to be determined.[389]

αα-Trehalases.—The effects of photo-oxidation and of treatment with diethyl pyrocarbonate on the activities of human- and porcine-kidney αα-trehalases have indicated that an imidazole group is present at the active site of each enzyme.[105]

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Acid Proteases.—The ability to bind concanavalin A was destroyed when the carbohydrate residues of acid proteases and renin from a *Mucor* species were oxidized with periodate ion.[117]

α-Lactalbumins.—The properties of denatured forms of α-lactalbumin and of lysozyme have been compared.[375]

The tyrosyl residues of α-lactalbumin from human milk have been nitrated with tetranitromethane; three components, which differ in the extent of nitration, were detected.[390] The effect of nitration on the activity of α-lactalbumin in the lactose synthase system was investigated.

Treatment of human α-lactalbumin with diethyl pyrocarbonate reduced its activity in the lactose synthase system, although incubation of the modified enzyme with hydroxylamine restored the activity.[391]

Bovine α-lactalbumin reacted with either 2-hydroxy-5-nitrobenzyl bromide or *N*-bromosuccinimide to yield derivatives in which two of the four tryptophanyl residues were modified.[392] The chemical reactivities and fluorescence of these derivatives helped to establish the conformation of α-lactalbumin. The affinity of both derivatives for D-galactosyltransferase was less than that of the original enzyme, although K_m and V_{max} for lactose synthesis were unchanged. Reduction of all the disulphide linkages with 1,4-dithiothreitol markedly changed the luminescence of bovine α-lactalbumin (see p. 405), whereas reduction of a single, labile disulphide linkage had little effect.[393]

Sulphatases.—Desialylization of sulphatase 'A' from ox liver with neuraminidase slightly modified the charge on the molecule, but did not alter any other properties.[394] The modified enzyme was able to hydrolyse cerebroside sulphate.

Immobilized Derivatives of Enzymes.—Techniques used to immobilize enzymes have been discussed.[395] A mathematical model has been developed to assess how enzyme-catalysed reactions that involve the release or consumption of hydrogen ions behave when the enzyme is immobilized.[396] The Proceedings of a symposium covering the analysis and regulation of immobilized enzymes have been published.[397]

A study of the mechanical properties ('mechanochemistry') of immobilized enzymes (*e.g.* chymotrypsinogen trapped within a polyacrylamide gel) has provided a means whereby the chemical processes at a molecular level can be controlled by mechanical compression and decompression of the supporting gel (*i.e.* by controlling the pore size).[398] Mechanical compression of a chymotrypsinogen–polyacrylamide gel resulted in a 20-fold increase in the diffusion-controlled tryptic activation of chymotrypsinogen, the reaction rate

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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* [a]	*Use of product*	*Ref.*
Acetobacter sp. cells	—	Chelation by zirconium hydroxide	Living immobilized cells for enzymic conversions; continuous production of acetic acid from ethanol	399
Achromobacter liquidum cells	—	Entrapment in polyacrylamide gel	Active immobilized L-histidine ammonia lyase for the continuous production of urocanic acid	400
Acid phosphatase	3.1.3.2	Reaction with concanavalin A	Active immobilized enzyme	102 [b]
Alanine amino-transferase	2.6.1.1	Reaction with acylazide-activated collagen		401
Alcohol dehydrogenase	1.1.1.1	Adsorption onto DEAE-cellulose	Active immobilized enzyme used to test a theoretical model for intraparticle diffusion	236
		Carbodi-imide-assisted reaction with copolymers of acrylamide and acrylic acid	Active immobilized enzyme	402
		Entrapment within polyacrylamide beads		
		Reaction with cyanogen bromide-treated copolymers of acrylamide and 2-hydroxyethyl methacrylate		
		Reaction with glutaraldehyde-treated polyacrylamide and with copolymers of acrylamide and methyl methacrylate	Active immobilized enzyme used to study the effect of a hydrophobic environment on enzymic reactions	403
		Reaction with glutaraldehyde-treated polyacrylamide beads	Active immobilized enzyme	402
Alkaline phosphatase	3.1.3.1	Coating on a platinum electrode and enclosure within nylon netting	Active immobilized enzyme used in the production of a phosphate electrode	404 [c]
		Hydrophobic interaction with cellulose esters and with phenoxyacetylated-dextran and other derivatives of glass	Comparison of methods for the hydrophobic immobilization of enzymes with retention of activity	209

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Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
L-Amino-acid oxidase	1.4.3.2	Glutaraldehyde-cross-linked to an amino-alkyl derivative of glass	Enzyme-reactor electrode for determining amino-acids	405
Aminoacylase	3.5.1.14	Glutaraldehyde-cross-linked to silanized inorganic supports	Active immobilized enzyme; resolution of racemic amino-acids using ceramic-based enzymes	406
Aminopeptidase (cytosol)	3.4.11.1	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; spectrophotometric determination of bound protein	42
α-Amylase	3.2.1.1	Adsorption onto a phenol-formaldehyde resin	Investigation of the effects of immobilization on the specificity of α-amylase	407
		Glutaraldehyde-cross-linked	Purification of rabbit immunoglobulin Ig anti-(porcine pancreatic α-amylase) antibody; solid-state immunoassay of α-amylase	368
		Reaction with activated, cross-linked poly-(4-methacryloxybenzoic acid)	Active immobilized enzyme	408
β-Amylase	3.2.1.2	Adsorbed onto starch	Isolation of the enzyme; immobilized enzyme	332
		Carbodi-imide-mediated reaction with acrylic acid–acrylamide copolymer	Active immobilized enzyme used in the continuous conversion of starch into maltose	409 [d]
Arylsulphatase	3.1.6.1	Glutaraldehyde-cross-linked with albumin on platinum	Enzyme electrode for a selective assay of sulphate ions	355
Aspartate amino-transferase	2.6.1.1	Diethyl adipimidate-assisted reaction with aminoethylcellulose	Active immobilized enzyme; steady-state kinetic analysis and stability studies of the immobilized enzyme	220
		Reaction with acylazide-activated collagen	Active immobilized enzyme	401
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme used in a micro-assay of L-aspartic acid	43 [e]
Carboxypeptidase C	3.4.12.1	Glutaraldehyde-cross-linked to amino-ethylcellulose	Active immobilized enzyme; studies of the substrate specificity of immobilized carboxypeptidase C	219
Catalase	1.11.1.6	Adsorption within the pores of controlled-pore titania	Active immobilized enzyme; stabilizer and activator of co-immobilized D-glucose oxidase	410 [f]

		Glutaraldehyde-cross-linked to an ill-defined support	Active immobilized enzyme; studies of the deactivation of immobilized catalase by hydrogen peroxide	411
		Reaction with derivatized silanized alumina or Kieselguhr	Active immobilized enzyme; studies of the deactivation of immobilized catalase by hydrogen peroxide	412
		Reaction with a diazo derivative of glass	Active immobilized enzyme for the direct analysis of D-glucose in blood using a mixed-bed reactor	413
Chymotrypsin	3.4.21.1	Adsorption onto DEAE-cellulose	Active immobilized enzyme used to test a theoretical model of intraparticle diffusion	236
		Carbodi-imide-coupling to an aminoalkyl derivative of polyacrylamide	Investigations of the immobilization of enzymes by covalent binding to amine supports	39
		Chelation by transition-metal hydroxides	Active immobilized enzyme	414
		Diazo-coupling to polyacrylamide	Active immobilized enzyme	39
		Glutaraldehyde-coupling to aminoalkyl derivatives of glass and cellulose	Investigations of the immobilization of enzymes by covalent binding to amine supports	39
		Immobilized within a poly(methacrylic acid) gel	Active immobilized enzyme; studies of the main principles of enzyme stabilization and of the increase in thermal stability on immobilization	415
		Radiation-induced cross-linking to agarose gel	Study of the thermodynamics of the irradiation process	416

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Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
		Radiation-induced cross-linking to DNA	Studies of the thermodynamics of the irradiation process and of the effects of radiation on DNA	416
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme	39
		Reaction with cyanogen bromide-treated aminoalkyl derivatives of polyacrylamide and glass	Investigations of the immobilization of enzymes by covalent binding to amine supports	39
		Reaction with periodate-oxidized macroporous agarose	Active immobilized enzyme	39
Chymotrypsinogen	see 3.4.21.1	Entrapment within polyacrylamide gel	Kinetic study of the tryptic activation of chymotrypsinogen in immobilized form; studies of the effects of mechanical forces on immobilized enzymes	398
Creatine kinase	2.7.3.2	Reaction with acylazide-activated collagen	Active immobilized enzyme	401
Dextranase	3.2.1.11	Chelation by transition-metal hydroxides	Active immobilized enzyme	414 [g]
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; comparison of the kinetic properties of immobilized dextranases	44
Escherichia coli cells	—	Chelation by zirconium hydroxide	Living immobilized cells for enzymic conversions	399
Esterase	—	Reaction with concanavalin A	Active immobilized enzyme	102 [b]
Ferredoxin hydrogenase	1.12.7.1	Reaction with aminoalkyl and succinyl derivatives of glass	Comparison of methods for the production of ferredoxin hydrogenase in an active immobilized form	417
β-D-Fructofuranosidase	3.2.1.26	Entrapment in poly(*N*-vinylpyrrolidone)	Active immobilized enzyme	418
		Reaction with vanacryl and covanacryl (polyaldehyde) polymers	Active immobilized enzyme; investigations of the immobilization parameters and the kinetics and stability of the immobilized enzyme	419
D-Fructose bisphosphate aldolase	4.1.2.13	Reaction with activated, cross-linked poly(4-methacryloxybenzoic acid)	Active immobilized enzyme	408

Fumarate hydratase	4.2.1.2	Entrapment in fibres of cellulose acetate	Continuous conversion of fumaric acid into L-malic acid	216
α-D-Galactosidase	3.2.1.22	Reaction with hydroxysuccinimide ester-activated nylon surfaces	Active immobilized enzyme	420
β-D-Galactosidase	3.2.1.23	Entrapment in cellulose fibres with optional glutaraldehyde-fixing	To show that enzymes are trapped in cellulose fibres	261
		Entrapment in a cross-linked poly-(2-hydroxyethyl methacrylate) gel	Active immobilized enzyme; kinetic studies of immobilized enzymes	421
		Entrapment in density-adjusted cellulose acetate or adsorption and cross-linking onto density-adjusted nylon	Active immobilized enzyme; alteration of the density of particles of the immobilized enzyme	422
		Entrapment in poly(*N*-vinylpyrrolidone)	Active immobilized enzyme	418
		Entrapment within the lattice of polyacrylamide gel	Active immobilized enzyme for the hydrolysis of whey and lactose in buffered solutions	423
		Glutaraldehyde-cross-linked to partially hydrolysed nylon tubing	Active immobilized enzyme for determining the flow kinetics of the enzyme immobilized in a tube	424
		Reaction with amino derivatives of porous glass by glutaraldehyde- or diazo-coupling or a carbodi-imide-assisted reaction	Active immobilized enzyme; comparison of the properties of free and immobilized β-D-galactosidase	425
		Reaction with hydroxysuccinimide ester-activated nylon surfaces	Active immobilized enzyme	420
Glucoamylase	3.2.1.3	Adsorption onto DEAE-cellulose		237, 238, 426

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Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
		Covalent attachment to porous particles of silica	Active immobilized enzyme for the continuous production of D-glucose from dextrin	427
		Entrapment in poly(*N*-vinylpyrrolidone)	Active immobilized enzyme	418
		Glutaraldehyde-cross-linked with gelatin, albumins, soybean protein, or casein with bentonite fillers, *etc.*	Active immobilized enzyme; studies of the binding of glucoamylase to inert proteins	428
		Reaction with azido derivative of carboxymethylcellulose	Comparison of the suitabilities of cellulosic and amylosic matrices for the immobilization of enzymes	175
		Reaction with copolymers of ethylene-maleic acid or styrene-maleic acid	Active immobilized enzyme	238 [h]
		Reaction with cyanogen-bromide-treated dialysis tubing	Active immobilized enzyme bound to an annular enzyme reactor	426
		Reaction with testosterone 3-(*O*-carboxymethyl)oxime	Enzymic immunoassay for testosterone	429 [i]
Glucoamylase (conjugated with ethylene-maleic acid or styrene-maleic acid copolymers)	see 3.2.1.3	Adsorption onto DEAE-cellulose	Active immobilized enzyme	238
D-Glucose isomerase	5.3.1.18	Adsorption onto Duolite A7 resin	Active immobilized enzyme used in the production of D-fructose-rich syrups	430
		Not given	Immobilized enzyme for the large-scale conversion of D-glucose into D-fructose by batch and continuous processes	431
		Entrapment in cellulose fibres with optional glutaraldehyde-fixing	To show that enzymes are trapped in cellulose fibres	261
D-Glucose oxidase	1.1.3.4	Adsorption onto clays	Studies of the adsorption and desorption of D-glucose oxidase on clays	432
		Adsorption within the pores of controlled-pore titania	Active immobilized enzyme; investigations of the effects of hydrogen peroxide and the flow rate on the enzymic activity	410 [j]
		Chelation by transition-metal hydroxides	Active immobilized enzyme	414
		Coating onto platinum electrode and enclosure within a nylon net	Active immobilized enzyme for the production of a phosphate electrode	404 [k]

Entrapment in a polyacrylamide gel	Active immobilized enzyme for testing a rate equation for the immobilized enzyme operating under pseudo one-substrate conditions	433
	Active immobilized enzyme for testing a rate equation for the immobilized enzyme acting on two limiting substrates	434
Glutaraldehyde-coupling to an alkylamino derivative of glass	Active immobilized enzyme; determination of heat changes in the proximity of immobilized enzymes with an enzyme thermistor, and the use of an enzyme thermistor in assays for metabolites	435
Interaction with concanavalin A	Active immobilized enzyme; investigation of the effect of complexation with concanavalin A on the enzymic activity	356
Reaction with agarose cyclic imidocarbonate	Purification of anti-(D-glucose oxidase) antibodies by immunoadsorption	61
Reaction with derivatives of attapulgites, Kieselguhr, *etc.*	Active immobilized enzyme; studies of the inactivation of the immobilized enzyme by hydrogen peroxide	436
Reaction with diazo derivatives of glass	Active immobilized enzyme for the direct analysis of D-glucose in blood using a mixed-bed reactor	413
Reaction with vanacryl and covanacryl (polyaldehyde) polymers	Active immobilized enzyme; investigations of immobilization parameters and the kinetics and stability of the immobilized enzyme	419

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Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
β-D-Glucosidase	3.2.1.21	Carbodi-imide-assisted reactions with copolymers of acrylamide and acrylic acid	Active immobilized enzyme	402
		Chelation by transition-metal hydroxides		414
		Entrapment within polyacrylamide beads		402
		Reaction with cellulose cyclic imidocarbonate	Active immobilized enzyme; comparison of the properties of free and immobilized β-D-galactosidase	240
		Reaction with cyanogen bromide-treated copolymers of acrylamide and 2-hydroxyethyl methacrylate	Active immobilized enzyme	402
		Reaction with a diazobenzoic acid-formaldehyde resin attached to nickel, cobalt, tin, iron, or aluminium	Active immobilized enzyme; magnetic immobilized enzyme	437
		Reaction with glutaraldehyde-treated polyacrylamide beads	Active immobilized enzyme	402
Glutamate dehydrogenase	1.4.1.2	Reaction with acylazide-activated collagen		401
D-Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; dissociation of the matrix-bound tetramer into enzymically active dimers	46
Hexokinase	2.7.1.1	Reaction with acylazide-activated collagen	Active immobilized enzyme	401
Inorganic pyrophosphatase	3.6.1.1	Hydrophobic interactions with cellulose esters and with phenoxyacetylated-dextran and other derivatives of glass	Comparison of various methods for the hydrophobic immobilization of enzymes with retention of activity	209
α-Lactalbumin	see 2.4.1.22	Reaction with agarose cyclic imidocarbonate	Purification of D-galactosyltransferase from skim milk	84
Lactate dehydrogenase	1.1.1.27	Adsorption onto DEAE-cellulose	Active immobilized enzyme used to test a theoretical model for intraparticle diffusion	236
		Adsorption onto benzylated, 2,3-dibromo-propan-1-ol-cross-linked agarose	Investigations of benzylated agar derivatives for the chromatography and electrophoresis of enzymes	41
		Reaction with acylazide-activated collagen	Active immobilized enzyme	401
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; investigations of conformational changes and matrix inhibition on the enzymic activity	48

Lysozyme	3.2.1.17	Reaction with activated, cross-linked poly(4-methacryloxybenzoic acid)	Active immobilized enzyme	408
		Reaction with alginic acid cyclic imidocarbonate	Active immobilized enzyme; catalysis of reactions using immobilized soluble/insoluble enzymes	179 [l]
Malate dehydrogenase	1.1.1.37	Reaction with acylazide-activated collagen	Active immobilized enzyme	401
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme for a microassay of L-aspartic acid	43 [m]
α-D-Mannosidase	3.2.1.24	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; comparison of the properties of the immobilized and free enzyme	49
Miscellaneous (unspecified)		Reaction with a graft copolymer of 4-nitrostyrene on polypropylene in which the nitro-groups were converted into isothiocyanate groups	Active immobilized enzyme	438
Monophenol monooxygenase	1.14.18.1	Interaction with immobilized concanavalin A	Active immobilized enzyme; investigations of the involvement of carbohydrate residues in the active site of the enzyme	47
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme	47
Penicillin amidase	3.5.1.11	Adsorption onto DEAE-cellulose	Active immobilized enzyme used to test a theoretical model for intraparticle diffusion	236
Penicillinase	3.5.2.6	Glutaraldehyde-coupling to aminoalkyl derivatives of glass	Active immobilized enzyme; determination of heat changes in the proximity of immobilized enzymes with an enzyme thermistor, and the use of an enzyme thermistor in assays for metabolites	435
		Reaction with a complex *N*-hydroxy-succinimide ester derivative of glass	Flowing-stream analyser for measuring penicillin in fermentation broths	439
Pepsin A	3.4.23.1	Carbodi-imide-assisted reaction with hydroxyalkyl methacrylate gels modified with 1,6-diaminohexane or 6-aminohexanoic acid	Active immobilized enzyme; studies of the kinetic properties and stability of immobilized pepsin	440

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Table 7 (*cont.*)

Enzyme	*E.C. No.*	*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
Pepsin	3.4.23.1/2/3	Reaction with agarose cyclic imidocarbonate	Purification of pepsin inhibitor from *Ascaris lumbricoides* by affinity chromatography	50
Peroxidase	1.11.1.7	Reaction with activated, cross-linked poly(4-methacryloxybenzoic acid)	Active immobilized enzyme; preparation of quinone derivatives	408
Phosphorylase	2.4.1.1	Entrapped in polyacrylamide gel by polymerization after gel electrophoresis	Active immobilized enzyme; investigation of a new means of immobilization	441
Propanediol dehydratase	4.2.1.28	Reaction with agarose cyclic imidocarbonate	Studies of the binding of cobaltamine and the subunit interactions of propanediol dehydratase (B_{12}-dependent)	398
Pseudomonas putida cells	—	Entrapment in polyacrylamide gel	Active immobilized L-arginine deaminase for the continuous production of L-citrulline	442
Pullulanase	3.2.1.41	Carbodi-imide-assisted reaction with acrylic acid-acrylamide copolymer	Active immobilized enzyme for the continuous conversion of starch into maltose	409 [n]
Ribonuclease	3.1.4.22/23	Reaction with agarose cyclic imidocarbonate		51
Ribonuclease I	3.1.4.22	Carbodi-imide-assisted reactions with copolymers of acrylamide and acrylic acid		
		Entrapment within polyacrylamide beads	Active immobilized enzyme	402
		Reaction with cyanogen bromide-treated copolymers of acrylamide and 2-hydroxyethyl methacrylate		
		Reaction with glutaraldehyde-treated polyacrylamide beads		
Ribulose bisphosphate carboxylase	4.1.19	Reaction with diazo derivatives of glass and glutaraldehyde-cross-linking with amino derivatives of glass	Active immobilized enzyme; determination of changes in the properties of ribulose bisphosphate carboxylase on immobilization	443
Saccharomyces cerevisiae cells	—	Chelation by zirconium hydroxide	Living immobilized cells for enzymic conversions	399
Subtilisin	3.4.21.14	Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme	39
		Glutaraldehyde-coupling to aminoalkyl derivatives of glass	Investigations of the immobilization of enzymes by covalent binding to amine supports	39
		Reaction with cyanogen bromide-treated aminoalkyl derivatives of glass		

Tannase	3.1.1.20	Glutaraldehyde-cross-linked to aminoalkyl derivatives of glass	Active immobilized enzyme; determination of the half-life of the immobilized enzyme	444
Trypsin	3.4.21.4	Carbodi-imide-assisted reactions with copolymers of acrylamide and acrylic acid	Active immobilized enzyme	402
		Chelation by transition-metal hydroxides		414
		Entrapment within polyacrylamide beads		402
		Glutaraldehyde-coupling to aminoalkyl derivatives of glass	Active immobilized enzyme; determination of heat changes in the proximity of immobilized enzymes with an enzyme thermistor, and the use of an enzyme thermistor in assays for metabolites	435
		Reaction with acylazide-activated collagen	Active immobilized enzyme	401
		Reaction with agarose cyclic imidocarbonate	Active immobilized enzyme; spectrophotometric methods for determining bound protein	42
			Purification of the isoinhibitors of trypsin from cows' colostrum by affinity chromatography	52
		Reaction with cyanogen bromide-treated copolymers of acrylamide and 2-hydroxyethyl methacrylate	Active immobilized enzyme	402
		Reaction with periodate-oxidized dextran	Kinetic study of a water-soluble, active immobilized form of the enzyme	283
		Reaction with glutaraldehyde-treated polyacrylic beads	Active immobilized enzyme	402
		Reaction with periodate-oxidized cellulose		217
Trypsinogen (reduced and *S*-carboxymethylated)	see 3.4.21.4	Reaction with agarose cyclic imidocarbonate	Study of how the refolding of trypsinogen depends on the endogenous disulphide bridges	171
Tryptophanase	4.1.99.1		Kinetic studies of coenzyme-binding and -dissociation on immobilized tryptophanase	53, 54

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[442] K. Yamamoto, T. Sato, T. Tosa, and I. Chibata, *Biotechnol. and Bioeng.*, 1974, **16**, 1589.
[443] J. Shapira, C. L. Hanson, J. M. Lyding, and P. J. Reilly, *Biotechnol. and Bioeng.*, 1974, **16**, 1507.
[444] H. H. Weetall and C. C. Detar, *Biotechnol. and Bioeng.*, 1974, **16**, 1095.

Table 7 (*cont.*)

Enzyme		*Matrix or macromolecule coupled* [a]	*Use of product*	*Ref.*
Urate oxidase	1.7.3.3	Glutaraldehyde-cross-linked and then coated onto a platinum electrode and enclosed within a nylon net	Active immobilized-enzyme used for the production of an oxygen-sensing electrode for determining uric acid	445
Urease	3.5.1.5	Carbodi-imide-assisted reaction with copolymers of acrylamide and acrylic acid	Active immobilized enzyme	402
		Coating onto Teflon-coated bar magnets and enclosure within a nylon net	Immobilized-enzyme stirrer; a specific enzyme electrode for urea	446
		Entrapment within polyacrylamide beads	Active immobilized enzyme	402
		Glutaraldehyde-cross-linked to an amino-alkyl derivative of glass	Enzyme-reactor electrode used in a determination of urea	447
			Active immobilized enzyme; determination of heat changes in the proximity of immobilized enzymes with an enzyme thermistor, and the use of an enzyme thermistor in assays for metabolites	435
		Hydrophobic interactions with cellulose esters and with phenoxyacetylated-dextran and other derivatives of glass	Comparisons of methods for the hydrophobic immobilization of enzymes with retention of activity	209
		Reaction with acylazide-activated collagen	Active immobilized enzyme	401
		Reaction with cyanogen bromide-treated copolymers of acrylamide and 2-hydroxyethyl methacrylate		402
		Reaction with glutaraldehyde-treated polyacrylamide beads		402

[a] Covalent coupling can be assumed unless otherwise indicated. [b] In affinity-electrophoresis media. [c] Immobilized together with D-glucose oxidase. [d] Immobilized together with pullulanase. [e] Immobilized together with malate dehydrogenase. [f] Immobilized together with D-glucose oxidase. [g] Immobilized with lysine and glutamic acid. [h] Active water-soluble derivatives. [i] Active water-soluble derivative. [j] Immobilized together with catalase. [k] Immobilized together with alkaline phosphatase. [l] Water-soluble or -insoluble derivative depending on the pH. [m] Coupled together with aspartate aminotransferase. [n] Immobilized together with α-amylase.

[445] M. Nanjo and G. G. Guilbault, *Analyt. Chem.*, 1974, **46**, 1769.
[446] G. G. Guilbault and W. Stokbro, *Analyt. Chim. Acta*, 1975, **76**, 237.
[447] G. Johansson and L. Ögren, *Analyt. Chim. Acta*, 1976, **84**, 23.

returning to the initial level after decompression of the gel. The increase in catalytic activity may be used to amplify weak mechanical effects.

Many preparations of immobilized enzymes and of enzymes derivatized by coupling to other macromolecules to give water-soluble and -insoluble forms have been reported during the past year. These derivatives and their applications are summarized in Table 7. Immobilized enzymes are referred to in this Table whether or not they are enzymically active.

The following information on immobilized glycoside and polysaccharide hydrolases and carbohydrate isomerases and oxidases is worthy of note. Hydrolysis of 4-nitrophenyl β-D-galactopyranoside by β-D-galactosidase attached to nylon tubing is largely diffusion-controlled at low substrate concentrations and low flow-rates, but diffusion control is less pronounced at higher values.[424] Guidelines were suggested for the design of open tubular heterogeneous enzyme reactors for industrial, biomedical, and analytical uses. β-D-Galactosidase attached to an activated nylon surface retained its enzymic activity.[420]

Immobilization of β-D-galactosidase either by entrapment in cellulose acetate or by adsorption with cross-linking onto nylon yielded materials that are unsuitable for use in fluidized bed reactors, since their densities are close to unity.[422] Heavier particles were obtained by incorporating tungsten or stainless steel.

Immobilization of β-D-galactosidase by entrapment in polyacrylamide gels did not affect the K_m value, activation energy, or pH optimum of the enzyme.[423] The kinetic behaviour of *E. coli* β-D-galactosidase trapped in a cross-linked poly(2-hydroxyethyl methacrylate) gel has been studied in a recirculation reactor system that uses 2-nitrophenyl β-D-galactopyranoside as a substrate.[421] The pH–activity profile of the gel-entrapped enzyme was bell-shaped at high concentrations of the substrate and, in contrast to that of the free enzyme, could be fitted to the titration curve of two ionizable groups having pK values 6.4 and 8.6. The immobilized enzyme had a higher pH optimum than the free enzyme and retained a larger proportion of its activity at pH >7.

No leakage of the enzymes was detected when glucoamylase, β-D-fructofuranosidase, or β-D-galactosidase were trapped within poly(*N*-vinylpyrrolidone) gels, although activity losses of $\leqslant 90\%$ occurred.[418] The gel-entrapped glucoamylase hydrolysed dextrin (mol. wt. 1.04×10^4) to the same extent as the free enzyme.

A novel, stable, water-insoluble coating on various transition metals (which can be ferromagnetic) has enabled β-D-glucosidase to be attached to them.[437]

The properties of α-D-mannosidase immobilized on agarose cyclic imidocarbonate are analogous to those of the free enzyme.[49]

Bacillus subtilis α-amylase immobilized on a phenol–formaldehyde resin displayed *exo*-activity, which might result from steric hindrance between the immobilized enzyme and substrates of high molecular weight (*e.g.* amylopectin).[407] Thus, cleavage of the peripheral D-glucosidic linkages produced small oligosaccharides only.

Kinetic studies have been completed on an immobilized enzyme system containing β-amylase and *Aerobacter aerogenes* pullulanase that is used for the continuous production of maltose from starch.[409]

Immobilization of *Brevibacterium fuscum* dextranase on agarose cyclic imidocarbonate reduced its specific activity by 70% but increased its pH stability, whereas its action pattern, thermal stability, and pH and temperature optima were unaffected.[44]

Highly-charged, water-soluble conjugates of glucoamylase and a copolymer of either ethylene or styrene and maleic acid have been prepared.[238] There is almost no steric hindrance between substrates and glucoamylase immobilized on a new carboxymethylcellulose support.[175] The pH optimum of the bound enzyme was shifted to a slightly higher value, but the thermal stability was not affected. The use of glucoamylase attached to porous silica for the continuous production of D-glucose from starches has been studied.[427] In pilot-scale runs, there was no appreciable loss of enzymic activity after 80 days of continuous operation. *Aspergillus* glucoamylase has been attached by ionic adsorption to an annular reactor formed by rolling up a sheet of DEAE-cellulose paper with a fibre-glass spacer.[426] The characteristics of this bound-enzyme reactor, which has a low pressure drop, were determined. Polyanionic conjugates of glucoamylase adsorbed onto cationic carriers showed a higher thermal stability than that of the native enzyme adsorbed onto identical carriers.[238]

The enzyme-catalysed isomerization of D-glucose to D-fructose has attracted a good deal of attention, particularly with the availability of immobilized forms of D-glucose isomerase.[431] The properties of D-glucose isomerase attached to a phenol–formaldehyde resin have been carefully studied; the immobilized enzyme possesses sufficient mechanical strength to stand up to the pressures of column flow, is active over broad ranges of temperature and pH, and is not so dependent on Mg^{2+} and Co^{2+} ions for activity.[430] An active immobilized form of D-glucose oxidase is also produced by precipitation of the enzyme with concanavalin A; this form could be reused without appreciable loss of enzymic activity.[356]

Modification of Gangliosides and Glycolipids and Uses of Modified Gangliosides and Glycolipids

Permethylated derivatives of carbohydrate-rich fractions that are not found in normal urine have been isolated from the urine of patients with types 1 and 2 G_{M_1}-gangliosidoses.[448]

'Tay–Sachs ganglioside' has been converted into 'ganglioside G_{A_1}' (33) by removal of the 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid residues with acid.[449]

$$\beta\text{-D-Gal}N\text{Ac}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow 1)\text{-ceramide}$$

(33)

The terminal, non-reducing 2-acetamido-2-deoxy-α-D-galactopyranosyl residue of a Forssman hapten can be oxidized by D-galactose oxidase.[450] Reduction of the oxidized residue with sodium borotritide gave (34). D-Galactosyl- and lactosyl-ceramides, asialo-G_{M_1}-ganglioside, and G_{M_1}-ganglioside were also labelled

[448] N. M. K. Ng Ying Kin and L. S. Wolfe, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 123.
[449] J. F. Tallman, R. O. Brady, J. M. Quirk, M. Villalba, and A. E. Gal, *J. Biol. Chem.*, 1974, **249**, 3489.
[450] M. Israel, G. Bach, T. Miyatake, M. Naiki, and K. Suzuki, *J. Neurochem.*, 1974, **23**, 803.

α-D-[6-^{3}H]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
(34)

in this way.[451, 452] G_{M_1}-ganglioside, asialo-G_{M_1}-ganglioside, and a globoside were also degraded to oligosaccharides by ozonolysis. The modified glycolipids and oligosaccharides were used as substrates in assessing the specificities of β-D-galactosidases.

Cerebroside sulphate labelled with tritium has been used as a substrate in an assay for cerebroside sulphatase activities using t.l.c.[453]

Modification of Carbohydrate-containing Antibiotics

Active immobilized forms of antibiotics have been prepared by treating the amino-groups of carbohydrate-containing antibiotics with cellulose *trans*-2,3-carbonate,[454] and by chelation to transition-metal hydroxides[455] and transition-metal derivatives of cellulose.[456]

[451] A. R. Rushton and G. Dawson, *Biochim. Biophys. Acta*, 1975, **388**, 92.
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